Lifestyle effects on ocular health

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Summary

Some lifestyle aspects may be a risk factor for certain diseases. However, short-term exposure of risk factors may not cause the disease but rather chronic exposure. In the case of eye health exposure to sunlight has been mentioned as a potential risk factor to pterygium, cataract, and macular degeneration for example. Dietary intake has been suggested as having a protective role in patients who are at risk of dry eye, cataract and age related macular degeneration. Poor dietary habits have also been linked as a potential risk factor in cataract but the literature is more restricted. Smoking is renowned as a risk factor in many systemic conditions and has been linked to ocular pathology.

The lifestyle effects that will be explored in this thesis are dietary intake, ultraviolet radiation exposure and whether someone is a smoker. The methods used to assess the effects of these three elements will be assessment of the tear film, lens function and macula pigment.

This thesis conveys the detrimental effects of smoking on tear film characteristics and a relationship was shown between the amounts that an individual smokes with the level of potential dry eye disease. The dietary intake was positively related to lower subjective symptoms of dry eye disease, and linked to amplitudes of accommodation, but there was no relationship found between diet and macular pigment. In one cohort used in this thesis a harmful effect of ultraviolet radiation exposure was seen with tear film. In the final experimental chapter, a transient effect of smoking was noted on tear film and amplitude of accommodation, and on pupil size.

There are indications for future work, however there, are some useful take home messages for patients and especially for clinicians involved in eye health or policy makers in the arena of public health.

Key words: Smoking, tear film, amplitude of accommodation, diet, transient effects on the eye

Dedication

For my parents

Acknowledgement

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Contents

LIST OF TABLES

List of Figures

Chapter 1

Introduction

Different lifestyle practices, such as smoking, diet, physical exercise, hot or cold climate exposure could have an impact on an individuals' health. These lifestyle factors may have a cumulative effect on the body to remain stable (Lipowicz *et al.*, 2013). The process of maintaining physiological stability in adaptation to environmental demands is called allostatic (Read and Grundy, 2012). This cumulative effect on the body may produce an allostatic load which is a cost of adaptation to cumulative stress and in biological perspective, the chronic activation of responsive physiological system is referred as stress (Clark *et al.*, 2007). Lifestyle factors such as smoking, diet, and exposure to sunlight may have cumulative and predisposing effects on the eyes, which mean they could an adverse effect on ocular health.

1.1 Existing knowledge associated with effect of lifestyles on ocular health

Smoking is capable of causing several types of cancers and many chronic health conditions as mentioned in figure number 1.1 below:

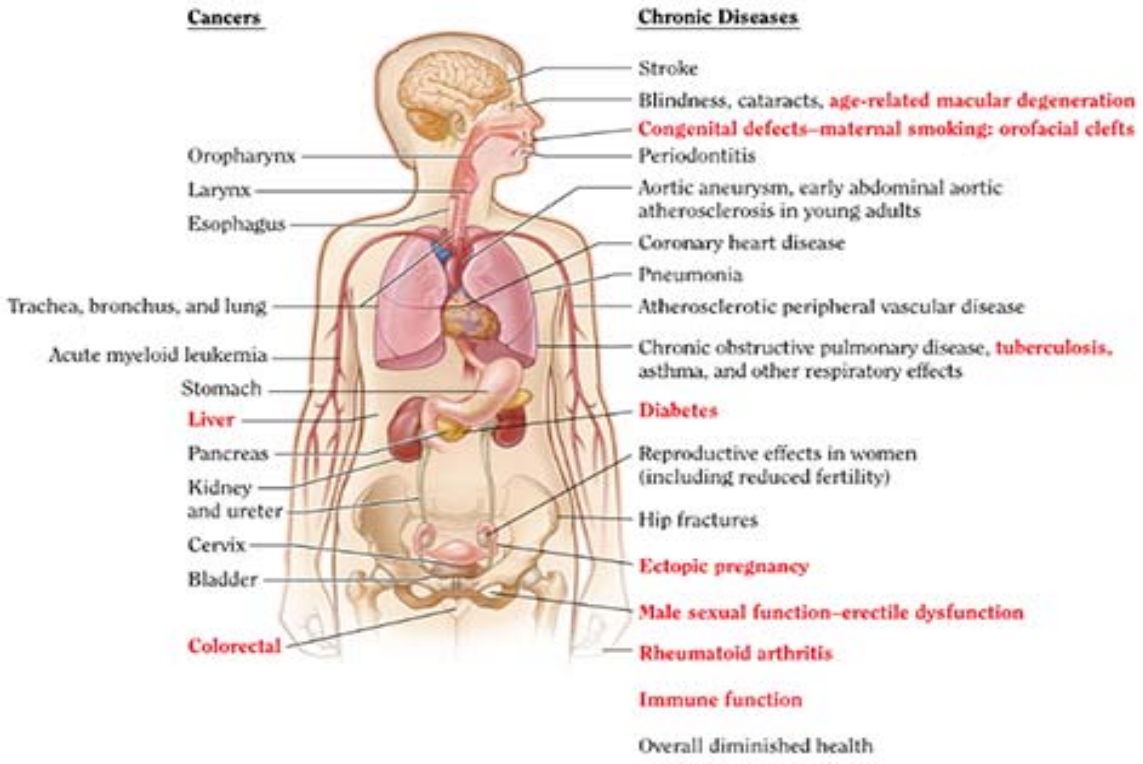


Figure 1.1: adverse effects of active smoking on health (Source: The Health Consequences of Smoking—50 Years of Progress: A Report of Surgeon General, CDC, 2014).

Smoking is associated with many ocular diseases and the association with eye diseases has been studied since the 1970s when the first survey on smoking association with cataract was

conducted (CDC, 2004). However, in the general population and among health care professionals, knowledge of ocular adverse effects of smoking is limited. Even organisations like WHO and CDC only suggest smoking as a risk factor for cataract and age–related macular degeneration of in their literature (WHO, 2010, CDC, 2014).

Kennedy *et al.* (2011) studied knowledge of individuals on the adverse effects of smoking on ocular health in Australia, Canada, America, & England. They found that Australians had the highest awareness, almost 50%, since they had a national campaign on ocular adverse effects, and other of countries had less than 13% who knew about the harmful ocular effects of smoking. Similarly, studies have found that level of awareness among individuals is less and people could halt smoking if they are adequately addressed on adverse effects of smoking on ocular health and its tendency to cause blindness (Bidwell *et al.*, 2005, Moradi *et al.*, 2007). Studies have revealed that awareness of smoking being a risk factor of eye diseases is limited in the public, especially when compared to the awareness of other diseases caused by smoking (e.g. lung cancer or heart diseases). However, people do have a fear factor of 'blindness' which can be used as a motivational factor for smoking cessation (Handa *et al.*, 2011, Ratneswaran *et al.*, 2014).

There are no studies presented in the existing literature, which can show any existing knowledge/awareness of public related to adverse effect of poor diet or sun exposure on ocular health. There are few studies in the literature, which show that existing knowledge of alcohol related health issues is limited in public but varies internationally. Countries such as UK, Morocco, and Australia have relatively higher awareness on alcohol related diseases compared to other countries due to national campaigns on alcohol related diseases (Bowden *et al.*, 2014, Scheideler and Klein, 2018).

1.2 Smoking related burden of disease

Tobacco use is one of the biggest public health threats to the world and according to World Health Organisation (WHO) estimates there are more than one billion smokers in the world (WHO, 2015). It is estimated that there are more than eight million deaths each year because of tobacco use (WHO, 2019). Amongst the eight million deaths, more than seven million are because of direct tobacco use and rest are believed to be related to exposure of passive smoking (WHO, 2019). The majority of world smokers (80%) are living in low- and middle-income countries (WHO, 2019).

In the UK, 11.2 million adults use tobacco every day. An additional 129,000 children also use tobacco daily (Anon, 2015). Every year in the UK, tobacco–caused diseases kill 115,650 people. This figure comprises 21.7% men and 16.2 % women (Anon, 2010). According to data published in 2013 by the Office for National Statistics, England (ONS, 2013) the total percentage of adults who smoked in 2010 was 20% compared to39% who smoked in 1980.

According the ONS (2013), current smokers smoked 12.7 cigarettes per day in England. Only in England, smoking contributed to 1.6 million adult hospital admissions in 2011/ 2012 with diseases caused by tobacco use and, this figure has increased from 1.1 million in 1996/97. These figures indicate that smoking/tobacco use is a public health threat to the UK. According to Public Health England (2015), smoking-related diseases cost the NHS England £ 2.6 billion in 2015.

1.3 Alcohol related burden of disease

Alcohol use is one of the leading risk factor for death and disability (Griswold *et al.*, 2018); however, its overall relationship with health remains complex given the possible protective effects of moderate alcohol consumption on some conditions (Balakrishnan *et al.*, 2009, Room *et al.*, 2005, Griswold *et al.*, 2018). Low to moderate alcohol consumption has some protective role on some health conditions, such as coronary heart disease (CHD) and diabetes in contrast; irregular heavy drinking increases the risk of CHD (Howard *et al.*, 2004, Ronksley *et al.*, 2011). An individual consumption and pattern of drinking can lead to many harmful effects on the human body organs. Cumulative consumption of alcohol can harm the body by acute intoxication leading to injuries and poisoning. Heavy drinking can lead to impairments, disabilities and self-harm (Rehm *et al.*, 2003).

According to the 'Opinions and Lifestyle survey' conducted by Office of National Statistics (ONS, 2018), 57 % individuals who are over 16 years drank alcohol in year 2017. This percentage equates 29.2 million population. According to an estimation by Public health England, in 2014, alcohol generated total cost to the society of approximately £ 21 billion in England and Wales. According to National Health Service (NHS) England, in 2017, there were 5,843 alcohol-specific deaths and 337,870 hospital admissions due to alcohol consumption (NHS, 2019). NHS spends more than £3.5 billion per year on alcohol related health problems (NHS, 2019).

1.4 Diet related burden of disease

Malnutrition in all its forms such as obesity, undernutrition, and other dietary risks (antioxidants or minerals deficiencies), is linked with poor health (Swinburn *et al.*, 2019). Suboptimal diet is a modifiable risk factor for many non-communicable diseases (NCD) such as type 2 diabetes, cardiovascular diseases, musculoskeletal disorders and some types of cancers (Afshin *et al.*, 2019). According to a global systematic evaluation of dietary consumption patterns conducted in 195 countries, 11 million deaths and 255 million disability-adjusted life years (DALYs) were associated with dietary risk factors in year 2017 (Afshin *et al.*, 2019).

Poor quality diet affects more than three million individuals in the UK (Wakeman, 2019). Poor diet is a behavioural risk factor, which has the highest impact on health budget. In the UK, poor diet related diseases cost the NHS £5.8 billion for the year 2006 -07 (Scarborough *et al.*, 2011). The scale of malnutrition in England is alarming according to department of Public Health England. According to the guidance published by Public Health England (2017), 63 % of adults in England were either overweight or obese in year 2015. The proportion of obesity is increasing in males and females. The prevalence of obesity increased from 14.9 % to 26.9 % between 1993 to 2015 (Health and England, 2017).

1.5 Ultraviolet radiation (UVR) related burden of disease

Australian Radiation Protection and Nuclear Safety Agency (ARPANSA, n.d) defines UVR as the portion of the electromagnetic spectrum between 100 nanometres (nm) and 400nm. Solar UVR exposure is responsible for approximately 1.5 million DALYs and 60,000 premature deaths in year 2000 (Lucas *et al.*, 2006). UVR related burden is mostly associated with cataracts and skin melanomas. The direct global burden of disease (GBD) attributable to UVR is relatively small compared to other lifestyles (e.g. smoking, obesity, alcohol) however, many environmental factors interacts with each other that can be responsible for bigger GBD (Lucas *et al.*, 2008). Rapid human migration around the world in last few hundred years increased the risk of melanomas, as dark pigmented skin at lower latitudes have low levels of melanoma and skin cancers, but at higher latitudes, dark pigmented skins are at higher risks of melanoma (Shaw and Pal, 2002). Increased industrialisation increased the risk of UVR related diseases by producing chlorofluorocarbons (CFCs) that react with ozone layer. The reaction results in the loss of stratospheric ozone layer with increasing levels of UVR reaching the earth (Lucas *et al.*, 2006).

In the present literature, some studies have already shown association of certain lifestyles (smoking, diet, and UVR) with ocular health. Studies that have shown lifestyle effects on tear film, accommodative ability and on macular pigment has been summarised below in this chapter.

1.6 Ocular effects of Smoking

Effect of smoking on the human body is widely studied but ocular effects of smoking is still not studied as extensively as its effects are studied on some other human body parts. In the existing literature, there are few studies that have shown a direct or indirect relationship of active smoking with many ocular conditions such as ocular inflammation (Lin *et al.*, 2010, Roesel *et al.*, 2011). Smoking is considered as an additional risk factor for gluacoma (Zanon-Moreno *et al.*, 2009, Fernandes *et al.*, 2015) and studies have concluded that smoking can

elevate intra-ocular pressure (IOP) of the eye (Mehra *et al.*, 1976, Kim *et al.*, 2015). Smoking is also associated as an environmental factor for polypoidal choroidal vasculopathy (Nakanishi *et al.*, 2010). Some studies reported that that progression and presence of diabetic retinopathy is positvely associated with smokers diabetic patients (Mühlhauser *et al.*, 1986, Katulanda *et al.*, 2014).

Some studies associated smoking with colour vision defect (Erb *et al.*, 1999, Arda *et al.*, 2015) and finally, maternal smoking is associated with starbismus (Hakim and Tielsch, 1992, Cotter *et al.*, 2011, Fernandes *et al.*, 2015). However, there are many studies conducted on the effect of smoking tear on the film abnormality/dry eye conditions, cataract formation and with age related macular degeneration (AMD). There are many studies which have concluded a negative effect of smoking on tear film, cataract formation and on AMD. Some of them are discussed below:

1.6.1 Tear film & Dry eye conditions

There are many studies conducted so far to examine adverse effects of smoking on the tear film instability and other conditions associated with deterioration of tear film. Studies conducted on the effect of smoking on the tearfilm dated back to late 1970s'. Basu *et al.* (1978) conducted a study on "the effect of cigarette smoke on the human tear film" and concluded that tear film break-up time (TBUT) could be decreased to forty percent in non–smokers due to exposure to passive smoke. Altinors *et al.* (2006) evaluated smoking's effects on the eye surface and concluded that smoking caused deteriorating effects on pre–corneal tear film by damaging its superficial lipid layer. Matsumoto *et al.* (2008) investigated the long-term cigarette smoking exposure on the tear film in otherwise healthy chronic smokers and compared it with non–smokers. The study revealed that chronic smoking adversely affects the eye and was responsible for the prominent quantitative and qualitative disturbance to the ocular surface. Rummenie *et al.* (2008) revealed from their study that even short-term passive exposure to cigarette smoke in healthy non-smokers could cause adverse effects on ocular health. El-Shazly *et al.* (2012) found that passive smoking has a strong tendency to develop dry eye conditions in children whose parents are smokers. Lower goblet cells density and squamous metaplasia (Aktaş *et al.*, 2017, Agrawal *et al.*, 2018) is also reported in smokers. Studies have reported high dry eye related subjective symptoms from smokers than non-smokers (Masmali *et al.*, 2016, Erginturk Acar *et al.*, 2017, Aktaş *et al.*, 2017).

1.6.2 Cataract development

Smoking association with cataract development is one of the most cited topics for its adverse effects on eye health. Different studies have concluded different results about the intensity of

smoking responsible for cataract development. Still it is evident from the shreds of evidence that smoking has a significant association with cataract development.

Christen *et al.* (1992) structured a study to find an association of cigarette smoking with cataract. The study established that high intensity of tobacco use was responsible for the development of cataract and concluded that smoking more than twenty cigarettes a day was positively linked with a development of cataract (mostly nuclear sclerosis and posterior sub–capsular cataract) in US population.

Lindblad *et al.* (2005) found a dose–response relationship between intensity of smoking and cataract extraction. They further observed that smoking cessation can reduce the risk of developing cataract but this requires a long time. In 2014, Lindblad and his associates (Lindblad *et al.*, 2014) again supported their observation derived from their previous study. They concluded that smoking cessation can decrease the risk of cataract extraction but it depends on the amount of smoking, heavier smoking habit could take decades to minimise the possibility of cataract removal. Similarly, a study (Wu *et al.*, 2010) conducted on an association of smoking and socioeconomic status with ARC; the study concluded that smoking and low socioeconomic status were associated with cataract and in every six nuclear sclerosis cases, one case was related to the smoking.

Lu *et al.* (2012) investigated cigarette smoking and body mass index as risk factors of age-related cataract and found that current smoking of cigarettes more than 30 per day has a positive association in developing age–related cataract (ARC). The study also concluded that if a person is obese and has a smoking habit than the risk for ARC is significantly high. Another study conducted by Jiang *et al.* (2015) found that Glutathione S- transferases M1 (GSTM1) genotype which was associated with age related cataract were higher in smoker patients.

1.6.3 Age–related macular degeneration (AMD)

Like smoking association with cataract, an association of smoking with AMD is also one of the most studied topics that show its adverse effects on eye health. Many researchers have drawn a definite conclusion on it. Christen *et al.* (1996) observed the relationship between cigarette smoking and the incidence of AMD in men. The study found that smoking increases the risk for AMD. The study found a dose–response association between smoking and AMD. The study reported that individuals with the smoking habit of twenty or more cigarettes per day have a greater risk for disease than non–smokers or with the smoking habit of fewer than twenty cigarettes per day.

A population based cross–sectional study on conducted by Cackett *et al.* (2008) observed a positive association between smoking and late AMD prevalence. The study further noted that high amount of smoking (more than five packs per week) was strongly associated with AMD. Hughes *et al.* (2007) concluded that an individual's risk for getting AMD is predictable by

knowing individual's haplotype data, and smoking status. The study further suggested that smoking cessation is the best options for those individuals who got high-risk genes and estimated that by a total elimination of smoking, individuals will have thirty–three percent fewer chances of getting severe AMD. Similarly, Lee *et al.* (2010) observed a significant effect of smoking on exudative AMD in the interaction analysis of genes (LOC387715 and HTRA1) and environmental factors.

1.6.4 Summary

The previous studies show that the main effects on the eye from smoking are to the tear film, the lens, and the macula. The tear film results are immediate and can be transient. The effects on the lens and the macula may be late stage pathologies that lead to cataracts and age-related macular degeneration respectively. However, in early stages, it may be that effects of smoking can be seen by assessing crystalline lens flexibility such as measurements of amplitudes of accommodation. In the macular region, early changes may be possible to see by measuring macular pigment.

1.7 Smoking effects on tear film characteristics

In the next section, details of studies that have investigated the effects of smoking on the tear film will be described followed by information regarding lens and macula studies relating to the consequences of smoking.

Table 1.1 displays studies which have mentioned effect of smoking on tear film:

Author(s)	**Study title**	**Sample size (N) and Study design**	**Diagnostic test used**	**Results**	**Conclusion**	**Strengths and limitations**
Basu *et al.* (1978)	**"**The effect of cigarette smoke on the human tear film"	N = 14 cross sectional	TBUT	Cigarette smoke exposure was associated with 35 to 40 % reduction in BUT.	This study demands further research on the chemical responsible for an alternation of three constitutes of the tear film.	This study being conducted is the first of its kind on this research topic.The study had performed only one test (TBUT) on the subjects. Other important and routinely conducted tear film analysis tests were missing. Secondly, the sample size of 14 subjects is small, and its results could not be applied on the larger scale.
Grus *et al.* (2002)	"Effect of smoking on tear proteins"	N = 105	• Basal secretory test	Tear protein patterns were different in	Electrophoretic analysis of tear	The main limitation of the study

		Smokers= 29 Severe smokers = 26 Control = 50 Cross – sectional study	• Subjective symptoms • Sodium dodecyl sulfate-polyacrylamide gel electrophoresis Digital image analysis	smokers and in severe smokers compared to control group.	protein model can help in giving insight to smoking – induced ocular surface diseases.	mentioned by authors was that the study could not distinguish between the direct influence of cigarette smoke on the tear film and the possible role of systemic concentrations of nicotine. Besides, respondent bias in giving the history of smoking and in providing subjective symptoms of the dry eye could alter the results. Finally, it would be better if the study did some analysis on the responses of subjective symptoms provided

						by smokers and non–smokers.
Satici *et al.* (2003)	"The effects of chronic smoking on the ocular surface and tear characteristics: a clinical, histological and biochemical study"	N = 81 Smokers = 44 Control =37	• Schirmer's test I • TBUT • Rose bengal staining scores • Impression cytology • Tear lysozyme concentration • Eye irritation symptoms • Eye irritation indices	There was no statistically significant difference found between smokers and non–smokers group for goblet cell density and rose bengal staining. Other test results showed abnormal results in smokers' group.	Chronic smoking has an adverse effect on ocular surface and some characteristics of the tears. Chronic smoking can cause weakness in the ocular defence.	The study enrolled smokers who smoked six or more cigarettes per day for at least one year, and the study was unable to tell about the average smoking years of smokers in the smokers group. Additionally, the study did not sub-divided the chronic smokers according to the intensity of smoking. As mentioned in the previous study of this literature review (Yoon *et al.* 2005) intensity of smoking

						can alter the results. Further, the study design has its disadvantages and could cause bias in the results (for example in reporting the symptoms scores and in giving history on on the numbers of cigarettes smoked etc.).
Yoon *et al.* (2005b)	"Effects of Smoking on Tear Film and Ocular Surface"	N = 55 Smokers =29 Non-smokers = 26 Prospective study	• Dry eye symptoms scoring • TBUT • Basal tear secretion test • Corneal sensitivity test • Keratoepitheliopathy scoring • Conjunctival impression cytology	No significant difference found between symptoms scores, goblet cell density, and keratoepitheiliaopathy score. Basal tear secretion and corneal sensitivity were lower in smokers, and	Smoking deteriorates tear film and ocular surface.	One of the strongest points of this study was; the study sub–divided the smokers subjects into three subcategories to determine the effects of smoking frequency on tear film and ocular surface. On the

				TBUT was shorter in smokers as well.		contrary, the cross-sectional design of the study has some disadvantages so the study's results could be affected by the bias factors (for example in reporting the symptoms scores and in giving history on the numbers of cigarettes smoked etc.).
Altinors *et al.* (2006)	"Smoking associated with damage to the lipid layer of the ocular surface"	N = 94 Smokers =60 Healthy=34 Prospective, comparative, interventional Case series	• Corneal & conjunctival sensitivity • Surface staining with fluorescein • TBUT • Schirmer's test I • Conjunctival impression cytology	In smokers; mean TBUT was 5.3seconds, conjunctival sensitivity was 26.2 mm, average central corneal sensitivity was 37.6mm. Schirmer's test I values and goblet cell	Smoking has adverse effects on lipid layer of pre–corneal tear film.	This study only compared chronic smokers with non-smokers. If study recruited some light and moderate smokers and then compared the intensity of smoking with their ocular

			• DR – 1 tear lipid layer interferometry	densities were same in both groups. DR–1 interferometry revealed high–grade of lipid layer changes.		conditions, then the results would be more validated by providing dose–response relationship among smokers. Additionally, the study tried to reduce the risk of bias in its methodology by selecting smokers regardless of their complaints and by excluding subjects with particular occupations, but the study did not mention any information obtained on passive exposure of smoking from non-smokers which

						could alter the outcome results.
Rummenie *et al.* (2008)	"Tear cytokine and ocular surface alternations following brief passive cigarette smoke exposure"	N = 12 prospective study	• TBUT • Ocular surface fluorescein staining • Rose bengal staining • Schirmer's test I • Conjunctival impression cytology • Conjunctival brush cytology	In passive smokers; TBUT, tear evaporation rate, tear lipid spread time and vital staining showed worse effects on exposure to smoke.	The study showed that even a shorter exposure to the cigarette smoke will have adverse implications for the eye health. Thes adverse effects were evident by an increase in tear inflammatory cytokines, lipid pre-oxidation products, and reduction of mucosal defence.	This study involved a variety of tests used for assessing tears and dry eye condition which increase the reliability of the results. On the contrary, there are some limitations of this study; at first, the study used twelve mg of tar cigarette for smoke exposure (as per one of the big brand used in Japan) but usually in the world, most of the cigarette brands have ten or less than ten mg of tar. Similarly,

						nicotine level used in the study was one mg, but most of the brands use 0.9 mg nicotine in their cigarettes. Secondly, the study sample was twelve participants. Both of these limitations could alter the outcome results of the research when applied to a larger population.
Matsumoto *et al.* (2008)	"Alternations of the tear film and ocular surface health in chronic smokers"	N = 15 Prospective study	• Tear evaporation rate • DR- 1 Lipid layer interferometry • Tear hexanoyl – lysine ELISA Analysis • TBUT	TBUT was found shorter in smokers, Hb–CO level was higher in smokers. Significant loss of goblet cells was recorded among the smokers. Conjunctival	Chronic smoking can induce disturbance to ocular surface health qualitatively and quantitatively.	This study had a variety of tests involved in dertmining the effects of chronic smoking on the ocular surface. There are some possible chances of

			• Ocular vital staining scores • Schirmer's test I • Impression cytology parameters • Brush cytology	neutrophil infiltration was significant in smokers. Tear evaporation rate was higher in smokers.		respondent bias in this study as for smokers, it was requested not to smoke at-least 6 hours prior to test(for breath and hameoglobin CO measurements), so it could be possible that smokers smoked cigarettes in that period but did not report it. In addtion, the small sample size of the study also has some limitation regarding its reliability and validity.
El-Shazly *et al.* (2012)	"Passive Smoking as a Risk Factor of Dry Eye in Children"	N =112 Cross-sectional study	• Dry eye symptoms • Visual symptoms • TBUT • Schirmer's test I	Among one hundred and twelve children, eighty were diagnosed with the	Passive smoking was found as an important risk factor for the dry eye in	The study tried to eliminate any possible risks of respondent based

			• Corneal fluorescein staining • Urinary cotinine and urinary cotinine creatinine values	dry eye. Among these seventy–six children were exposed to passive smoking. Results revealed that cotinine/creatinine ratio and numbers' of cigarettes/ per day were important determinants of dry eye.	children (especially male children).	bias factors in reporting the history of smoking exposure by proving it clinically with urinary cotinine and urinary cotinine and creatinine test. Still, the cross–sectional study design limitations are the main limitations of this study.
Thomas *et al.* (2012)	"The effect of smoking on the ocular surface and the precorneal tear film"	N =101 (51 smokers & 50 non-smokers) Cross-sectional study	• TBUT • Surface staining with fluorescein • Corneal + conjunctival sensitivities • Schirmer's test II • Questionnaire	TBUT and corneal & conjunctival staining were found lower in the smoking group. Punctaute staining was observed higher in the smoking group, but Schirmer's test II results were same in both groups.	The study found a strong association between smoking and tear film instability.	Due to the geographical location of the research, only male participants were enrolled in the study. The study did not explain the questionnaire used in detail nor the study analysed the

						questionnaire in detail. The study failed to assess any indoor air pollution exposure or any remote exposure to smoke which could alter the results. Additionally, due to the location of the study (India), other exposures like exposure to the sun (UV radiations), temperature, etc. could also alter the results of the research.
Sayin *et al.* (2014)	"Effects of chronic smoking on central corneal thickness, endothelial cell, and dry eye parameters"	N = 102 Smokers' = 49 Non-smokers= 53 Cross-sectional study	• TBUT • Schirmer's test 2 • Central corneal thickness • Specular microscopy	TBUT and Schirmer's test scores were much lower in the smokers' group compared to the control group. There	The study concluded that smoking affects Schirmer's test scores, TBUT and hexagonal cells of	On of the main limitation of this study was that this study only involved heavy smokers as participants (>30

				was no significant difference observed in the mean CCT, endothelial cell density and size. Smokers had a lower % of hexagonal cells compared to the control group.	the corneal endothelium.	cigarettes /day) who smoked for at–least ten years. Unlike to most of the ocular diseases associated with smoking that show their effects usually after decades,dry eye syndrome is a short-term effect of an adverse effect of smoking.So if the study could involve light and moderate smokers as research participants, then the results would be more reliable compared to present results. Also, cross-sectional study design limitations

						are also applicable to this study.
Hua *et al.* (2014)	"Discrepancy between subjectively reported symptoms and objectively measured clinical findings in dry eye: a population based analysis"	N = 2262 Population-based cross-sectional study	• Patient– reported symptoms • TBUT • Schirmer's scores (Schirmer's test II)	Factors influencing dry eye symptoms are mainly gender, smoking, and age. Diagnostic test results showed remarkable difference among different groups.	Development of dry eye syndrome is related to many factors. Pre- clinical phase concept will be helpful in screening patients with or without dry eye signs.	The study sample size was large, which increases the reliability of the research results. On the contrary, this study was unable to do some of the necessary dry eye evaluation tests Like evaluating surface staining, impression cytology, tear osmotic pressure and surface microscopy, etc. which was acknowledged by the study's authors as well and mentioned by them in their limitation

						section of the survey.
Masmali *et al.* (2016)	"Assessment of Tear Film Quality among Smokers Using Tear Ferning Patterns"	N = 65 smokers = 35 non-smokers = 30 case controlled comparitive study	• McMonnies questionnaire • PRT test • Tear Ferning gradings • TBUT	Subjective symptoms scores and tear ferninng grades were significantly higher in smokers compared to non-smokers. Mean TBUT was lower in smokers compared to non-smokers. Strong correlations found between Mc Monnies scores and PRT and between Mcmonnies scores and Tear ferning grades.	Cigarette smoking could affect tear film quality of the eye.	The study use tear ferning patterns for evaluating tears quality between smokers and non-smokers. This method is not used before to examine tears of smokers and non-smokers. The study sub divided its smoker participants in to four further gradings according to smoking duration. However, it was unclear that that smoking duration was self reported by participants or the study calculated

						pack smoking years exposure for each participant. The sample size is small and larger sample size could strenghtens the study's results. Self reported bias from participants can affect the results.
Aktaş *et al.* (2017)	" Impact of Smoking on the Ocular Surface, Tear Function, and Tear Osmolarity"	N = 101 smokers = 50 non-smokers = 51 prospective case control comparitive study	• Tear osmolarity test • TBUT • Schirmer test 1 • Corneal sensitivity • Conjuctival impression cytology • OSDI Scores	Mean TBUT, corneal sensitivity and goblet cell density was statistically lower in smokers compared to controls. Tear osmolarlity and OSDI scores were higher in smokers compred to non-smokers.	Smoking results in increased tear osmolarity and it damages the tear film,	The study only recruited heavy smokers (20 or more cigarettes per day) for the comparison and calculated pack smoking years for each smoker participant. Unlike to most of the ocular diseases associated with smoking that show their effects

						usually after decades,dry eye syndrome is considerablly a short-term effect of smoking. So if the study could involve light and moderate smokers as research participants, then the results would be more relaibe and comparitive and the reader could see the difference of results with due increase of smoking intensity. Self reported bias could affect OSDI results. Finally, the study itself mentioned limitations related to

						tear osmoloarity test.
Agrawal *et al.* (2018)	"Effect of smoking on ocular surface and tear film: A clinico- pathological study"	N = 100 cross-sectional study	• OSDI questionnaire • TBUT • Schirmer test 2 • Conjuctival impression cytology	Mean TBUT and Schirmer test 2 measurements were significantly lower in smokers compared to non-smokers. On impersion cytology, more smokers (20%) showed grade 2 metaplasia and grade 3 metaplasia (8%) compared to non-smokers (2%) and (0 %) respectively.	Cigarette smoking is a significant risk factor for dry eyes and ocular suface disorders charaterized by loss of goblet cells and sqaumous cell metaplasia. Severity of dry eye is positively associated with smoking intensity.	The study sub divided its smoker participants into mild, moderate and heavy smokers according to the smoking intensity that gives a clear picture of some results drawn from the study. However, participants division crietria is not explained clearly. It would be ideal if study did divide its participants according to smoking pack years (which was not used). The cross-sectional study

						designs has also some limitations.
Alanazi *et al.* (2019)	"Assessment of the Tear Evaporation Rate in Chronic Smokers Using Delfin VapoMeter"	N = 240 Smokers = 120 Non-smokers = 120 Observational case-control study	• OSDI questionnaire • DelfinVapoMeter	Smokers had a higher OSDI scores and tear evaporation rate compared to non-smokers	Smokers had higher tear evaporation rate. Vapometer can be used as a reliable tool to study eye dryness.	Participants enrolled in the study were age-matched. Only male smokers were enrolled in the study that limits its results implication to public. The study location (Middle East) had other environmental condition such as humidity and high temperature that can also alter results.

1.7.1 Patient-reported Symptoms

Patient-reported symptoms and history for dry eye evaluation are an important factor in establishing whether or not patient have dry eye disease (Agarwal, 2006) According to Holly (1993) dry eye diagnosis can be divided into four groups:

- The first based on clinical presentation (e.g. signs and symptoms).
- The second relates to tear film composition (e.g., tear film composition, osmolality, and tear film dynamics).
- The third refers to tear film (e.g. TBUT, evaporation and lipid abnormalities)
- The fourth relates to ocular surface (e.g. surface staining, impression cytology, and surface microscopy).

Almost every study (except Basu et al. 1978) includes patient-reported symptoms/questionnaire, but some studies do not provide sufficient analysis of this (Grus *et al.* 2002 & Thomas *et al.* 2012). Most of the studies (Satici et al 2003; Yoon et al. 2005; Altinors' et al. 2006; Matsumoto et al .2008; Sayin et al. 2014 & Hua et al. 2014) in the present literature used customized dry eye questionnaire to evaluate the dry eye symptoms in smokers and non-smokers participants.

Some studies (Masmali et al. 2016; Aktas et al. 2017; Agrawal *et al.* 2018 & Alanazi et al. 2019) used comprehensive form of dry eye evaluation questionnaire (e.g. OSDI and MacMonnies dry eye questionnaires). There was a consistency in results obtained from different forms of questionnaire used. All studies included in this literature review showed that DES related symptoms were more prevalent in smokers compared to non-smokers.

1.7.2 Tear film break–up time (TBUT)

An effect of smoking on TBUT is evident from literature. Every study included in this literature review had conducted TBUT test except Alanazi et al. 2019.

Basu *et al.* (1978) were among one of the first researchers to investigate effects of smoking on human tear film. Some studies in the present literature (Basu *et al.* 1978; Rummenie *et al.* 2008 & El-Shazly *et al.* 2012) investigated the passive effect of smoking on the tear film of healthy non-smokers participants. In terms of TBUT, all studies conducted on the passive effect of smoking on the tear film found an inverse effect on TBUT.

There was a consistency in TBUT results in other studies of this literature review that were conducted to evaluate tear film parameters difference in smokers and non-smokers. All of the

studies concluded with a lower TBUT in smokers compared to non-smokers. Thomas *et al.* (2012) and Agrawal *et al.* (2018) further found a dose-response relationship between cigarettes smoked per day and TBUT. Both studies found a significant decrease in TBUT with an increase in daily intensity of smoking. It can be concluded that smoking adversely affect the pre–corneal tear film stability by affecting the lipid layer of the tear film and contributes to dry eye condition secondary to increased evaporation. The reduced TBUT will lead to dry eye symptoms in these subjects (Agarwal, 2006).

1.7.3 Schirmer's test

In the present literature, studies found three types of Schirmer's test results. Seven out of 15 studies (Yoon *et al.* 2005; Matsumoto *et al.* 2008; El–Shazly *et al.* 2012; Sayin *et al.* 2014; Hua *et al*. 2014; Aktas *et al.* 2017 & Agrawal *et al.* 2018) have reported a significantly lower tears secretion among smokers' compared to non–smokers.

Satici *et al.* (2003) have reported increased tear secretion among smoker participants in their study while investigating effects of chronic smoking on the tear film and ocular surface. They reported smokers had a Schirmer's test value of was 30.3 ± 16.7mm compared to non–smokers' 23.8 ± 12.4mm ($p > 0.05$) per five minutes. In contrast, Altinors' *et al.* 2006 and Thomas *et al.* (2012) did not find any significant difference ($p < 0.05$) in Schirmer's test results between smokers and non-smokers. Thomas *et al.* 2012 studied a dose-response relationship between numbers of cigarettes smoking per day and Schirmer's test score. The study found no relationship between Schirmer test scores and the number of packs of cigarettes smoked per day ($p < 0.05$).

Rummenie *et al.* 2008 found no significant change in tears secretion volume at baseline/ before exposure (19.2 ± 12.0 mm), after five minutes (19.3 ± 10.71 mm) and after 24 hours of passive exposure to smoke (18.9 ± 9.2 mm) in healthy non-smokers. Although, most of the studies in this literature showed a decrease in Schirmer test scores but the results are not as consistent as it was for TBUT and further studies are needed to evaluate the effect of smoking on tears production.

1.7.4 Ocular staining

Fluorescein staining (FS) is an important indicator of dry eye parameters. Like TBUT and Schirmer's tests, many studies (Altinors' *et al.* 2006, Matsumoto *et al.* 2008, Rummenie *et al.* 2008, El–Shazly *et al.* 2012 & Thomas *et al.* 2012) had shown an increase in FS in smokers. In contrast, Yoon *et al.* (2005) & Aktas *et al.* (2017) had shown no significant difference FS in smokers compared to normal subjects.

Altinors *et al.,* (2006) found that that FS grades in smoker's group were higher than in control group the results were statistically significant. Matsumoto *et al.* (2008) found similar results that mean FS grades for smoker's group was significantly higher compared to a control group. Rummenie et al. (2008) in their pre and post smoking exposure study found a significant difference in FS grades at four different time intervals. FS grades were 0.6 ± 0.6 before five minutes smoking exposure, 2.3 ± 2.3 after five minutes to smoke exposure and after 24 hours, this grade was 1.6 ± 2.0.

El –Shazly *et al*. (2012) reported similar results as mentioned by Matsumoto *et al.* (2008) and Altinors' *et al.* (2006) on FS. The study showed that exposure to smoking has increased FS grades, and there is a significant statistical difference between the smoker and non–smoker groups. Thomas *et al.* (2012) have shown that 56.9%of smokers had punctuated corneal staining while there was no staining observed in the control group (non–smokers) and the difference between two group was found to be statistically significant ($p < 0.0001$).

In contrast, Yoon *et al.* 2005 reported no significant difference ($p = 0.7$) in FS grades between smokers and non–smokers group. Finally, a recent study conducted by Aktas *et al.* (2017) supported this and found no significant difference in Oxford gradations of smokers and non-smokers).

Fluorescein staining is the most widely used diagnostic test for evaluating dry eye and most studies show smoking to contribute to corneal and conjunctival staining.

Some studies (Satici *et al.* 2003 & Rummenie *et al.* 2008) used Rose Bengal as ocular staining agent. Satici et al. (2003) found no significant difference in RB staining between smokers' and control group. Conversely, Rummenie *et al.* (2008) found significant differences in staining when they examined exposure to passive smoking for non–smokers at three different times.

In this review of the literature, two studies performed Rose Bengal staining but the results from them are not sufficient to establish an association of smoking with RB staining. The literature did not show any studies that used Lissamine Green staining to identify dry eye in smokers.

1.7.5 Conjunctival cytology

The review of literature shows conflicting results when looking at the adverse effect of smoking on epithelial cells and goblet cell densities. Satici *et al.* (2003), Yoon *et al.* (2005), and Altinors' et al. (2006) did not show any significant difference in goblet cell densities of smokers and non-smokers. In contrast, studies conducted by Matsumoto *et al.* (2008), Rummenie *et al*.

(2008), Aktas *et al*. (2017) and Agrawal *et al.* (2018), had shown that smoking decreased the goblet cell density. Regarding evaluating squamous cell metaplasia, all studies have shown squamous metaplasia in smokers except one study (Altinors' et al. 2006) which shows no significant association of squamous cell metaplasia with smoking. These all studies have used Nelson *et al.* (1983) grading classification for impression cytology.

1.7.6 Tears osmolarity

Alanazi *et al.* (2019) found that heavy smokers had significantly higher osmolarity rate compared to non-smokers (control group). A similar trend of result was found by a study conducted by Aktas *et al.* (2017) that showed a higher tear osmolarity values for heavy smokers (305.3 ± 9.8 mOsm/L) compared to non-smokers (301.1 ± 7.0 mOsm/L). The study also found that based upon tears osmolarity results, 40% of its smoker's participants had a diagnosis of dry eye.

1.8 Effect of smoking on crystalline lens

As discussed above in the section of smoking effects on ocular health, the association of smoking with cataract development is one of the widely discussed side effects of smoking on the ocular health. Unlike to its effects on tear film, which are less time consuming, an association of smoking with cataract formation is a long time prospect, which reveals its results in decades. However, the lens parameters like lens flexibility and its power to accommodate can indicate its adverse effect on the lens at early stages. There is only a little amount of literature present on the association of smoking with presbyopia or its connection with the amplitude of accommodation.

A South Indian population-based study conducted by Nirmalan *et al.* (2006) to find the prevalence of presbyopia in the state of Andhra Pradesh. The study collected demographic and risk behaviour information from participants to find any association with presbyopia. The study revealed that smoking has no significant relationship with presbyopia by using multivariate analysis. The primary variables were female gender (OR1.4, 95% CI: 1.1 – 1.8), rural residence (OR 1.5, 95% CI: 1.2 -1.8), alcohol consumption (OR 0.8, 95% CI: 0.6 -0.9), nuclear opacity of lens greater than LOCS III grade 2 (OR 4.8, 95 % CI: 1.4 – 16.8) and hyperopia (OR 3.6, 95 % CI: 1.3 – 2.1) were associated with more presbyopia.

There are studies present in the literature, which were conducted on the topic of presbyopia. Most of them were carried out to find the prevalence of presbyopia in different ethnic populations (Duarte *et al.*, 2003, Carnevali and Southaphanh, 2005, Burke *et al.*, 2006, Babu and Amaresh, 2015). These studies did not investigate the association of smoking with the

onset of presbyopia either as a primary outcome or as a confounding factor (except Nirmalan *et al.* 2006).

Khalaj *et al.* (2014) conducted a cross-sectional study to find out prevalence of presbyopia among the smoking population. The study showed that smokers had an earlier onset and progression of presbyopia than non–smokers. The study population was subdivided into two categories cigarette smokers as samples (n = 152) and healthy participants as controls (n = 152). This study's results revealed that the prevalence of presbyopia among smokers was higher than in healthy subjects at various ages ($p = 0.001$). In particular, the results showed that from 36 years to 38 years old 19 smokers needed glasses for near task compared to none in non–smokers. Similarly, in 39 – 40 years age group 85 smokers were in need of near glasses compared to none in healthy subjects. This study further showed that prevalence of onset age of addiction to smoking was also significantly correlated with the onset of presbyopia ($p = 0.02$).

Ide *et al.* (2012) found a positive association of smoking with decreased amplitude of accommodation when they investigated smoking habit as a lifestyle factor while studying two different types of accommodators as a biomarker for ageing and lifestyle. Ide *et al.* (2012) found a significant correlation between age and accommodative amplitude among smokers and non-smokers. The study revealed that amplitude of accommodation was significantly lower in smokers groups (mean ±SD 4.9 ± 2.7 D) compared to non-smokers (mean ± SD 6.9 ± 3.1 D, $p < 0.001$).

In the literature, few studies (Roberts and Adams, 1969, Bardak *et al.*, 2017) reported transient effects of smoking on accommodative ability. Roberts and Adams (1969) observed that smoking immediately induced a reduction of 1.25 D (at least) in AoA of their study participants. The participants returned to normal AoA within five minutes of cessation. The study used a 3 mm fixed artificial pupil to avoid variation in depth of focus and monocular measurement of AoA avoided fusional interference. In contrast, Bardak *et al.* (2017) found a significant increase in objective accommodation after smoking at each accommodative stimuli (ranged 0-5) and the increment was significant at 2D and at 3D of stimuli ($p < 0.05$). Both studies had mentioned nicotine as a responsible agent for the short change in AoA and suggested that nicotine rapidly changed into less toxic substance in human body and this may account for the return to normal values of AoA after few minutes of smoking exposure.

1.9 The association of smoking with macular pigment optical density

Age–related macular degeneration (AMD) is a serious public health problem and one of the leading cause of age–related irreversible blindness (Congdon *et al.*, 2004, Evans *et al.*, 2004). The association of smoking as an environmental risk factor for AMD is well established and smoking is among one of the modifiable risk factors for AMD. Studies have shown a positive association of smoking with AMD (Hughes *et al.*, 2007, Cackett *et al.*, 2008, Kawasaki *et al.*, 2008, Zhang *et al.*, 2011, Jonasson *et al.*, 2014, Lechanteur *et al.*, 2015, Marazita *et al.*, 2016, Merl-Pham *et al.*, 2016).

Retinal carotenoids (lutein, zeaxanthin, and meso–zeaxanthin) and their association with the onset of maculopathies are one of the important research topics in ophthalmology field (Berendschot *et al.*, 2000, Nolan *et al.*, 2007). The lutein and meso–zeaxanthin together formed a yellowish pigment in the human retina and known as “macular pigment” (de Kinkelder *et al.*, 2011) and typically located in ganglion cell layer and the inner plexiform layer of the retina. The amount of macular pigment (MP) assessed as macular pigment optical density (MPOD). Its high concentration is in the macular region (Kirby *et al.,* 2010). The MP protects retina by acting as a blue light filter (λ 320 to 450 nm) (Snodderly *et al.*, 1984, Sharpe *et al.*, 1998, Algvere *et al.*, 2006). The MP also acts as anti–oxidant and helps to reduce the oxidative stress (Khachik *et al.*, 1997).

The human body is unable to produce the retinal carotenoids and it can only be supplemented by diet (Bone *et al.*, 1985). Food like egg yolk, maize, kale, and spinach are some nice source of a high molar percentage of lutein and zeaxanthin (Howells *et al.*, 2011). Several studies e.g. (Hammond, 2002, Rock *et al.*, 2002, Bone *et al.*, 2003, Nolan *et al.*, 2007) have shown a positive association of lutein, zeaxanthin, and meso–zeaxanthin with MPOD results in humans. Other than dietary variables, age, sex, smoking, exposure to ultraviolet light and drinking habits are main factors that can alter MPOD results (Trieschmann *et al.*, 2007). The established risk factors for AMD are often associated with decreased macular pigment (Nolan *et al.*, 2007, Kirby *et al.*, 2010).

Table 1.2 summarizes studies included in this literature review that has been conducted on the effect of smoking on MPOD as below:

Authors' & publication date	**Study title**	**Study design and sample size**	**Clinical tests**	**Results**	**Conclusion**	**Strengths and limitations of the study**
Hammond *et al.* (1996)	"Cigarette Smoking and Retinal Carotenoids: Implications for Age-related Macular Degeneration"	N = 68 34 smokers 34 non–smokers Case – control study	• MPOD measurement by psychophysical method Dietary Assessment of usual carotenoids intake by Health habit and history questionnaire (HHHQ)	The mean MP od smoking group was 0.16 (SD= 0.12) compared to non–smokers who has mean MP of 0.34 (SD= 0.15) p <0.0001. In a dose–response relationship MP density and smoking frequency was found inversely related (r= - 0.498, p < 0.0001)	This study concluded that smoking causes a reduction in MP density and smokers might be at the risk of having macular diseases. The epidemiological data identified smoking, as a risk factor for AMD is consistent with this hypothesis.	This study does not provide any indications of clinical tests (e.g., VA, and full routine eye check-up) performed on participants. Although, the response of HHHQ questionnaire was analysed by the software program (HHHQ Diet System Analysis Software by Block et al. 1994). Still there are chances of respondent related bias, as the study did not confirm those responses clinically by

						performing serum carotenoid analysis. Finally, due to small sample size, these findings are unreliable on the wider scale.
Hammond *et al.* (2002)	"Macular Pigment Optical Density in a South – western Sample"	N = 217 Population based cross – sectional	• MPOD measurement by psychophysical method 1°, 460-nm test stimulus. Personal data questionnaire that includes information on personal information, medical history, smoking history, iris colour, dietary intake "Likert – scale questions."	The average MP density was 0.22 ± 0.13. The MP density slightly declined with age ($r = -0.14$, $P = 0.02$). In women, the average MP density was lower than men were ($p<0.05$). The MP density (average) was also low in light–coloured irises compared to dark coloured irises ($p <0.009$). Heavy smokers also had a	The MP density found lower in Southwestern sample compared to those in Northeast sample. The study found that MP density was 13 % lower in women and 18 % lower in individuals with light coloured irises than in dark coloured. The association of smoking with MP density was found significant in only	One of the limitations of this study was the reference point for MPOD measurement was 4^o from retinal eccentricity, but some preliminary data showed (Bieber and Werner, 1999) that people with old age might have a secondary spatial density peak of high MP density.

				little MP density compared to light smokers ($p<0.0045$) and to non–smokers ($p<0.034$).	heavy smokers (>10 cigarettes). The heavy smoker's MP density is almost 25 % lower compared to subjects in the control group.	
Nolan *et al.* (2007)	"Risk factors for age-related maculopathy are associated with a relative lack of macular pigment"	N=828 Cross- sectional study	• Detailed questionnaire (including questions on, demographic, lifestyle, smoking, drinking, medical history and family history) • Food frequency questionnaire • Visual acuity • Fundus photography • Iris photography	The study reported a statistically significant age-related decline in MP optical density ($r2 = 0.082$, $p < 0.01$). Smoking had a positive statistically significant association with low MPOD levels. Current and past smokers had low MPOD levels than non–smokers ($p <$	The study concluded that the increase in age, family history of ARM and tobacco use were associated with the lack of MP. This association was positively associated in the absence of retinal pathology, and in advance of disease onset. The study supports the	The study sample size was large, and it tried to reduce bias factor in selecting participants as well. In the study's limitations, the study was unable to confirm the self-reported cholesterol levels by participants and the study has not investigated any use of statins among those who reported high cholesterol levels.

			• Serum carotenoids analysis MPOD measurement with heterochromatic flicker photometry.	0.01). Subjects with a confirmed family history of age–related maculopathies (ARM) had low MPOD level compared to those who had no known family history of the disease (P <0.01).	hypothesis that the enhanced risk that these variables represent for ARM may be attributable, at least in part, to a parallel deficiency of macular carotenoids.	
Kirby *et al.* 2010	"A Central Dip in the Macular Pigment Spatial Profile Is Associated with Age and Smoking"	N= 484 Single visit study	• Detailed demographic questionnaires (including VA, refraction, height, weight, BMI, smoking status, ethnicity, iris colour, alcohol consumption, family and personal history of eye diseases and ocular	In the older subjects (the mean ± SD 46.9 ± 12 years) there was a significant presence of central dip MP spatial profile Whereas the average age of participants with a typical MP spatial profile was 41.8 ± 12 years; P ±	A central dip was observed in smokers and in an older age group that may represent undesirable features of macular pigmentation.	There are high numbers of participants in this study, and the study performed many tests including the detailed history that contribute to its strength. As this study is a single visit study, so it has its limitation.

			and dermatological sun sensitivities). • Food frequency questionnaire • Fundus and iris photography • MPOD with a customised version of heterochromatic flicker photometry (cHFP). • High–performance liquid chromatography	0.004) and in the current smoker as well (P ± 0.031). In the men study found age–related decline in MPOD (0.25°retinal eccentricity(r = -0.146, P = 0.049).		
Dietzel *et al.* 2011	"Determinants of Macular Pigment Optical Density and its Relation to Age-Related Maculopathy: Results from	N = 369 Follow-up examination of the prospective Muenster Aging and Retina	• Interview by using standardized risk factor questionnaire, which includes information related to; smoking, lifestyle, medical history,	MPOD at 0.5° and 2.0° between pairs and within single eyes was strongly associated (P < 0.001). Smoking and body mass	The study did not confirm the hypothetical inverse association between MPOD and ARM stage.	This study had high numbers of participants that increase the reliability and validity of results. The study could focus on spatial distribution of MP,

	the Muenster Aging and Retina Study (MARS)"	Study (cross-sectional study)	history on vitamin supplements. • Physical examination includes height, weight, pulse rate, blood pressure, and blood sample for genetic and biochemical analysis • Pupil dilation • Fundus photography MPOD measurement with auto-fluorescence method.	index showed moderately inverse relations with MPOD at 2.0°. There was positive association found between age and MPOD in both eccentricities. Use of L/Z vitamin supplements raised MPOD. Measurement of baseline serum was significantly associated with MPOD.		which in terms gives more focused conclusion instead of MPOD measurement in two different eccentricities. Furthermore, cross-sectional study design has its advantages and disadvantages.
Obana *et al.* 2011	"Macular Pigment Changes in Pseudophakic Eyes Quantified with Resonance Raman Spectroscopy"	N= 259 Prospective comparative case series	• Cataract surgery (phacoemulsification) • Post-operative procedures on days 1, 4, 7, and 14 and months 1, 2, 3, 4, 6, 12, 18, and 24 respectively.	There was no significant difference found in baseline characteristics until six months. Up to 1 year no significant	Cataract surgery with clear IOLs can induced decrease in MPOD levels as it transmits higher intensities of blue	This study could suffer from selection bias as while asking the participants for their IOL choice those who were unable to make decisions on their own;

			• Visual acuity • Intra – ocular pressure • Raman MPOD scoring • OCT images	difference in average MPOD results. After one year MPOD, levels were higher in yellow–tinted IOLs. On day one postoperatively multiple regression, older age, and diabetes were correlated with lower MPOD levels; 1 year postoperatively and after that, however, lower MPOD levels were associated with clear IOLs rather than yellow–tinted IOLs.	light than yellow-tinted IOLs.	the examiner chooses IOLs for them in order to keep equality of numbers using clear and yellow IOL. Secondly, among in 259 subjects only 18 subjects were current smokers. Perhaps this may be the reason that study found no significant association between smoking and MPOD levels. Additionally, if double blinded randomised control trial design was adopted than study could get rid of bias problems.
Raman *et al. (*2012)	"Association of macular pigment optical density with	n= 33 (case) 29 (controls)	• Detailed questionnaire (including questions	High risk for AMD was seen in smokers (P=0.032),	The study found an inverse association between wet AMD	The sample size of the research study was small to validate its

	risk factors for wet age-related macular degeneration in the Indian population"	Case – control study	related to demographic, lifestyle, smoking, drinking, medical history) • Semi–quantitative food frequency questionnaire • 'The lifetime ocular UV exposure data collection performed by using Melbourne visual impairment project model.' • A comprehensive eye examination • Pupil dilation • Retinal photographs MPOD measurement by MPOD densitometer that	also smokers have lower MPOD level than non–smokers (mean (95% CI)) (0.16 (0.09–0.23) vs 0.28 (0.22–0.34), adjusted P=0.026). UV exposure had a significant effect on MPOD levels. Subjects with low UV exposure had high MPOD and vice versa (0.46 (0.38–0.54) vs 0.17 (0.01–0.33), P=0.01). Low dietary intake of carotenoids was associated with low MPOD values.	and MPOD. Smoking, UV exposure, and obesity (established risk factors for AMD) had an inverse association with MPOD. While study concludes that dietary intake of carotenoids had a positive association with MPOD.	results on a large scale. Furthermore, age matching between cases and controls was done with ±5 year's difference. An age inclusion criterion for this study was ≤ 50, so risk factors for AMD and factors affecting MPOD on younger population are not clear. The study might be affected by bias factor as dietary intakes of carotenoids were recorded on subject's response and the study was unable to perform the serum concentration of carotenoids to confirm the answers.

			uses heterochromatic flicker photometry (HFP).			
Murray et al. 2013	"Macular pigment in ophthalmic practice; a survey"	N = 56 Survey	• Visual acuity • Routine eye test • MPOD using heterochromatic flicker photometry • Information smoking, gender, family history of AMD and diabetes • awareness of AMD • Iris colour	The overall average MPOD value was 0.400±0.165 (with centre only approach). There was not statistical difference found in increased age with decreased MPOD values (r=−0.17). MPOD values of dark irises were higher than light irises (r=0.28, p<0.05). The MPOD values of type II diabetic participants were 27 % lower than in non-diabetic	This MPOD technique provides similar results and seconds the findings of earlier studies. More than one measurements may be required for a small number of population (similar to other HFP-based methods).	This study did not provide details on questions asked to participants, and it failed to explore the dietary intake questions in research subjects. The study did not compare the MPOD values with diet. Secondly, the percentage of smokers (9/56) was quite less to establish any relationship of smoking with MPOD values.

				participants (r=0.29, p<0.05).		
Abell *et al*. 2014	"The use of heterochromatic flicker photometry to determine macular pigment optical density in a healthy Australian population"	N =201 Cross– sectional	• Food frequency questionnaire • Visual acuity • OCT • Psychophysical MPOD scanning	Age significantly predicted the MPOD results. Those participants who had completed the dietary questionnaire revealed that high diet scores were correlated with high MPOD score. MPOD scores were not affected by gender, iris, and smoking status.	The study has determined a mean MPOD value for healthy subjects in a population south of the equator, providing a reference point for future studies on Caucasian samples.	The study itself detailed its limitation on sampling bias that "the study could not rule out sampling bias in this study by selecting healthy patients who are unlikely to have risk factors or comorbidities and therefore not representative of the general population." The MPOD scanning technique used in this study has its limitation in giving predictive values. In addition to above, dietary responses from research participants may induce respondent bias by under–reporting

						or over–reporting the questionnaire. So the study results would be more validated if study performed serum analysis of subjects to clinically prove their responses.
Obana *et al.* 2014	"Effect of age and other factors on macular pigment optical density measured with resonance Raman spectroscopy"	N =144 Cross- sectional study	Pre-operation • Visual acuity • Axial length • Phacoemulsification Post operation • Auto-refraction • Visual acuity • IOP • BMI • Dilated fundus examination • OCT	The average macular pigment RRS Raman counts were 4,375±1,917 (SD). On multiple regression Analysis, results revealed that age and axial length were significantly correlated with low MPOD values (regression coefficient of	Age acted as an important patient parameter in lower MPOD levels. The study revealed that axial length was also a negative predictor of MPOD levels.	This study revealed the results of participants above 50 years. Effect of age and other factors on younger age was not discussed in this article and remained unknown.

			• MPOD measurement using Raman spectroscopy	−59 for age and −404 for axial length, respectively). No significant correlations were observed for other factors.		
Huntjens *et al.* 2014	"Macular Pigment Spatial Profiles in South Asian and White Subjects"	N = 54 South Asian & 19 White Cross- sectional study	• Lifestyle health questionnaire • Visual acuity • Macular assessment profile test by using heterochromatic flicker photometry (HFP).	Central MPOD was significantly greater in South Asian (0.56 ± 0.17) compared with white subjects (0.45 ± 0.18; P=0.015). Integrated MPOD up to 1.8° (i.e., mean MPOD [MPODav(0–1.8)]) Was also significantly improved in South Asian (0.34 ± 0.09) compared to white	MPOD Atypical profiles were higher in South Asians and resulted in increased integrated MPOD up to 1.88, and may, therefore offer enhanced macular protection from harmful blue light.	This study did not discuss the association of smoking with MPOD results in detail and focused on the macular comparison of macular pigment spatial profiles between white and South Asians.

				subjects (0.27 ± 0.10; P =0.003). Mean MPOD (0–1.8) was significantly increased in all subjects presenting a ring-like profile (0.35 ± 0.08) or central dip profile (0.39± 0.09), compared with typical exponential profiles (0.28 ± 0.09; P < 0.0005). The study observed a statistically significant association between ethnicity and spatial profile type (P=0.008).		

Ji *et al.* (2015)	"Macular pigment optical density in a healthy Chinese population"	N = 441 Cross-sectional	• Demographic and lifestyle data • MPOD measurement at 7° eccentricity by one wavelength reflectometry • Lens Opacities Classification System III (LOCS III)	A significant inverse relationship of MPOD with age. Participants with lens opacities had lower MPOD scores compared with participants with no lens opacities. The MPOD values were not associated with sex, BMI, or smoking status.	In Chinese population, MPOD values within 7° of eccentricity was found to decrease with increasing age, and lens opacities had an impact on these measurements.	The study used a new one-wavelength reflectometry method to measure MPOD values. However, the study did not compare its results with other methods of measuring MPOD to find the reliability of results obtained. Smoker's participants (33 out of 441) were low in numbers. Different dietary habits could also alter the outcome but this was not measured.

In the present review of the literature some studies did not find any association of smoking with MPOD levels. Obana *et al.* (2011) did a prospective comparative case series to investigate changes in MPOD level after cataract surgery. In multiple regression analysis, other important parameters (e.g. age, sex, smoking, IOP, glaucoma, BMI, pre and post-operative, VA and refractive error, IOL, and its type) were analysed. In context of smoking, the study revealed that smoking caused a non–significant decrease in MPOD levels. One of the possible reasons for not finding a significant relationship between smoking and MPOD could be due a low number of smoker participants (31 out of 259) enrolled overall.

Abell *et al.* (2014) and Obana *et al.* (2014) did not find any significant association of smoking, with MPOD. Both studies found age as a negative predictor for MPOD and did not find any association of other risk factors such as smoking, gender, diabetes with MPOD. Both studies designs were cross-sectional and had low numbers of smoker participants (eight participants out of 144 in Obana *et al.* 2014 and 22 participants 201 participants in Abell *et al.* 2014 study). Similarly, Huntjens *et al.* (2014) compared macular pigment spatial profiles of South Asian (n = 54) and White subjects (n = 19). The study found no significant difference in MPOD values between smokers and non-smokers participants. The participation of subjects who were smokers (n = 8) in this study was lower (three out of 54 South Asian participants and 5 out of 19 White participants).

Murray *et al.* (2013) and Ji *et al.* (2015) conducted surveys in England and China to find out risk factors associated with MPOD. Neither Murray *et al.* 2013 nor Ji *et al.* (2015) found any association of smoking with MPOD. One of the possible reason for not finding any significant association between smoking and MPOD could be due to restricted numbers of smokers participants enrolled in the study. Ji *et al.* (2015) had only 33 smokers out of 408 participants. Murray *et al.* (2013) had nine smokers out of 56 participants. All studies (mentioned above) considered smoking either as a risk factor or as a confounding factor.

In this present literature review, studies like Raman *et al.* (2012), Dietzel *et al.* (2011), Kirby *et al.* (2010), Nolan *et al*. (2007), Hammond *et al.* (2002), & Hammond *et al.* (1996) have shown an inverse relationship between smoking and MPOD levels.

Hammond *et al.* (1996) conducted a direct study to find out the effect of smoking on retinal carotenoids in 68 participants (34 smokers and 34 non-smokers) as a primary outcome. The study concluded that the mean MP optical density in smokers was 0.16 (SD = 0.12) compared to the average MP optical density of non-smokers which were more than two folds of smokers group 0.3 (SD = 0.1), and this difference was statistically significant ($p < 0.0001$). The study also found an inverse dose–response relationship between MP density and smoking

frequency (r= -0.5, p < 0.0001). There was a difference in dietary intake between smokers and non-smokers participants. Smoker's participants consumed a significantly higher amount of fats compared to non-smokers.

Hammond (2002) and Nolan *et al.* (2007) found a consistent result to Hammond *et al*. (1996) in their cross-sectional design studies. Hammond (2002) found lower MPOD values in individuals living in a high light environment. There was a significant difference (p <0.004) found in low MP density of heavy smokers than in light smokers (0.18 ± 0.09 and 0.25 ± 0.09, respectively) with those who never smoked (0.23 ± 0.09, p < 0.03). There was no relationship found between cumulative exposure to cigarettes (or smoking years) with MP density. Nolan *et al.* (2007) study had comparatively higher participation of smokers (n = 155), ex-smokers (n = 205) and non-smokers (n = 405). The study found a statistically significant difference in MP density between smokers and non-smokers, and even with past smoker and non-smokers (p < 0.01). The frequency of smoking was found to be inversely associated with MPOD levels in current smokers even after adjusting the dietary intake of L and Z (r = - 0.2, p < 0.01).

Kirby *et al.* (2010) found no significant difference in MP density values (current smokers= 85, past smokers = 127 & never smoke= 272) at any retinal eccentricities nor in MPOD area which was attributed to smoking status (p>0.05). However, the study found a significant relationship between cigarette smoking and MP spatial profiles. There was a higher percentage of current smokers (18.8%) with the central dip in MP spatial profile compared to past smokers (14.2%) and never smokers (8.8%) with statistical significance (p= 0.03). On the binary logistic regression for MPOD spatial profile to known and putative risk factors for AMD, cigarette smoking showed a significant relationship (p= 0.02). In addition to above, the logistic regression output revealed that with age adjustment, never smokers were less likely to have a central dip in MP spatial profile than current smoker (p= 0.005).

Dietzel *et al.* (2011) conducted a study on the follow–up participants of (n =369, current & past smokers = 126, never smoked = 243) the prospective Muenster Aging and Retina Study and measured MPOD values at 0.5^{o} and 2.0^{o} from the centre of the fovea. In the context of smoking association with MPOD values, the study revealed that smoking was associated with low MPOD values at 2.0^{o}. Women and those who never smoked showed higher average MPOD values than those who were men and current or former smokers. Dietzel *et al.* (2011) did not evaluate the MPOD values for smokers and past smokers separately, and their study did not investigate any possible association between smoking frequency and MPOD values.

Raman *et al.* (2012a) found that smoking was significantly associated with increased risk for AMD with unadjusted odds ratio is 3.5 (95% CI 1.1–11.5, p = 0.03). There was no significant dose-response relationship found with other cigarettes smoking measures (e.g. years of

smoking or pack of cigarette smoked per year). In multivariate analysis, (whilst keeping age and gender constant) smoking showed an OR of 3.9 (95% CI 1.2–12.0, p = 0.02). In the unadjusted and adjusted mean MPOD values with all variable of AMD, smoking showed significant association with AMD (adjusted p = 0.02). This finding was also similar to the study by Hammond *et al.* (1996), which showed that smokers had fifty–three per cent lower average MPOD values than non–smokers. Similarly, this study had demonstrated an inverse relationship between smoking frequency and MPOD (r= - 0.4, p = 0.04) which is also consistent with previous studies results done by Hammond *et al.* (1996) and Nolan *et al.* (2007).

1.10 Impact of diet on the tear film

Vitamin A, vitamin D, omega–6 and omega–3 rich diet are considered as a nutritional supplement for the prevention and treatment of dry eye syndrome (DES).Vitamin A is an essential element in maintaining epithelial tissues and which may contributes to evaporative form of dry eye (Foulks *et al.*, 2007). Vitamin D, which is a fat-soluble vitamin; it is produced in the skin after exposure to sunlight. It is well known that vitamin D is good for bone health but less known that it has the potential in maintaining epithelial cell health (Bikle, 2010). Similarly, essential fatty acids (EFAs) for humans are alpha-linolenic acid, and linolenic acid that is known as omega–3 and omega–6. These EFAs may have a protective role in enhancing pre-corneal lipid layer and thus may provide support in retarding tears evaporation (Srinivasan and Yip, 2007). The main sources of omega–6 are corn oil, sunflower oil, peanut oil, cereals, egg, and in whole wheat grains. Similarly, omega–3 EFAs are commonly found in fish, fish oil, green leafy vegetables, and nuts and in beans (Roncone *et al.*, 2010). Table 1.3 highlights studies that show the effect of omega-3 and omega-6 on the tear film:

Author's and Publication date	Study title	Sample size and study design	Clinical tests	Results	Conclusion	Strengths and limitations
Larmo *et al.* (2010)	"Oral sea buckthorn oil attenuates tear film osmolarity and symptoms in individuals with dry eye"	N = 100 RCT design	• Tear film osmolarity • TBUT • Schirmer test • OSDI scores	No significant difference was observed in the mean values of OSDI scores, TBUT, and Schirmer's test (1) results between treatment and placebo groups. An increase in tear osmolarity was seen in both groups. Treatment group had significantly less increase in tear osmolarity compared to placebo group (p = 0.04).	Sea buckthorn oil reduced the increase in tear film osmolarity in cold sessions and it is helpful in reducing dry eye symptoms.	Selecting a double-blinded RCT study design gives the strength to study results. Dye eye has different types and many causative factors and the study did not aimed to recruit study participants of any specific subtype.
Wojtowicz *et al.* (2011)	"Pilot, prospective, randomized, double-masked, placebo-	N = 36 RCT design	• OSDI score • Fluorophotometry • Schirmer's test	Seventy percent participants from treatment group and seven percent	Intake of Omega-3 had shown no effect in meibum lipid composition or	A double masked randomisation was done which gives strength to the study.

	controlled clinical trial of an omega-3 supplement for dry eye"		• Tear evaporation rate • Meibomian gland secretion samples • TBUT	participants from placebo group became asymptomatic in the end of trial. Schirmer's test and fluorophotometry showed increased tear secretion in treatment group. No trend was observed between groups for other parameters.	aqueous tear evaporation rate. However, it increased tear volume production.	However, the study itself mentioned that the study was statistically underpowered.
Brignole-Baudouin *et al.* (2011)	"A multicentre, double-masked, randomized, controlled trial assessing the effect of oral supplementation of omega-3 and	N = 138 Multicentre RCT design	• Dry eye symptoms • TBUT • Schirmer's test • Corneal fluorescein staining • Lissamine green test	Although not statistically significant but participants who received the treatment of fatty acids showed a trend of improvement in DES signs and symptoms. There was a significant	Omega-3 and omega-6 supplements can reduce expression of HLA –DR conjunctival inflammatory marker and can be beneficial for reducing dry eye symptoms.	Bausch and Lomb sponsored this study and one of the authors of the study was an employee of Bausch and Lomb that shows a conflict of interest. On the other side, the study used a refined and objective method of

	omega-6 fatty acids on a conjunctival inflammatory marker in dry eye patients"			reduction observed in HLA-DR positive cells in treatment group (p = 0.02) compared to placebo group.		assessment (HLA-DR) that provides strength compared to subjective end points (such as patient reported symptoms.
Kawakita *et al.* (2013)	"Effects of dietary supplementation with fish oil on dry eye syndrome subjects: randomized controlled trial"	N = 27 RCT design	• Subjective symptoms • TBUT • Schirmer's test (I) • Fluorescein staining • Rose Bengal staining	TBUT and subjective symptoms of "eye pain scores" were improved after 12 weeks in fish oil supplement group. There was no significant difference observed in other test parameters.	Fish oil supplementation can be effective in DES treatment.	A double masked randomisation was done which gives strength to the study. However, the study dropout rate was high (16 out of 43 participants) left a small sample size.
Bhargava *et al.* (2016)	"Short-Term Omega 3 Fatty Acids Treatment for Dry Eye in Young and Middle-Aged Visual	N = 522 RCT design	• Dry eye symptoms • TBUT • Schirmer's test (I) • Conjunctival impression cytology	Omega-3 fatty acid group showed a significant improvement in subjective symptoms, TBUT and in Nelson gradations for	Consumption of 2400 mg/ day of omega-3 fatty acid supplement improves symptoms, conjunctival cytology, and tears stability but not tear production in	Apart for RCT study design, this study had a larger sample size, and a younger study population (compared to other studies). In contrast, this study focused on

	Display Terminal Users”			conjunctival impression cytology compared to placebo group.	symptomatic VDT users.	VDT user related DES, there could be a possibility of different results attained from other DES factors (such as smoking, old age).

All of the studies included in table 1.3 were double-blinded RCT style studies. RCT methodology is considered as the gold standard in eliminating selection related bias and this style gives a high internal validity (Stang, 2011). All of the above-mentioned studies commonly conducted three clinical tests (i.e. symptoms scores, TBUT and/or Schirmer's test scores).

In terms of patient-related subjective symptoms results, two studies showed no significant improvement in DES symptoms. Larmo *et al.* (2010) used OSDI to evaluate the DES symptoms while Brignole-Baudouin *et al.* (2011) used customised DES symptoms (dryness, burning sensation, photophobia and stinging). Both studies found an improvement in DES symptoms but the difference was not statistically significant. In contrast, some studies such as Wojtowicz *et al.* (2011), Kawakita *et al.* (2013) & Bhargava *et al.* (2016) observed a significant improvement in DES symptoms after ending of trial supplementation.

In terms of TBUT results, Bhargava *et al.* (2016) & Kawakita *et al.* (2013) found a significant increase in TBUT in the treatment group after ending trial supplementation time period. Other studies did not show any improvement in TBUT reading after ending trial time period. In contrast, Larmo *et al.* (2010) & Wojtowicz *et al.* (2011) found no significant improvement in TBUT readings. The study duration of Bhargava *et al.* (2016) was shorter than rest of the studies (45 days compared to 90 days). The dropout rate was higher in Kawakita *et al.* (2013) study. These factors could be the possible reasons for having mixed results with context to TBUT measurements.

Another common but important clinical measurement was Schirmer's test scores and it is related to tears production. Out of five only one study Wojtowicz *et al.* (2011) found an increased in tears secretion at the end of the trial period in the treatment group. In contrast, other studies included in this literature review did not find any significant change in Schirmer's test scores. The result related to TBUT and Schirmer's test scores is still inconsistent in this review.

Table 1.4 shows studies conducted on the effect of vitamin D on tear film as below:

Author's and Publication date	Study title	Sample size and study design	Clinical tests	Results	Conclusion	Strengths and limitations
Galor *et al.* (2014)	"Effect of a Mediterranean dietary pattern and Vitamin D levels on dry eye syndrome"	N = 247 Cross-sectional study	• Food Frequency Questionnaire • Dry Eye Questionnaire 5 • Tear osmolarity test • TUBT • Corneal staining • Schirmer test (II) • Measurement of serum vitamin D levels	Adherence to Mediterranean diet (MeDi) was not associated with a positive effect on DES. Higher levels of vitamin D were associated with decreased DES symptoms.	Adherence to MeDi was not linked with any beneficial effect on DES. There was a small but favourable effect of high vitamin D levels on DES symptoms.	The study had a large sample size. In contrast, there were many limitations associated with this study such as potential bias factor in dietary assessment and confounding factors such as smoking status, exposure to sunlight and socioeconomic status.

Kurtul *et al.* (2015b)	"The association of vitamin D deficiency with tear break-up time and Schirmer testing in non-Sjögren dry eye"	N = 55 Prospective clinical study	• TBUT • Schirmer test (I) • OSDI scores • Serum level of vitamin D measurement	The controlled group had higher TBUT, Schirmer test (I) and serum vitamin D levels compared to vitamin D deficient group ($p > 0.05$ for all). DES symptoms were detected in all participants of study group compared to 15 % in controlled group.	The study demonstrated that vitamin D deficiency decreases the TBUT and Schirmer test values. Lower vitamin D levels may be associated with DES in non-Sjögren syndrome.	The study added a value in the existing literature by showing the link between vitamin D deficiency in non- Sjögren syndrome. In contrast, the sample size is small and further studies with larger sample sizes are required to strengthen these findings.
Jee *et al.* (2016)	"Serum 25-Hydroxyvitamin D Levels and Dry Eye Syndrome: Differential Effects of Vitamin D on Ocular Diseases"	N = 16,396 Survey/ cross-sectional design	• serum 25-hydroxyvitamin D levels analysis • Blood pressure measurement • Blood glucose measurement	After adjusting for confounding factors such as smoking, sun exposure, age, sex, diabetes and hypertension, there was no significant relationship found between DES and	The current study did not support any association between serum vitamin D levels and DES. The beneficial effect of serum 25-hydroxyvitamin D may be more effective for diabetic	Large sample size and cross-sectional study design are the strengths of this study. However, cross-sectional study design has limitations of its own kind. The study did not explain clearly, what kind of

			• Semi-structured interview • Full ophthalmic examination	serum vitamin D levels.	retinopathy and age-related macular degeneration compared to DES and cataract.	ocular surface test it had used. DES was also self-reported.
Yildirim *et al.* (2016)	"Dry eye in vitamin D deficiency: more than an incidental association"	N = 98 Case- control study	• Schirmer (I) test • TBUT • OSDI score • Stanford Health Assessment Questionnaire (HAQ) • Fatigue severity scale (FSS) • Visual analogue scale-pain (VAS-pain).	Participants with vitamin D deficiency had significantly lower Schirmer and TBUT test values. There was a moderate but negative correlation between OSDI scores and vitamin D levels. There was weak but negative correlation found between FSS and TBUT, and Schirmer test values. No significant correlation found between HAQ	Vitamin D deficient patients showed signs of DES and impaired tear function that indicates the protective role of vitamin D in DES. Vitamin D deficient patients should be evaluated for DES.	The present study strengthen the findings of previous studies that showed a positive effect of vitamin D on ocular surface by conducting different tests (subjective and objective) of tear film parameters. One of the main limitation of this study is inclusion of female gender only. To apply the results on general population further studies are required

				scores and DE parameters.		with the inclusion of both genders.
Jeon *et al.* (2017)	"Are Serum Vitamin D Levels Associated With Dry Eye Disease? Results From the Study Group for Environmental Eye Disease"	N = 740 Cross-sectional study	• Serum vitamin D levels • OSDI scores	There was no significant association found between DED and serum vitamin D levels.	The findings of the current study did not support any association of DED with serum vitamin D levels.	Large sample size and cross-sectional study design are main strengths of the current study. In contrast, limitation associated with cross-sectional study design can limit its results' implication. Secondly, the study did not measure any clinical test to assess dry eye disease. Only OSDI scores were used to categorize DED.
Demirci *et al.* (2018)	"Dry Eye Assessment in Patients With Vitamin D Deficiency"	N = 60 Cross-sectional study	• TBUT • Schirmer test (I) • OSDI score	Participants with vitamin D deficiency had higher, OSDI scores, high tear osmolarity values	The study concluded that vitamin D deficiency is associated with tear hyperosmolarity and	The study strengthens the previous studies results that showed positive association

			• Fluorescein staining using Oxford scale scoring system • Tear osmolarity	and had higher Oxford scale scores compared to normal participants ($p > 0.05$ for all). TBUT and Schirmer (I) test values were lower in vitamin D deficient group compared to normal group ($p = 0.001$).	tear film abnormalities.	between vitamin D deficiency and DES. The study self-reported some of its limitations such as small sample size, lack of screening for inflammatory markers (such as IL-6, TNF, alpha and MMP-9) and lack of a control group consisting of participants of Dry eye with vitamin D deficiency.

All studies included in this literature review were observational studies. Three most common clinical tests (i.e. dry eye questionnaire/symptoms, TBUT, and Schirmer's test values).
Jee *et al.* (2016) and Jeon *et al.* (2017) did large sample surveys on the association between serum 25-hydroxyvitamin D levels and dry eye syndrome (DES). Jee *et al.* (2016) used a customised DES related questionnaire while Jeon *et al.* (2017) used OSDI. Both studies did not associate vitamin D levels with DES. In contrast, Galor *et al.* (2014), , Kurtul *et al.* (2015b), Yildirim *et al.* (2016) and Demirci *et al.* (2018) studies have consistent results in terms of patient-related symptoms. All of these studies suggest vitamin D levels are positively correlated with dry eye symptoms. Jee *et al.* (2016) did not explain clearly in their methodologies on what ocular surface test they have used while and Jeon *et al.* (2017) subdivided their study population in to normal or DED participants on the basis of OSDI scores but failed to use any other clinical test that can confirm DED objectively.

There is an inconsistency in TBUT results, Galor *et al.* (2014) did not find any statistically significant difference between intake of the Mediterranean dietary pattern (MeDi) or vitamin D levels with TBUT values. Jee *et al.* (2016) and Jeon *et al.* (2017) did not explain the nature of clinical test used clearly. However, other studies included in this literature review showed a decreased TBUT in the vitamin D deficient group. Those studies that showed a positive effect of vitamin D levels on TBUT usually measured serum levels of vitamin D instead of estimating vitamin D levels from dietary intake. Measuring vitamin D from dietary intake can be biased compared to serum analysis.

Similarly, there is an inconsistency in Schirmer's test I results. Galor et al. (2014) did not show any significant effect of vitamin D levels and MeDi on Schirmer I test values. In contrast, other studies have shown a positive effect between vitamin D intakes on Schirmer's test values. One of the possible reasons for the discrepancy of results could be due to the difference of methods used in evaluating vitamin D levels. So far, there are no RCT styles studies conducted on effect of vitamin D levels on DES. Although RCT style studies are expensive and time-consuming but such studies, results are more reliable.

Two studies have also shown an association between vitamin D levels and ocular surface fluorescein staining. However, the results are not consistent. Galor *et al.* (2014) found no significant association of conjunctival staining with vitamin D intake. In contrast, Demirci *et al.* (2018) reported that ocular surface fluorescein staining were significantly higher in the vitamin D deficient group ($p > 0.05$). The possible reason in the inconsistency of results would be due

to the fact that Galor *et al.* (2014) did not divide their study population based on vitamin D deficiency but Demirci *et al.* (2018) divided their population into two groups vitamin D deficient group and normal participants group.

1.11 Dietary intake effect on accommodation facility

So far, there are no studies, which were conducted to investigate effects of diet on accommodation facility of human lens but there are evidence which states that dietary intake can influence on the age related cataract (ARC). Many studies have done so far on this topic but the data have been inconsistent. Cataract is one of the main causes of blindness in the world and its prevalence is high in the developing world (WHO, 2010). Approximately 70 percent of elderly population aged above than 79 years have lens opacities. According to McCusker *et al.* (2015), one of the first prospective studies on the association of vitamins with risk factor of cataract development was conducted by Sperduto *et al.* (1993b) in nutritionally deprived population of rural China named as “Linxian cataract studies”, which showed that multivitamins and minerals supplements was significantly associated with 36 percent of reduction in nuclear cataract. A meta-analysis by Zhang *et al.* (2015b) have shown that dietary and supplementary intake of vitamin E, and high level of serum tocopherol may associated with low risk of age related cataract (ARC).

Appleby *et al.* (2011) did a study on diet association with cataract risk in a large population. Total 26,750 volunteers of aged above than 40 years were selected from a hospital database from England and Scotland. The study observed a significant relation between diet and cataract. The study observed a progressive decrease in cataract risk from high meat eaters to low meat eater, fish eating participants, vegetarian, and vegans. The study found that incidence of cataract decreases from 0.9 (0.8, 1.1) in moderate meat eaters to 0.6 (0.3, 0.9) in vegetarians and vegans (95% CI, $p < 0.001$) after adjusting multivariable. Mares *et al.* (2010) found a negative association between healthy diet scores and prevalence of nuclear cataract in US women. The multivariate-adjusted odds ratio showed that high healthy diet score was strongest modifiable predictor of low prevalence of nuclear cataract. High versus low quintile for diet score was 0.6 (95% confidence interval, 0.4-0.9).

Abdellah *et al.* (2019) conducted a case control study on 325 participants with cataract and 380 control participants to observe relationship between serum vitamin D levels and ARC. The study observed that a significant decrease in vitamin D levels in cataract participants (7.6 ± 5.5 ± 11.2 ng/mL) versus controls (18.5 ± 9.6 ng/mL). Individuals with nuclear cataract had significantly lower vitamin D levels compared to individuals with cortical and posterior sub-capsular cataracts ($p < 0.001$). Similarly, some studies have shown other dietary elements such as acids omega-3, antioxidants, and lutein zeaxanthin (L/Z) association with cataract.

Sedaghat *et al.* (2017) observed different nutrient patterns and risk of cataract in Iranian population in their case control study and found antioxidants pattern (beta and alpha carotene, vitamin A and vitamin C) and omega-3 pattern significantly reduced the risk of cataract ($p < 0.05$).

Mamatha *et al.* (2015) conducted a hospital-based study in Indian population to find out risk factors for nuclear and cortical cataract. The study found that individuals who had higher intake of L/Z and β-carotene were associated ($p < 0.001$) with a lower risk of nuclear and cortical cataracts. However a few studies did not show any significant association of diet or dietary elements with cataract. In the AREDS (AREDS, 2001a) trail of cataract analysis, there was no significant association of diet found on the progression of cataract or on visual acuity. In antioxidant group the lens related events were 33 percent whereas in the placebo group it was 34 percent (OR = 0.9, p = .5) and similarly, there was no significant difference in visual acuity score (OR = 1.03, p = .89) between groups. Jee *et al.* (2016) also did not find any protective effect of serum 25-Hydroxyvitamin D effect on cataract (OR = 0.7, 95% CI: 0.6–1.0). Finally, Sedaghat *et al.* (2017) did not find any protective association of polyunsaturated trans-fatty acids (PUFA) nutritional pattern on cataract and in fact found that fatty pattern The fatty acid pattern elevated the risk of cataract (OR=1.9, 95%CI: 1.1-3.8).

1.12 The association of diet with macular pigment optical density

Dietary intake of antioxidants is considered as a primary prevention for macular degenerations (Jampol and Ferris III, 2001) and many large RCT style studies have reported the preventive effects of vitamin C and E, zinc and β carotene including AREDS study in 2001. It is suggested that antioxidants prevent macular degeneration by reducing the oxidative stress and damage to retina that may occur in retina due to constant exposure to light especially blue light (Seddon and Hennekens, 1994). Lutein (L), zeaxanthin (Z) and meso-zeaxanthin are present in central macula and their percentage decreases with increase in the retinal eccentricity Howells *et al.* (2011) and together they are called as macular pigment. The human body cannot synthesise carotenoids and thus the main source of these carotenoids is from diet. Some of the main source of lutein and zeaxanthin are egg yolk, maize, spinach, and kale (Schalch, 1992). In the literature, some studies have shown a positive relationship of dietary and serum intake retinal carotenoids and MPOD (Rock *et al.*, 2002, Bone *et al.*, 2003, Trieschmann *et al.*, 2007, Arnold *et al.*, 2013).

Table 1.5 summarises some studies conducted on the effect of dietary L/Z intake and MPOD values as below:

Author's and Publication date	Study title	Sample size and study design	Clinical tests	Results	Conclusion	Strengths and limitations
Curran-Celentano *et al.* (2001)	"Relation between dietary intake, serum concentrations, and retinal concentrations of lutein and zeaxanthin in adults in a Midwest population"	N = 280 Cross-sectional study	• Health questionnaire • Blood sampling • Food frequency questionnaire • MPOD measurement	Average MPOD was 0.21 ± 0.13. Dietary and serum intakes of L and Z were significantly correlated ($r = 0.21$, $p < 0.001$). L and Z values were related with variation in MPOD ($r = 0.25$, $p < 0.001$).	The study found an association of MPOD with dietary and serum L and Z intakes. Further studies should include MPOD measurement to understand retinal carotenoids role in diseases like AMD.	The current study used the cross-sectional study design to explore the relationship between diet and serum intakes of L/Z and MPOD measurement. In contrast, biological factors such as individual's absorption profile and day-to-day variations in blood concentration can limit its implication on general population.
Trieschmann *et al.* (2007)	"Changes in macular pigment optical density and serum	N = 136 Case-control study	• MPOD measurement by	There was a significant increase in MPOD value in the	The current study found that L and Z Supplementation,	The current study was the first study to use two-wavelength

	concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study"		Autofluorescence method (AF) • Serum L and Z measurement • Antioxidants and L and Z supplementation	intervention group over the period of supplementation (12 weeks) compared to no significant increase in the control group. Participants with low baseline MPOD were more likely to show either a dramatic rise, or no rise in MPOD compared to those subjects who had medium to high baseline MPOD values.	combined with co-antioxidants, lead to in an increase of MPOD at 0.5° eccentricity in most participants. Some participants did not have any increase in MPOD value despite an increase in serum levels of Land Z, indicating intestinal malabsorption of these carotenoids.	AF technique for evaluating MPOD values in an AMD population. In contrast, the study medication "Ocuvite Lutein™" was obtained from a commercial company, and the study did not show any "conflict of interest" or any information related to funding. Thus increasing the risk of potential bias.
Nolan *et al.* (2007)	"Risk factors for age-related maculopathy are associated with a relative lack of macular pigment"	N = 828 Cross-sectional study	• MPOD measurement with heterochromatic flicker photometry • Serum levels of L and Z	There was a statistical age-related decline in MPOD. Smokers and ex-smokers had lower MPOD values.	The study concluded that the increase in age, family history of ARM and tobacco use were associated with the lack of MP.	The study sample size was large, and it tried to reduce bias factor in selecting participants as well.

			• Food frequency questionnaire	Participants with family history of AMD had lower MOPD values. Dietary and serum levels of L and Z were positively related to MPOD (r = 0.185–0.230, p < 0.01). In multiple linear regression models only dietary and serum L was found to be a positive predictors of MPOD.	This association was positively associated in the absence of retinal pathology, and in advance of disease onset. The study supports the hypothesis that the enhanced risk that these variables represent for ARM may be attributable, at least in part, to a parallel deficiency of macular carotenoids.	In the study's limitations, the study was unable to confirm the self-reported cholesterol levels by participants and the study has not investigated any use of statins among those who reported high cholesterol levels. The study used L and Z as a confounding factors and did not investigate it as a primary outcome.
Kirby *et al.* (2010)	"A Central Dip in the Macular Pigment Spatial Profile Is Associated with Age and Smoking"	N= 484 Single visit study	• Detailed demographic questionnaires • Food frequency questionnaire	In the older subjects (the mean ± SD 46.9 ± 12 years) there was a significant presence of central dip. Current smokers	A central dip was observed in smokers and in an older age group that may represent undesirable features	There are high numbers of participants in this study, and the study performed many tests including the detailed history that

			• Fundus and iris photography • MPOD heterochromatic flicker photometry (HFP). • High–performance liquid chromatography	had significantly lower MPOD values. In men, the study found age–related decline in MPOD (0.25°retinal eccentricity(r = -0.146, p = 0.049). There was no significant relation found between dietary intake of L and Z. However, serum level of L and Z were positively associated with MPOD values at all eccentric levels.	of macular pigmentation.	contribute to its strength. As this study is a single visit study, so it has its limitation.
(Richer *et al.*, 2011a)	"Randomized, double-blind, placebo-controlled study of zeaxanthin and visual function in patients with atrophic	N = 60 RCT design	• MPOD measurement by heterochromatic flicker photometry	The participants were divided into three groups (i.e. 8 mg Zx group (n = 25) and 8 mg Zx plus 9 mg lutein (L) group	In older male population with AMD, Zx formula helped in foveal elevation of MPOD associated with cone. The L	The current study results make a biological sense on the retinal distribution and predominance of Zx

	age-related macular degeneration The Zeaxanthin and Visual Function Study (ZVF)"		• Low and high contrast visual acuity • 10^0 Yellow Kinetic visual fields • Contrast sensitivity • Glare recovery • 6^0 blue cone Chroma Test	(n = 25) and 9 mg L (n = 10) placebo group. MPOD values significantly increased in all three groups. The Zx group participants had a significant increase in visual acuity (1.5 lines).	enhanced parameters associated with rods.	in foveal region. One of the limitation of the study is the low presence of female participants (n = 3).
Dietzel *et al.* (2011)	"Determinants of Macular Pigment Optical Density and Its Relation to Age-Related Maculopathy: Results from the Muenster Aging and Retina Study (MARS)"	N = 369 Longitudinal study	• MPOD measurement via Autofluorescence measurement • Risk factor questionnaire • Body mass index (BMI) • Serum analysis for L and Z • Blood measurement	MPOD was measured at two foveal eccentricities 0.5 and 2.0 degrees. Smoking and BMI were negatively associated with MPOD at 2.0°. Serum level of L and Z were positively associated with MPOD. There was a	Smoking, BMI, and age exert have a weak effect on MPOD. L serum levels (mostly due supplements) had a positive impact on foveal MPOD.	The study had a large number of participants. It runs for a longer duration (2.5 years). Limitations associated with the study are associated with its design. The self-reported study's limitation was inability to study

				slight but significant increase in MPOD with increase in age.		spatial distribution of MP profiles.
Raman *et al.* (2012a)	"Association of macular pigment optical density with risk factors for wet age-related macular degeneration in the Indian population"	N = 62 Case – control study	• Detailed questionnaire (including questions related to demographic, lifestyle, smoking, drinking, medical history) • Semi–quantitative food frequency questionnaire • 'The lifetime ocular UV exposure data collection performed by using Melbourne visual impairment project model.'	High risk for AMD was seen in smokers (P=0.032), also smokers have lower MPOD level than non–smokers (mean (95% CI)) (0.16 (0.09–0.23) vs 0.28 (0.22–0.34), adjusted P=0.026). UV exposure had a significant effect on MPOD levels. Subjects with low UV exposure had high MPOD and vice versa (0.46 (0.38–0.54) vs 0.17 (0.01–0.33), P=0.01). Low dietary intake of carotenoids	The study found an inverse association between wet AMD and MPOD. Smoking, UV exposure, and obesity (established risk factors for AMD) had an inverse association with MPOD. While study concludes that dietary intake of carotenoids had a positive association with MPOD.	The sample size of the research study was small to validate its results on a large scale. Furthermore, age matching between cases and controls was done with ±5 year's difference. An age inclusion criterion for this study was ≤ 50, so risk factors for AMD and factors affecting MPOD on younger population are not clear. The study might be affected by bias factor as dietary intakes of

			• A comprehensive eye examination • Pupil dilation • Retinal photographs • MPOD measurement by heterochromatic flicker photometry (HFP).	was associated with low MPOD values.		carotenoids were recorded on subject's response and the study was unable to perform the serum concentration of carotenoids to confirm the answers.
Abell *et al.* (2014)	"The use of heterochromatic flicker photometry to determine macular pigment optical density in a healthy Australian population"	N = 201 Cross– sectional	• Food frequency questionnaire • Visual acuity • OCT • Psychophysical MPOD scanning	Age significantly predicted the MPOD results. Those participants who had completed the dietary questionnaire revealed that high diet scores were correlated with high MPOD score. MPOD scores were not affected by gender,	The study has determined a mean MPOD value for healthy subjects in a population south of the equator, providing a reference point for future studies on Caucasian samples.	The study itself detailed its limitation on sampling bias that "the study could not rule out sampling bias in this study by selecting healthy patients who are unlikely to have risk factors or comorbidities and therefore not representative of the

				iris, and smoking status.		general population." The MPOD scanning technique used in this study has its limitation in giving predictive values. In addition to above, dietary responses from research participants may induce respondent bias by under–reporting or over–reporting the questionnaire. So the study results would be more validated if study performed serum analysis of subjects to clinically prove their responses.
Alassane *et al.* (2016)	"Relationships of Macular Pigment	N = 433	• MPOD measurement by	Mean MPOD was significantly higher in	Plasma L levels were associated with	The study presents its findings were

	Optical Density With Plasma Lutein, Zeaxanthin, and Diet in an Elderly Population: The Montrachet Study"	Cross-sectional study	Autofluorescence method • Plasma L and Z levels • Self- reported food frequencies questionnaires	female than male participants were. MPOD was positively correlated with plasma L and Z levels. Participants with alcohol intake had lower MPOD than non-alcoholic participants.	MPOD after adjusting for potential confounding factors in elderly population.	reported in a simple but comprehensive way that was easy to read and follow. The study self-reported its limitations that include potential bias from self-reported food frequency questionnaire, only Caucasian ethnic participants, and not evaluating MP spatial profiles.
Li *et al.* (2018)	"Macular pigment and serum zeaxanthin levels with Goji berry supplement in early age-related macular degeneration"	N = 114 RCT style	• MPOD measurement by heterochromatic flicker photometry • Serum levels of L and Z • Slit lamp bimicroscopy • Visual acuity	Participants who were taking Goji berry supplement had an increase in serum Z level and in mean MPOD compared to control participants after 90 days of	Goji berry supplementations for 90 days improve serum Z levels and MPOD. It may be an effective therapeutic intervention for preventing AMD progression.	The study design is double blinded RCT that have its own benefits. The sample size is small and the intervention time was limited to 90 days. Study ethnic population was

			• Questionnaires • Optical coherence tomography • Colour fundus photography	supplementation. No increase in serum L levels. The treatment group had a relative increase in visual acuity after 90 days of consuming Goji berry supplement.		limited to Chinese. It may be possible that results would be different with other ethnic background participants who have different nutritional status.

High dietary intake of antioxidants (especially L/Z) is associated positively with MPOD values. In the present literature review, ten studies were included. Eight out of ten studies were observational studies and two studies were RCT style. In terms of dietary association with MPOD levels, some studies, Curran-Celentano *et al.* (2001); Nolan *et al.* (2007); Raman *et al.* (2012a) & Abell *et al.* (2014) analysed the correlation between dietary intake of L and Z with MPOD. In contrast, Kirby *et al.* (2010) did not find any relationship between dietary intake of L and Z and MP spatial profiles. All of the studies that analysed the dietary relationship with MPOD were cross-sectional design nature.

All of the studies listed in table 1.5 above conducted serum analysis of L and Z levels. Most of the studies observed that both serum L and serum Z level were positively associated with MPOD levels. However, few studies i.e. Nolan *et al.* (2007) found only serum L level was positively and significantly associated with MPOD levels. Contrarily, Li *et al.* (2018) found serum Z level were positively associated with MPOD levels.

Another important comparison of the studies described in the literature review above can be done based on MPOD measuring technique. There are various objective techniques of measuring MPOD (Canovas *et al.*, 2010). These techniques are divided into two methods i.e. optical methods (such as Autofluorescence spectrometry) and psychophysical methods (such as heterochromatic flicker photometry). Six out of 10 studies used heterochromatic flicker photometry. One of the main limitations of this method is that MPOD results rely on patient cooperation (Curran Celentano *et al.*, 2002). Some studies in this literature review involved elder participants such as (Alassane *et al.*, 2016, Dietzel *et al.*, 2011), results of these studies could be of low reliability than those studies in which Autofluorescence spectrometry method was used.

Apart from L/Z, there is growing evidence of a protective impact of poly-unsaturated trans-fatty acids (PUFA) on MPOD. Long-chain omega-3 may favour the retinal accumulation of L/Z to increase MPOD (Mares *et al.*, 2006, Johnson *et al.*, 2008). Arnold *et al.* (2013) observed in their RCT study “The LUTEGA study” that supplement containing L/Z and omega -3 long-chain PUFAs had significantly increased the serum concentration of L/Z and elevated MPOD levels in AMD individuals taking the treatment compared to those who were taking placebo treatment. The study found that participants who received a double dose of supplements had significantly better plasma fatty acid profiles compared to participants who received a single dose. Merle *et al.* (2017) conducted an RCT study in participants whom parents were diagnosed with neurovascular AMD. The study found that high MPOD was significantly associated with higher level of plasma docosapentaenoic acid (DPA) ($\beta = 0.03$, 95% CI: 0.003, 0.05; $p = 0.03$) after multivariate adjustment. Plasma alpha-linolenic, eicosapentaenoic, and docosahexaenoic acids were not significantly associated with MPOD.

1.13 UVR/ sunlight exposure association with ocular health

Ultraviolet radiation (UVR) is an electromagnetic radiation from 100-400 nm of waveband. The visible waveband is 400-700 nm (WHO, 2002). The UV spectrum is divided into three sub bands: UV-A which has a wavelength of 315-400 nm, UV-B (280-315) and UV-C that has wavelength of 100-280 nm. The UV radiation reaching the earth surface is largely composed of UV-A (WHO, 2002). Different eye structures absorb UV radiation. These are; cornea, aqueous humour and crystalline lens (McCarty and Taylor, 2002). According to Young and Sands (1998), human cornea absorbs the UV of wavelength below 300 nm and lens absorbs light under 400nm and lens ability to absorb UV light changes throughout the human life.

The main source of UVR is the sun and due to ozone depletion and climate changes, diseases associated with UVR are increasing (McKenzie *et al.*, 2003). In addition, lifestyle changes has increase leisure activities that are performing under sun exposure or UVR intense environment (Yam and Kwok, 2014).

1.13.1 Effect of Ultraviolet light on tear film

So far, there are no studies, which directly investigated the role of UVR exposure in the disturbance of tear film. In the literature, there are many studies, which have shown an association of UVR with eyelid carcinomas e.g. basal cell carcinoma & squamous cell carcinoma (Gallagher *et al.*, 1995, Rosso *et al.*, 1996, Naldi *et al.*, 2000). UVR association and with corneal and conjunctival diseases like pterygium (Moran and Hollows, 1984), pinguecula (NORN, 1982), photo-keratitis (Cullen, 2002) and with climatic droplet keratopathy (Gray *et al.*, 1992) is seen in the literature.

1.13.2 Effect of UVR on the lens

In the literature, there is a burden of epidemiological studies evidence showing an inverse relationship between sunlight and different types of cataract (HILLER *et al.*, 1977, Delcourt *et al.*, 2000, Katoh *et al.*, 2001). Similarly, many animal studies have shown an inverse relationship of UVR on the ocular lens (Fris *et al.*, 2008, Mody *et al.*, 2006, Galichanin *et al.*, 2014). Neale *et al.* (2003) conducted a case-control study in Australian population to find any association of sun exposure with nuclear cataract. Those participants who had a grade two or high nuclear opacity were considered as cases and those who have nuclear opacity less than grade two were randomly selected as controls. The study observed a strong and positive association of occupational sun exposure at the age of 20–29 with nuclear cataract (odds ratio = 5.9; 95% confidence interval = 2.1–17.1).

West *et al.* (1998) investigated on the association of sunlight with different forms of cataract in the older American population. Although, the study found low exposure to sunlight when compared to other studies that calculated sun exposure in occupational workers still, the study revealed that sunlight exposure was significantly associated with cortical cataract (odds ratio [OR], 1.10; 95% confidence interval [CI], 1.02- 1.20). There was no significant difference found between difference races and sex.

Until today, there have been no studies conducted to investigate how UVR affects accommodation facility. As mentioned above, there is evidence that supports the long exposure to sunlight/UV-B especially in younger ages could contribute in formation of cataract. It will be a point of interest to investigate whether UVR or sun exposure has any adverse effect on accommodative power of lens.

1.13.3 Effect of UVR on MPOD

Many animal and case report studies have linked sunlight exposure with diseases of retina and choroid e.g. AMD, uveal melanoma (Youn *et al.*, 2010, Roduit and Schorderet, 2008, Jhappan *et al.*, 2003). However, epidemiological studies have shown a mix result of sunlight exposure association with AMD. Yam and Kwok (2014) conducted a literature search on association of sunlight and AMD. The review found that most of the epidemiological studies (Taylor *et al.*, 1992, Cruickshanks *et al.*, 1993, Khan *et al.*, 2006) did not find any relationship. The follow-ups of Beaver Dam Eye Study (Cruickshanks *et al.*, 2001, Tomany *et al.*, 2004) found a positive association of sunlight with early AMD. There was a significant association between time spent in sunlight (in teenage time) and development of AMD (odds ratio [OR] 2.2; 95% confidence interval [CI] 1.0 – 4.8). Although, Taylor *et al.* (1992) did not find any association of UV-B or UV-A with AMD but their study found a significant association between blue light exposure and AMD (OR= 1.3, CI 1.0 – 1.8).

The association of UVR exposure to AMD is a known risk factor and many epidemiological studies have indicated an inverse relationship between them. Still few studies tried to investigate this relationship. Raman *et al.* (2012a) found that smoking, UVR exposure, and a low dietary intake of carotenoids was inversely linked with MPOD in Indian subjects
However, in another study conducted to determine mean MPOD value in participants with wet AMD Raman *et al.* (2012b) did not find any significant association of UVR exposure with MPOD results.

1.14 Gaps in the existing literature

The literature review for this thesis shows the following gaps in the current literature.

- Few studies presented in the literature that have shown the effect of smoking on AoA or on presbyopia.
- No study has shown any effect of UVR or sunlight on the tear film.
- No study has shown any effect of UVR or sunlight on AoA.
- No study has shown any effect of diet on AoA.
- Effect of smoking on MPOD is still unclear.

This thesis will try to help fill some of the gaps in the literature by conducting research studies.

1.15 Research objectives

- To evaluate the effects of smoking on the tear film.
- To evaluate the impact of smoking on the accommodative ability of the eye
- To assess the effects of smoking on the macular pigment
- To investigate the impact of dietary elements on the tears, accommodation and macular pigment.
- To investigate the effect of UVR on tears, accommodation and macular pigment.
- To examine the transient effects of smoking on tear film and accommodative ability of the eye.

Success in fulfilling these research objectives will help to generate recommendations for the public and eye care professionals alike.

Chapter 2

Effect of smoking on the tear film, amplitude of accommodation and MPOD in the UK cohort

To explore the research objectives listed in section 1.14 this first chapter will explore the relationship between smoking and the tear film, smoking and AoA, and smoking and MPOD in the UK cohort of the data. This will help to establish if smoking has notable ocular effects.

2.1 Introduction

The literature review discussed how an allostatic load caused by different lifestyles (such as smoking habit and poor nutrition) could contribute adversely to ocular health including its effect on the tear film. There is sufficient literature that shows that smoking has an adverse effect on the tear film (Sayin *et al.*, 2014, Hua *et al.*, 2014, Masmali *et al.*, 2016, Agrawal *et al.*, 2018). There are many theories on the mechanisms by which smoking can affect the tear film, the most prominent is based on the peroxidation of the lipid layer of the tear film (Altinors *et al.*, 2006, Thomas *et al.*, 2012).

It is believed that the ocular surface is exposed to over one hundred trillion short-lived radicals in the gas phase and even more in the tar phase in which the radicals are longer lived (Pryor, 1997). The chemical composition of cigarette smoke is complex with over 4000 chemicals being present, including free radical species, aldehydes, peroxides, epoxides, nitrogen oxides, peroxyl radicals, and other pro-oxidants (Miller *et al.*, 1997). These chemicals may contribute to the disease process. Studies have shown lipid layer damage in smokers. Altinors *et al.* (2006) found an uneven spread of tear film over the corneal surface rendering it unwettable and damaging the pre-corneal tear film by lipid peroxidation process.

Another possible mechanism by which smoking damages the tear film is by ocular surface epithelial damage. This is because smoke directly interacts with the ocular surface. Studies (Satici *et al.*, 2003, Yoon *et al.*, 2005a) have shown that smokers have a high level of squamous metaplasia in the conjunctival surface epithelium compared to non-smokers. Potentially this caused by the toxic interaction of cigarette smoke with conjunctival epithelial surface resulting in inflammation.

Studies have shown that smoking is associated with cataract (Kelly *et al.*, 2005, Lindblad *et al.*, 2005, Wu *et al.*, 2010, Ye *et al.*, 2012). A review of the literature showed that smoking intensified the cataract formation and its cumulative dose is associated with an increased need for cataract extraction (Lindblad *et al.*, 2014). Smoking is a modifiable risk factor for cataract formation; Lindblad *et al.* (2014) also found that smoking cessation could decrease the risk of cataract formation, which is a slow process. Since showing an association with smoking is a long-term prospect, lens parameters such as its accommodative ability to focus objects or lens

flexibility have been used as an early surrogate sign for the adverse effects of smoking on the crystalline lens (Ainsbury *et al.*, 2016).

Research is limited on the topic of smoking and its association with presbyopia or with the amplitude of accommodation (AoA). A survey conducted by Nirmalan *et al.* (2006) did not find any association of smoking with presbyopia in a South Indian population. A study by Khalaj *et al.* (2014) found that in an Iranian population the prevalence of presbyopia was higher and earlier in smokers than non-smokers in an age-matched study. The onset age of presbyopia was significantly correlated with onset age of smoking. A positive smoking association is also reported with decreased AoA by Ide *et al.* (2012) who found that AoA of smokers was significantly lower than AoA of non-smokers in an age-matched study.

In the present literature, there are contradicting results on the effects of smoking on the macular pigment optical density (MPOD). Some studies have shown a negative association of smoking with MPOD such as studies conducted by (Hammond *et al.*, 1996, Hammond, 2002, Nolan *et al.*, 2007, Kirby *et al.*, 2010, Raman *et al.*, 2012a). On the contrary, many studies presented with no significant association of smoking with MPOD values (Yu *et al.*, 2012, Murray *et al.*, 2013, Obana *et al.*, 2014, Abell *et al.*, 2014, Ji *et al.*, 2015).

2.2 Study aim

The purpose of this study is to find out the effects of smoking on tear film, AoA, and MPOD in the UK cohort of data.

2.3 Methods

The study design was a prospective cross-sectional study. Smoker and non–smoker participants were recruited from Aston University and were students, staff, and visitor of Aston University. The Aston University research ethics committee approved the study, and the research followed the tenets of the Declaration of Helsinki. Participants received a participant information sheet, which detailed what the research entailed. If they were happy to proceed, written informed consent was obtained.

2.3.1 Selection criteria

The subjects were selected on the following criteria.

Inclusion criteria:

- Aged between 18 to 50 years old
- No contact lens use
- Subjects able to give written informed consent
- Regular cigarette smoker of one or more cigarettes per day

- Non-smokers
- LogMAR visual acuity of 0.0 or better

Exclusion criteria:

- Any active ocular disease / condition
- Systematic disease condition (e.g. hypertension or diabetes)
- Known dry eyes condition

2.3.2 Study instruments

Following instruments were used in this research study.

1. Tearscope (EASYTEAR®view+, Trento, Italy) for analysing the tear break-up time (TBUT) and lipid layer non–invasively.
2. Keratograph 5M (Oculus optigerate GmbH, Wetzlar, Germany) with tear film scan software for measuring TBUT non–invasively and lipid layer non– invasively.
3. Slit lamp (CSO SL990, Firenze, Italy) for measuring TBUT invasively
4. Bio fluoro fluorescein strips (Biotech Vison care).
5. RAF near point rule (Clement Clarke Ltd, Essex United Kingdom) for measuring amplitudes of accommodation (AoA).
6. CSO computerised visual acuity chart (Costruzione Strumenti Oftalmici, Firenze, Italy) for measuring defocus curves.
7. Topcon phoropter VT-SE (Hanson Instruments, Redditch, United Kingdom) measuring an amplitude of accommodation by defocus curves.
8. Macular pigment screener (MPS9000 / MPSII, Tinsley Instruments, Essex, UK) for analysing MPOD values.

2.3.2.1 EASYTEAR®view+ Tearscope (Trento, Italy)

The EASYTEAR®view+ dacrioscope (commonly known as tearscope) is a relatively new and special instrument that has been designed and optimized to facilitate non-invasive observation of the ocular surface of the human eye. It helps to access and diagnose issues related to dry eye. It has three special adjustable LED light sources (white, blue and infrared) which are calibrated not to dazzle the eye of the patient. It also uses an innovative ‘light dispersion optical

system' that forms a calibrated and constant colour rendering that reduces any alternation and drying of the tear film during the examination. With the help of white LEDs, it creates significant corneal reflection permitting in vivo evaluation of interference fringes that allows observation of the tear film layer thickness and the performance of NIBUT. Blue LEDs are used to observe fluorescein evaluation of contact lenses (particularly scleral and mini-scleral lenses) and to perform BUT. Infrared LEDs are used to evaluate the integrity and operation of the meibomian glands.

This instrument was used in a dimly lit room, for TBUT, white LEDs lights were used with a 4x magnifying lens to view the corneal surface. An appropriate gird was then rolled in the form of the cylinder through the hole of dacrioscope in such a way that two edges of the gird are positioned on the top near the notch.

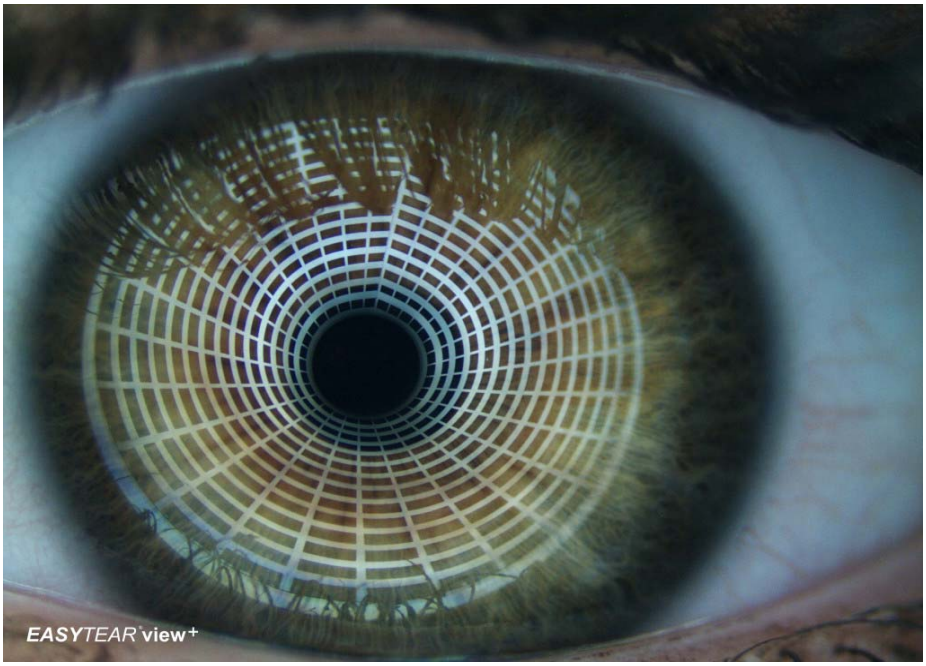


Figure 2.1: measuring tear break up time with dacrioscope www.easytearviewplus.com.

Tear lipid layer evaluation, an additional accessory (ISCOPE-MV500) five mega-pixels camera with 3.5" LCD screen was integrated with dacrioscope with magnification from 5x ~ 200x. It captured photographs of tear lipid layer and made small videos of tear lipid layers were recorded for every participant. Later, those photographs and videos were used to grade lipid layer according to the instruction and grading provided by the company mentioned in the instrument manual.

The dacrioscope was inserted on the accessory attachment holder of the slit lamp with the help of mounting bracket. The participant was examined by having him rest his head on the chin guard of the slit lamp. The slit lamp's illumination system was turned off and moved aside

so that it will not hinder usage and viewing of dacrioscope. The white LEDs light were used and the dacrioscope was moved closer to the eye to focus on the plane of the tear film. The image was blurred slightly to observe different colour interference fringes, which can vary from grey marble colour fringes to mix of brown and blue fringes, depending on the thickness and uniformity of the lipid layer.

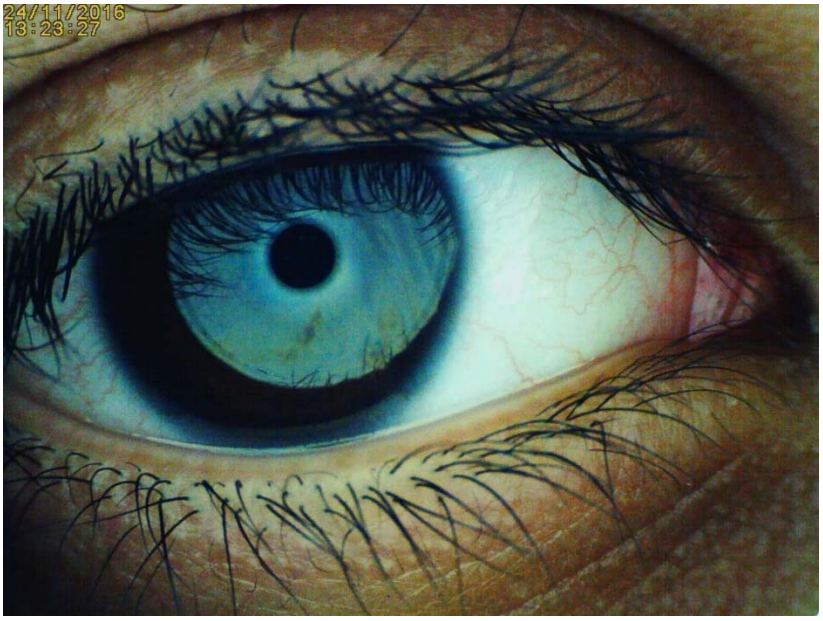


Figure 2.2: an example of tear film lipid-layer captured by ISCOPE-MV500 camera (Latif 2019)

2.3.2.2 Keratograph 5M (Oculus optigerate GmbH, Wetzlar, Germany)

The Keratograph combines keratometry measuring processes with topographic mapping. It is an illumination system, which has a special reflector illuminating a Placido bowl, which contains a series of 22 white concentric rings, and thus images obtained are reflected from this Placido bowl from the patients' eye. Besides being an advanced corneal topographer, it also contains additional imaging modalities to measure the anterior ocular surface. These include infra-red measurements of the meibomian glands at 840nm, evaluation of the tear break up time non-invasively, measuring the amount of bulbar and limbal hyperaemia, tear meniscus height, and dynamic evaluation of the lipid layer. It also has a built-in video recorder and software to analyse the data and can be used for future reference when evaluating the ocular surface after treatment or post-surgical interventions.

Like the dacrioscope, this test was also conducted in a dimly lit room to minimise reflections from the Placido bowel of Keratograph. Participants were positioned with their chins on the chin rest and the outer canthus aligned with the reference bar. Participants were asked to focus on the red light located directly in the centre of the concentric rings. All subsequent

measurements were taken from this reference point. The following procedures described below are in the order of the protocol used in this thesis.

Non-invasive Keratograph break up time (NIKBUT)

The non-invasive break-up time is evaluated and displayed in a colour-coded map of the cornea. Once aligned the patient is asked to look at a central red light and required to blink twice consecutively by the clinician. The second blink triggers the program to initiate the recording and it will continue until one of the two events occurs; either the patient blink or there is a significant amount of distortion recorded on the reflected image of the Placido rings. The information is then encoded by the software, and displayed to the clinician. The tear break up time indicates the quality of the tear film. A tear break up time of more than ten seconds is usually related as normal. Different colours coded on the corneal map indicate the stability of tear film. Red-coded area indicates an unstable tear film whereas; green-coded area shows stable tear film.

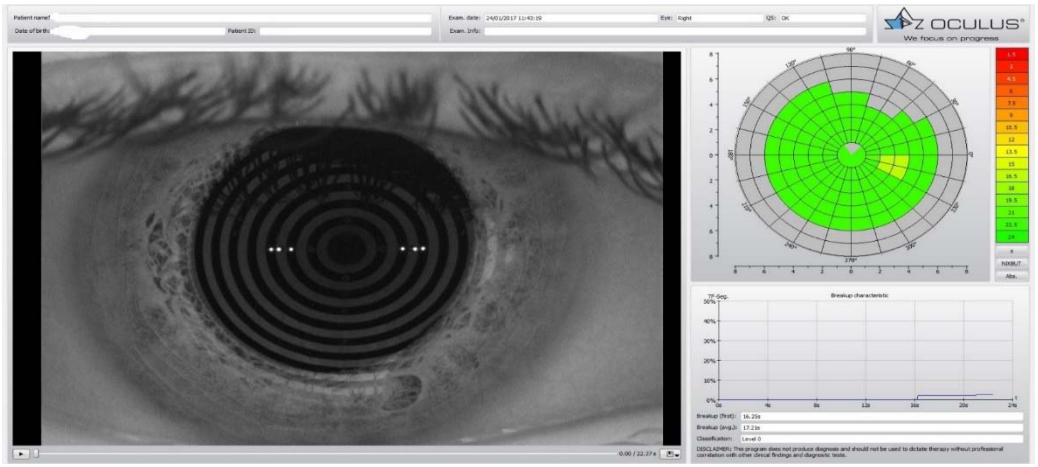


Figure 2.3a: showing colour coded corneal map and non-invasive Keratograph tear break-up time in seconds (Latif 2019)

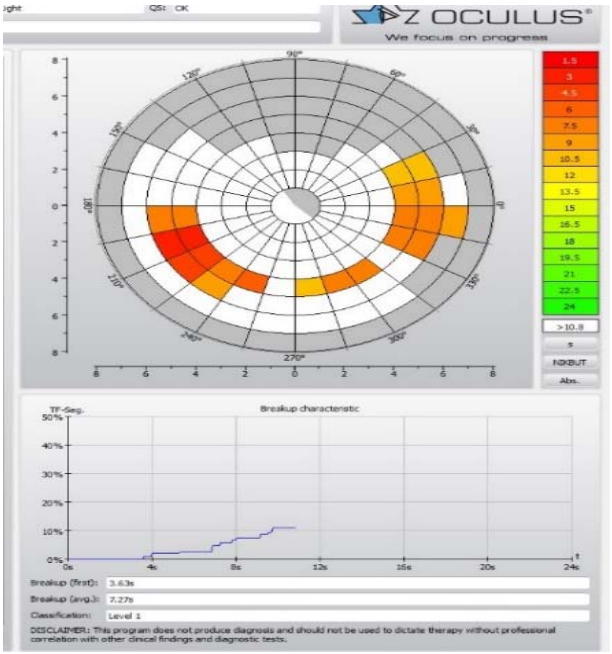


Figure 2.3b: *an example of decreased non-invasive Keratograph tear break-up time in seconds (Latif 2019)*

Dynamic lipid layer evaluation

The evaluation of the lipid layer is performed with the patient looking directly at the centre of the Placido disc. Once aligned, the measurement commences, and the clinician should advise the patient to blink normally as to reveal the spread and formation of the lipid layer across the corneal surface. This measurement is usually recorded to assess the dynamic behaviour of the tear film, as well as indicating of the thickness of the lipid layer. If the interference pattern displays colours and structures, it is regarded as normal; however, if no colours or structures are visible, it could be an indication of early tear film evaporation. Recordings can range from 5(s) to 10 (s) or longer if the clinician deems so. There is also the ability to capture images within the video recording for analysing images later on.

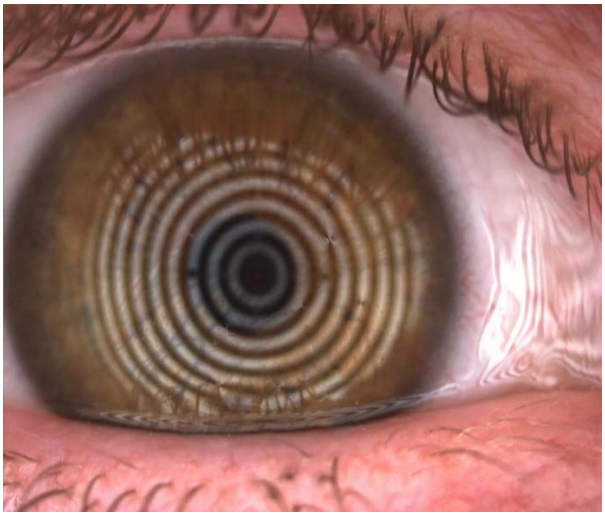


Figure 2.3c: an example of a lipid layer taken from Keratograph K5M (Latif 2019).

2.3.2.3 Slit Lamp (CSO SL990) & Bio fluoro fluorescein strips (Biotech Vision care, Luzern Murbacherstr, Switzerland)

The slit lamp bio-microscope provides the examiner with a stereoscopic view of the eye. It is an important tool in the assessment of signs, making diagnoses and for monitoring the effects of treatment and continuing prognosis of many ocular complaints. The slit lamp bio-microscope can be used to assess the eye's anatomy in detail, by varying the illumination and magnification, as well as with the use of filters topical drugs and stains (Kotecha, 2014).

Bio Fluoro fluorescein strips are fluorescein sodium strips used to stain cornea and conjunctiva to aid diagnosis of different eye conditions including detecting injuries, corneal abrasions, foreign bodies in the eye and contact lens fitting etc. These strips are sterilised with Ethylene Oxide. Each strip contains 1 mg of fluorescein sodium I.P.

Before starting an examination, the slit lamp was cleaned with alcohol, wipes and examiner washed their hands with soap and air-dried them in front of participants. This was done to ensure safe and hygienic practice minimising any infection spread. Slit-lamp eye pieces were focused separately and interpupillary distance was adjusted to get a stereoscopic view. Participant's chair was adjusted for their comfort. Participant's forehead and chin were pressed firmly against the rests to level the lateral canthi with the slit-lamp markers. Slit-lamp magnification was set to 10 x and cobalt blue filter was used, and in order to enhance the contrast yellow filter was used. Before performing TBUT, room light was dimmed and the strip was moistened with sterile saline solution. The Moisten strip was then placed along the bulbar conjunctiva and the participant was then asked to blink several times. The participant was then asked to stop blinking and a stopwatch calculated the time between last blink and first formation of black spot or an involuntary blink.

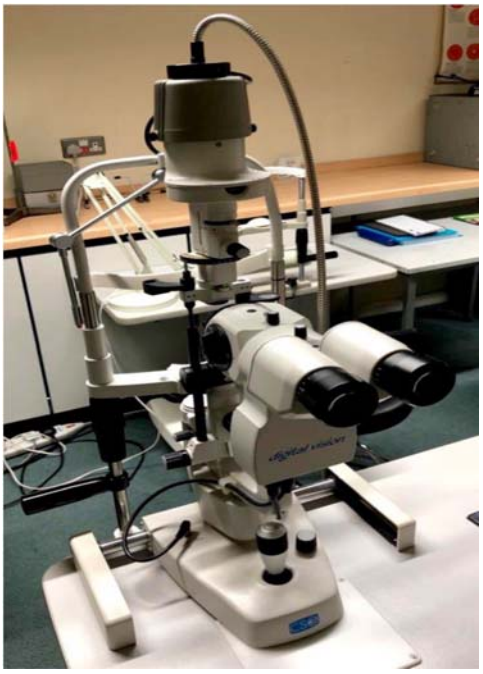


Figure 2.4: Slit lamp CSO SL990, (Latif 2019).

2.3.2.4 RAF Binocular Gauge (Clement Clarks Ophthalmic, Essex United Kingdom)

RAF rule is an instrument used to measure convergence and AoA both monocularly and binocularly. For the AoA test, each participant was asked to wear their distance correction (if any), then letters on the box were shown to the participant starting from 36 cm, and gradually the box was brought closer to the participant. The participant was asked to report when words became blurred. The distance from the chart to the participant's spectacle plane was measured in Dioptres. The box was then brought closer to increase the blur and then gradually brought back from the participant's eye. The participant was then asked to report when the letters became clear, that distance was recorded in Dioptres (D). An average of both readings in Dioptres was taken as a final AoA reading. RAF rule was cleaned with alcohol wipes before its use on each participant.

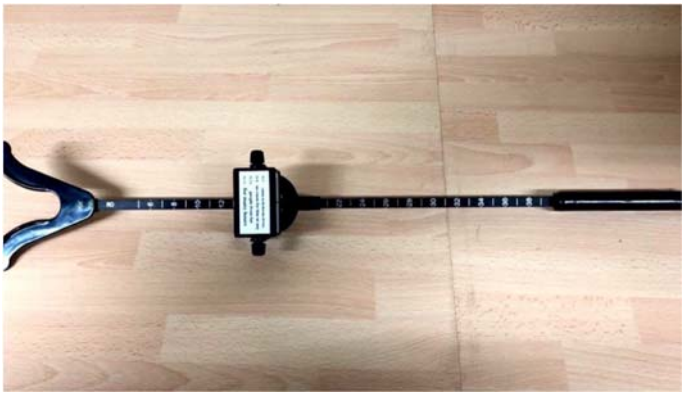


Figure 2.5: RAF rule (Latif, 2019)

2.3.2.5 CSO computerised vision chart (Mod.CVC03, Firenze, Italy)

The CSO chart is a computerised visual acuity chart with a wide range of different test available to use. It has 19" HD LCD screen with 1280 x 1024 resolution, with maximum contrast of 500:1 and with a maximum lightness of 280 cd/m^2 with remote control access. Optotypes are easily adjusted according to the room size. It has a selectable visual acuity notation, e.g. LogMAR, Snellen fractions, or decimal with automatic randomisation to prevent memorisation. Its' visual acuity range is from 1.30 to -0.30 in LogMAR progression with crowding feature. It can also be used as a contrast sensitivity chart by controlling the contrast threshold from 99 % to 0.6 %. Some other salient features are tests for binocular vision, colour vision test, test for sphero–cylindrical correction, low vision acuity tests, and Amsler's Grid.

For this research, visual acuity was measured in LogMAR progression with Landlot's C rings with possible five different positions and with randomisation.

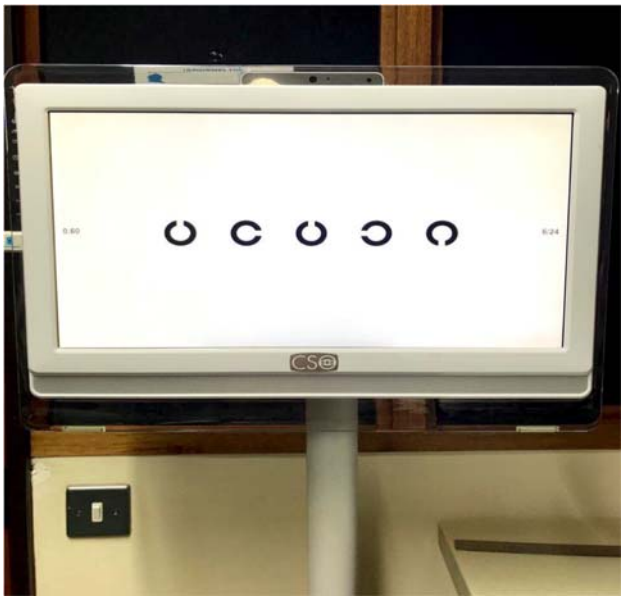


Figure 2.6: CSO computerised vision chart displaying five different positions of Landlot's C rings (Latif 2019)

2.3.2.6 Topcon vision meter (VT-SE, Hanson Instruments, Redditch, United Kingdom)

It is also known as a manual phoropter. It is used as an aid for refraction. It offers optimum comfort while facilitating clinical examination. Some of the features of this phoropter are; lens range from -19D to +16.75 in 0.25D steps, cylindrical lenses up to -6.00D. There are built-in cross cylinders, rotary prisms, and a wide selection of auxiliary lenses. It has an automatic near-point convergence mechanism. For this research project, this instrument was used to perform subjective refraction (if needed) and for determining defocus curves on the participants.

Figure 2.7: Topcon vision meter VT-SE Japan, (Latif, 2019)

2.3.2.7 Macular pigment screener MPS9000/MPSII (Tinsley Instruments, Essex, UK)

The MPS II is a computerised instrument for measuring a macular pigment optical density of an individual. It uses low and specific intensity light wavelengths at calibrated intensities to measure participant's heterochromatic flicker response. It is simple to use and does not require any advanced computer skills for its operation. An individual needs to look into the instrument via an eyepiece at the stimulus light and is asked to press a button when he/she sees a light flicker. The target background luminance is kept at 250cdm-2 to reduce detection by rods or short wave cones significantly. The machine has an internal microprocessor, which controls the intensity of the light and the test program sequence. The MPSII software has a powerful database, Where MPOD results are recorded, and this database is handy to monitor the progress of an individual from time to time. The MPSII have two test modes, i.e. standard test mode and detailed test mode.

For this study, only the standard test model was used which is a subset measure of detailed test mode, which measures the central region of macular pigment. The macular index is calculated from the participant's age and the central test run. An algorithm is made from the participant response to the test. It analyses the shape of the graph and test values. The trial has its self-reported reliability index with three possible outcomes, namely accept, caution, and reject, according to the confidence limits of the data.

Before using the instrument on the participant, the investigator made sure that the apparatus was cleaned with an alcohol wipe and there was no dust/debris on the screener especially on the optical area. Dim light conditions were used for operating MPSII screener. The participant was seated on the chair, and MPSII screener was brought closer to the right eye. The participant was asked to look into the lens to see three dots. The participant was then asked to focus on the middle dot, which will light blue-green colour and was asked to press the button once he /she felt the middle dot flickered. The test usually ran for 60 seconds to 120 seconds to get an MPOD value. The average of three values was used for the analysis.

Figure 2.8: Macular pigment screener MPS9000 / MPSII, (Latif, 2019)

2.3.4 Sample size calculation

The maximum sample size was calculated using G*Power 3.1(Faul *et al.*, 2007) using a two way paired t-test to show a medium effect size with 80% power and an alpha level of 0.05. The maximum number of subjects required was 128 (64 smokers and 64 non-smokers), and therefore 131 subjects (65 smokers and 66 non-smokers) were recruited to ensure adequate statistical power and allow for attrition.

2.3.5 Experimental procedure

A study advertisement and research participant information sheet were distributed to the participants before their arrival, and an appointment was made via email. On the day of appointment, full study procedure was explained to the participants and all queries related to the procedure were answered before taking informed consent.

Only the right eye was examined in this experiment. Subjective refraction was done if the participant's visual acuity was less than 0.0 LogMAR with the help of Topcon vision meter/phoropter after taking an estimation of refractive error with the help of Nidek OPD Scan III. The endpoint criterion was a maximum plus sphere and minimum minus cylinder power maintaining the best visual acuity. In most cases, the participant's glasses or prescription was used as a starting point of refraction. TBUT was measured non-invasively with Keratograph 5M and Tearscope and invasively on Slit lamp (Haag Streit) with the help of fluorescein strips. Three readings were recorded from each instrument in dim light conditions. Additionally, a single measurement reading was obtained for tear meniscus height (TMH), average pupil diameter (PD) and tear film lipid layer from the Keratograph 5M machine.

Three readings for AoA was recorded with RAF near point rule in bright light conditions. Subjective clear vision range was calculated by performing a defocus curves technique. LogMAR visual acuity was recorded with Landlot's C chart by different lenses (range from +1.50 Ds to –1.50 Ds) in a randomised manner. A single letter was shown to the participant with four different directions and the participant was asked to tell the direction of the letter. Finally, macular pigment optical density (MPOD) measurements were recorded; three measurements were recorded for each participant in dim light conditions. An approximately 30 second's gap was given for each measurement where three repeated measurements were taken and a gap of one minute was taken between different instruments.

2.3.6 Baseline data collection

An initial lifestyle questionnaire was completed by participants, which contained questions related to diet, smoking status and drinking status, OSDI questionnaire (for symptoms related to dry eye condition), near working hours, and sunlight exposure. A copy of the questionnaire is attached in Appendix 1.

Ocular Surface Disease Index (OSDI) was chosen to use as a dry eye questionnaire. It is a validated and reliable instrument for measuring the severity of dry eye disease (Henderson R, 2013). This questionnaire is a 5–category Likert scale containing 12 questions that investigate symptoms, triggers and consequences of dry eye (Brewitt and Sistani, 2001). These questions are used to assess the level of discomfort and how this condition can interfere with daily living tasks. In total 12 questions, five are related to ocular symptoms, four are related with functional tasks, and the remaining three are related to environment triggers. There are many other dry eye questionnaires available to use such as McMonnies Dry Eye Index, National Eye Institute Visual Functioning Questionnaire and Ocular Comfort Index. Studies have shown that OSDI scores correlated significantly with the above mentioned dry eye questionnaires (Tsubota *et al.*, 2007).

2.3.7 Passive smoking exposure levels

The Study divided its participants into three different groups according to their exposure to passive smoke. These sub groups are mentioned as below:

1. No exposure to passive smoke – those participants who were non-smokers and had no close contact with a smoker (e.g. no smoker close family members, friends, and room or house sharer).
2. Infrequent exposure to passive smoke – those who themselves were non-smokers but did have close contact with smokers (e.g. smoking family member or friend, smoker(s) present in a house/room).
3. Frequent exposure to passive smoke – this category included participants who were smokers.

2.3.8 Cigarette smoking per day gradations

The study participants were divided into three categories according to their cigarette consumption per day, i.e. non-smokers, light/mild smokers, and heavy smokers. Non-smokers were those participants who did not smoke any cigarette (actual non–smoker participants of the study). Light/mild smokers were those participants who smoked between one to ten cigarettes per day and heavy smokers were those who smoked more than ten cigarettes per day.

2.3.9 Smoking pack year gradations

Smoking pack-years, values were calculated using a free online smoking pack-year calculation website https://www.smokingpackyears.com/ for each participant to convert lifetime exposure to smoke into a numerical value. Participants were divided into three categories, i.e. non-smokers, smokers smoked less than ten pack-years and smokers smoked more than ten pack years in their lifetime.

2.3.10 Ethnicity classifications

The study participants were divided into three ethnic groups, i.e. Asians (Indo–Pak origin), White and Others (Chinese, Arab, Kurdish, Persian and Mixed race). The study ethnic classification was not according to standard classification detailed by the Office of National Statistics (ONS) because of the high number of Indo-Pak subjects and the fact that in later chapters a comparison would be made with subjects from Pakistan. Apart from Asian and White participants, participants from other ethnic groups were lower in number. For analysis purposes, smaller ethnic groups' subjects were grouped and represented under the 'Other' category.

2.3.11 Statistical analysis

All measurements from a case report form (CRF) was noted down in a Microsoft Excel spreadsheet, which was later exported to an SPSS spreadsheet. Statistical analysis was performed by using SPSS 23.0 statistical package program for Windows (SPSS Inc., Chicago, IL, USA). Normality was confirmed for the data sets using Shapiro–Wilk test p = 0.05. The parametric data underwent parametric tests (such as T-test, ANOVA, and ANCOVA). Non-parametric data underwent a non-parametric statistical analysis Man-Whitney U test and Kruskal Wallis H test. A value of less than 0.05 was considered as statistically significant.

2.4 Results

A total (N) 131 participants (65 smokers and 66 non-smokers) were enrolled in this study. Female participants were 40 (30.5 % of N) and male participants were 91 (69.5% of N). Mean age for the male participant was 25.1 ± 7.2 years old (range 18 to 47, median 22.0 years) and the mean age for females was 23.4 ± 5.4 years old (range 18 to 44, median 22.0 years). There was no significant difference observed between male and female ages (U =1710.0, p= .5). There was no significant age difference observed between smokers and non-smokers (U =1799.0, p= .1). Table 2.1 shows baseline descriptive data for smokers and non-smokers as below:

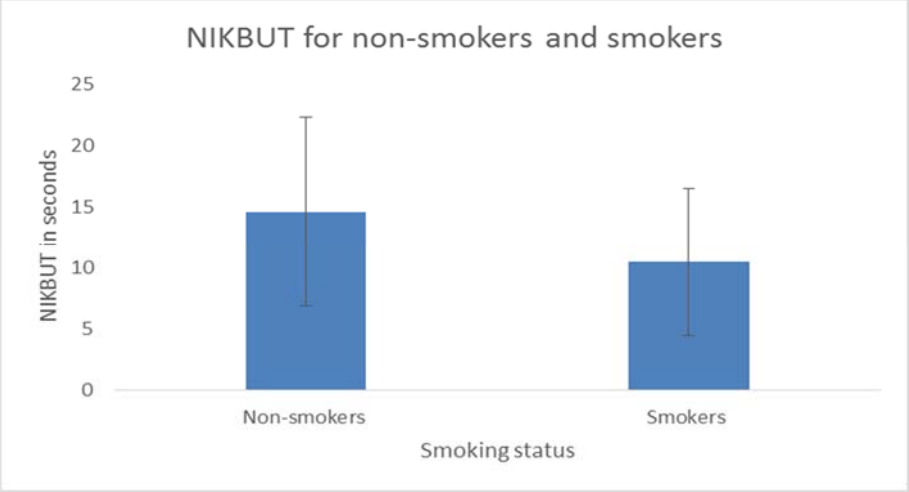
	Smoking status								P-value
	non-smoker				smokers				
	Mean	Standard Deviation	N	Median	Mean	Standard Deviation	N	Median	
Age (years)	25.5	7.0	66	23.5	23.7	6.4	65	21.0	0.5
NIKBUT (seconds)	14.6	7.7	66	14.2	10.5	6.0	65	8.6	0.002*
NITBUT (seconds)	13.9	5.5	66	12.5	9.6	3.9	65	8.9	0.001*
NAFLBUT (seconds)	11.8	6.4	66	10.6	6.7	2.9	65	6.2	0.001*
OSDI Scores	10.3	11.2	66	6.3	19.5	14.8	65	16.7	0.001*
Tear meniscus (millimetres)	0.3	0.1	66	0.3	0.3	0.1	65	0.3	0.2
Pupillometry (millimetres)	5.6	0.9	66	5.7	5.6	1.0	65	5.6	0.8
AoA (Dioptres)	9.7	2.6	66	9.4	9.9	2.1	65	10.1	0.1
MPOD value	0.4	0.1	66	0.4	0.4	0.1	65	0.4	0.8

**p value significant*
Table 2.1: Baseline data for smokers and non-smokers

2.4.1 Tear film analysis

2.4.1.1 Non-invasive Keratograph break-up time (NIKBUT)

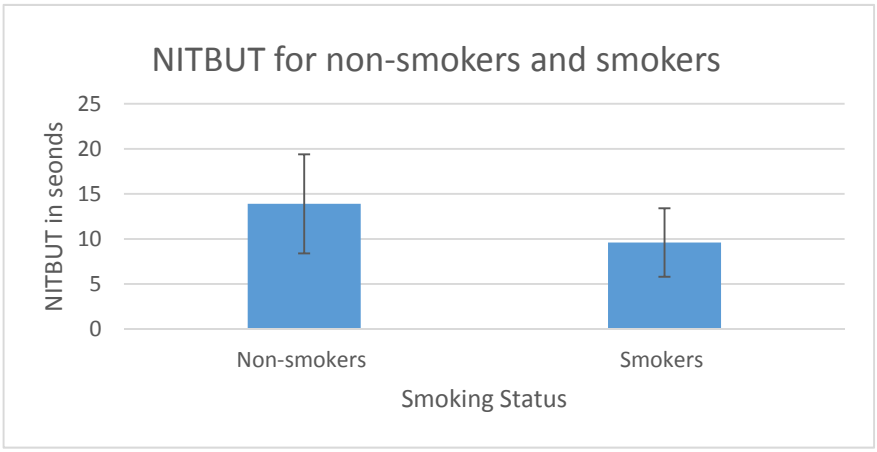
The mean NIKBUT for non-smokers was 14.6 ± 7.7 seconds (s) which was numerically higher than the mean NIKBUT for smokers was 10.5 ± 6.0 (s). A Mann–Whitney U test (Shapiro–Wilk test, $p < 0.05$) was used to find any significant difference, and the test indicated a significant difference between NIKBUT of non–smokers (Median = 14.1) and NIKBUT of smokers (Median = 8.60), $U = 1489.0$, $p = .002$, $r = -.26$. A graphical representation of means and 95 % confidence intervals are displayed in figure 2.9.



**p value = .002*
Figure 2.9: non-invasive Keratograph tear break-up time for non-smokers and smokers

2.4.1.2 Non-invasive Tearscope break up time (NITBUT)

The mean NITBUT for non-smokers was 13.9 ± 5.5 (s) which was numerically higher than the mean NITBUT for smokers' 9.6 ± 3.8 (s). A Mann–Whitney U test (Shapiro–Wilk test, $p < 0.05$) was used to find out any significant difference. The test indicated a significant difference between NITBUT of non–smokers (Median = 12.5) and NITBUT of smokers (Median = 8.9), U= 891.0, p >.001, r = - .41. A graphical representation of means and 95 % confidence intervals are displayed in figure 2.10.

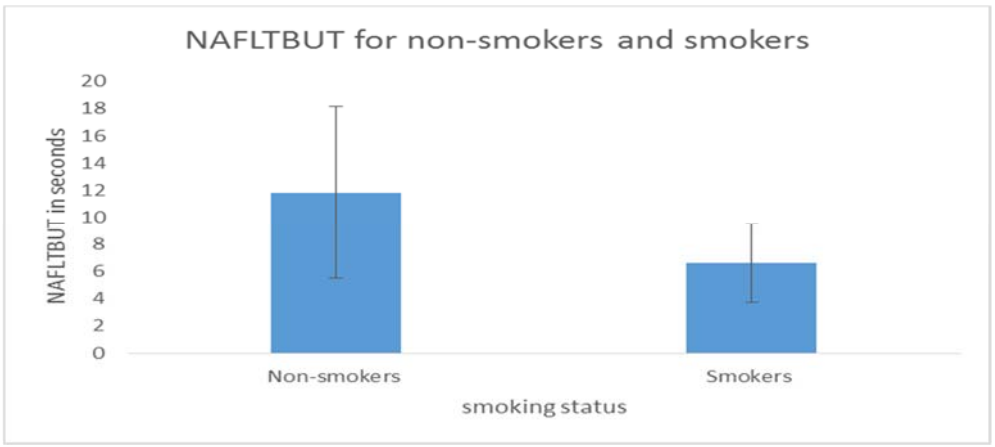


**p value = 0.001*

Figure 2.10: showing non-invasive Tearscope break-up time (NITBUT) for non-smokers and smokers.

2.4.1.3 Fluorescein tear break up time (NAFLTBUT)

The mean NAFLTBUT for non-smokers was 11.8 ± 6.3 (s) which was numerically higher than the mean NAFLTBUT for smokers' 6.6 ± 2.9 (s). A Mann–Whitney U test (Shapiro–Wilk test, $p < 0.05$) was used to find out any statistical significant difference. The test indicated a significant difference between NAFLTBUT of non–smokers (Median = 10.5) and smokers (Median = 6.2), U = 947.5, p >.001, r = - .48. A graphical representation of means and 95 % confidence intervals are displayed in figure 2.11.



**p value = 0.001*

Figure 2.11: showing fluorescein tear break-up time for non-smokers and smokers

2.4.1.4 Keratograph K5 M lipid layer thickness (K5 lipid layer thickness)

The mean K5 lipid layer thickness for non-smokers was 80.9 ± 32.9 nanometres (nm) which was numerically higher than the mean K5 lipid layer thickness for smokers 71.3 ± 23.9 nm. A Mann–Whitney U test (Shapiro–Wilk test, $p < 0.05$) indicated no significant difference between K5 lipid layer thickness of non–smokers (Median = 80.0) and K5 lipid layer thickness of smokers (Median = 80.0), $U = 1880.0$, $p = .21$.

2.4.1.5 Tearscope lipid layer thickness in nm (TS lipid layer thickness)

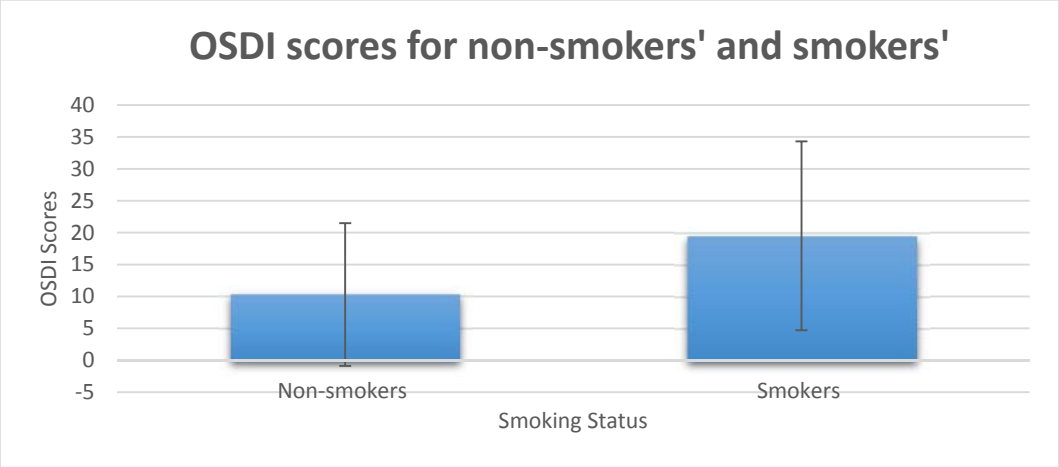
The mean TS lipid layer thickness for non-smokers was 85.7± 47.7 nm, which was numerically higher than the mean TS lipid layer thickness for smokers 70.6 ± 22.2 nm. A Mann–Whitney U test (Shapiro–Wilk test, $p < 0.05$) indicated no significant difference between TS lipid layer thickness of non–smokers (Median = 80.0) and TS lipid layer thickness of smokers (Median = 60.0), $U = 1404.5$, $p = .33$.

2.4.1.6 Tear meniscus height (TMH)

The mean TMH for non-smokers was 0.3 ± 0.1 millimetres (mm), which was marginally lower than mean TMH of smokers 0.3 ± 0.1 mm. A Mann–Whitney U test (Shapiro–Wilk test, $p < 0.05$ for both smoking status) indicated that TMH for non–smokers was not significantly lower (Median = 0.2) than smokers (Median = 0.3), $U = 1876.0$, $p = .276$.

2.4.1.7 Ocular Surface Disease Index scores (OSDI scores)

The mean OSDI scores for non-smokers was 10.3 ± 11.2 which was numerically lower than the mean OSDI scores for smokers' was 19.5± 14.8. A Mann–Whitney U test (Shapiro–Wilk test, $p < 0.05$) indicated that non–smokers had significantly lower OSDI scores (Median = 6.2) than smokers (Median = 16.6), $U = 1268.5$, $p = .001$, $r = - 0.35$. A graphical representation of means and 95 % confidence intervals are displayed in figure 2.12.



**p value = 0.001*

Figure 2.12: showing mean Ocular Surface Disease Index scores for smokers and non-smokers

2.4.1.8 Correlation between smoking pack years and TBUT

Spearman ranked correlations (r_s) was used to derive any correlation between different methods of TBUT with smoking years after doing normality check by Shapiro–Wilk test ($p < 0.05$ for all three methods, i.e. NIKBUT, NITBUT, NAFLTBUT).

There was a weak but negative correlation found between NIKBUT and smoking years, $r_s(129) = -.23$, $p = 0.007$. Similarly, a weak negative correlation was found between NITBUT and smoking years, $r_s(129) = -.30$, $p = 0.001$ and between NAFLTBUT and smoking years, $r_s(129) = -.33$, $p = 0.001$ respectively.

Figure 2.13 (a): correlation between smoking pack-years and non-invasive Keratograph break-up time

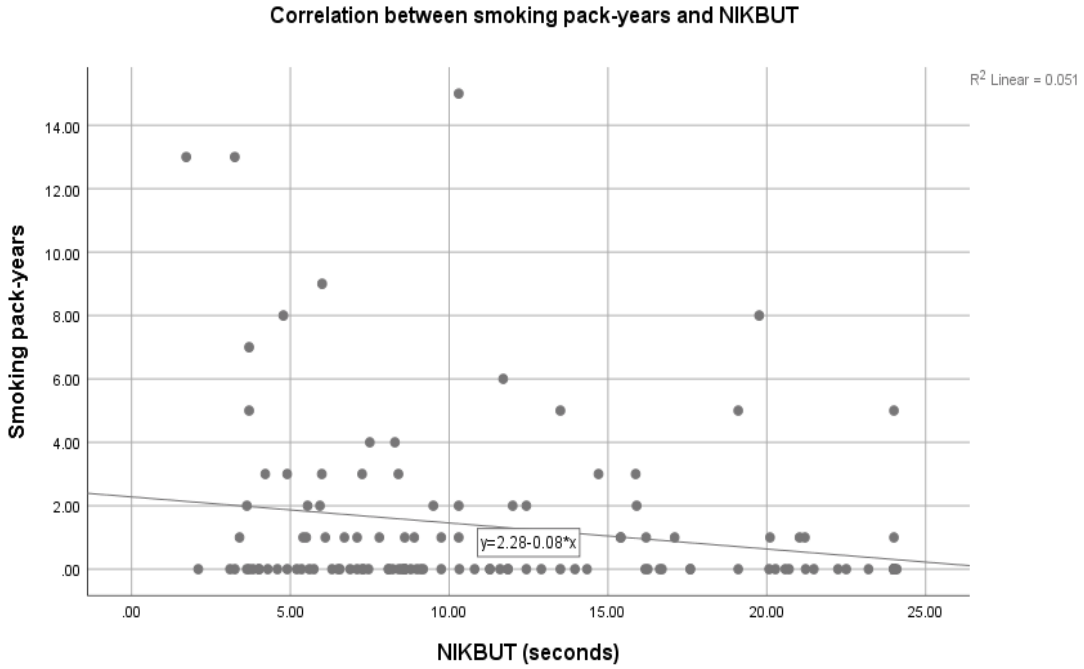


Figure 2.13 (b): correlation between smoking pack-years and non-invasive tearscope break-up time

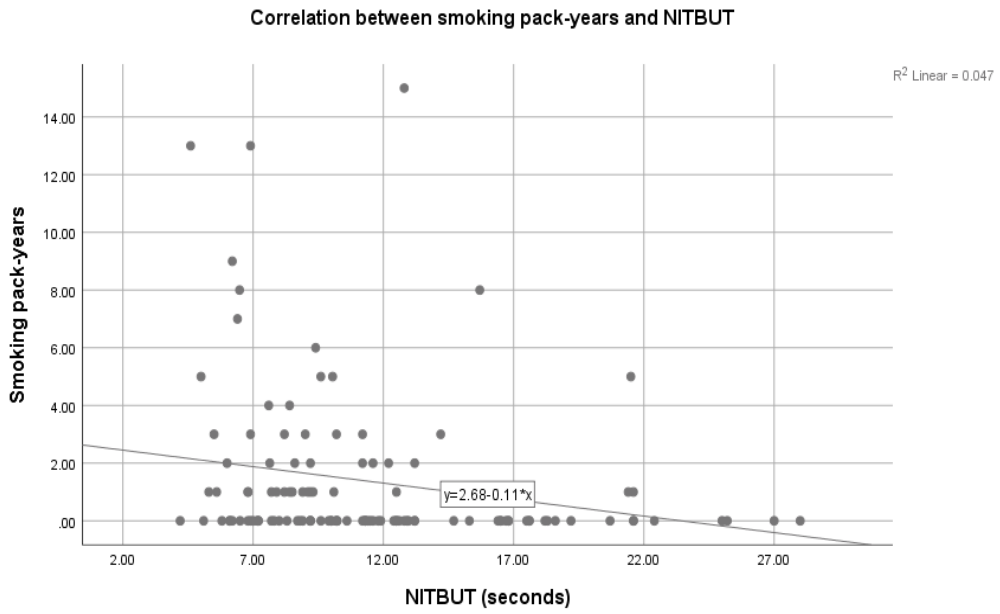
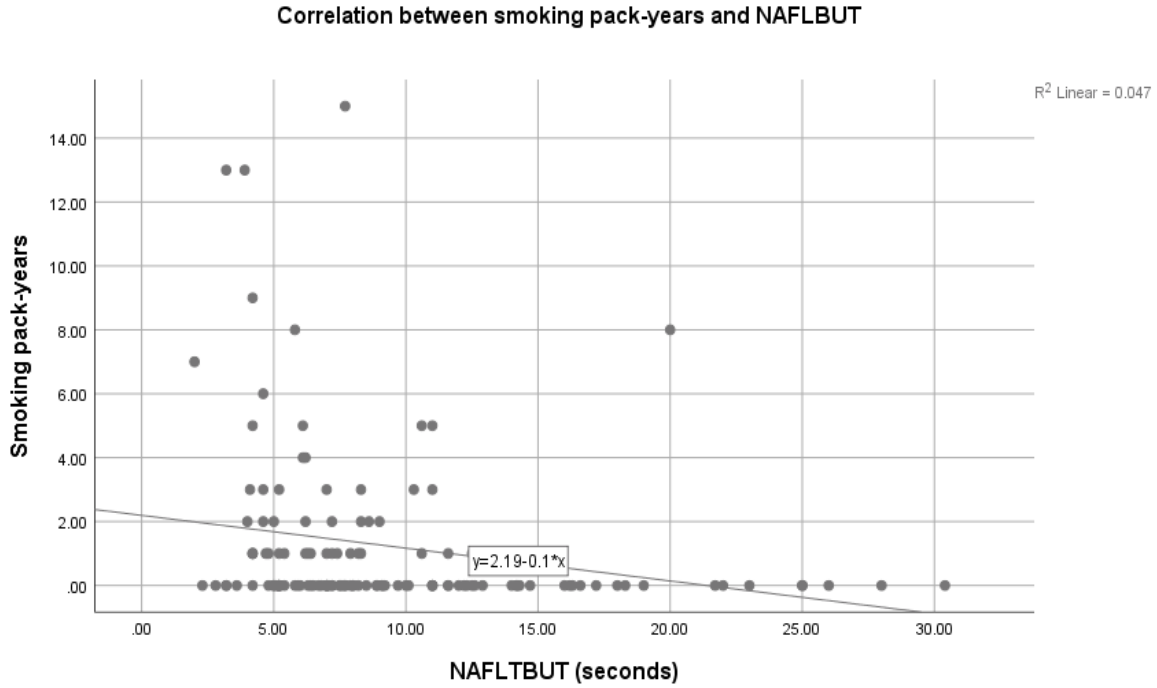


Figure 2.13 (c): correlation between smoking pack-years and invasive fluorescein tear break-up time



2.4.1.9 Correlation between cigarettes smoked per day and TBUT

Spearman ranked correlations (r_s) was used to derive any correlation between different methods of TBUT with cigarettes smoked per day after doing normality check by Shapiro–Wilk test ($p< 0.05$ for all three methods, i.e. NIKBUT, NITBUT, NAFLTBUT). There was a weak but negative correlation found between NIKBUT and cigarettes smoked per day, r_s (129) = -.30, p = 0.001. There was a moderate but a negative correlation was found between NITBUT and cigarettes smoked per day, r_s (129) = -.41, p = 0.001 and between NAFLTBUT and cigarettes smoked per day, r_s (129) = -.47, p = 0.001 respectively. Figure number 2.14 (a) is showing correlation between cigarettes smoked per day and NIKBUT. Figure 2.14 (b) is representing a correlation between cigarettes smoked per day and NITBUT and figure 2.14 (c) is representing a correlation between cigarettes smoked per day and NAFLTBUT respectively.

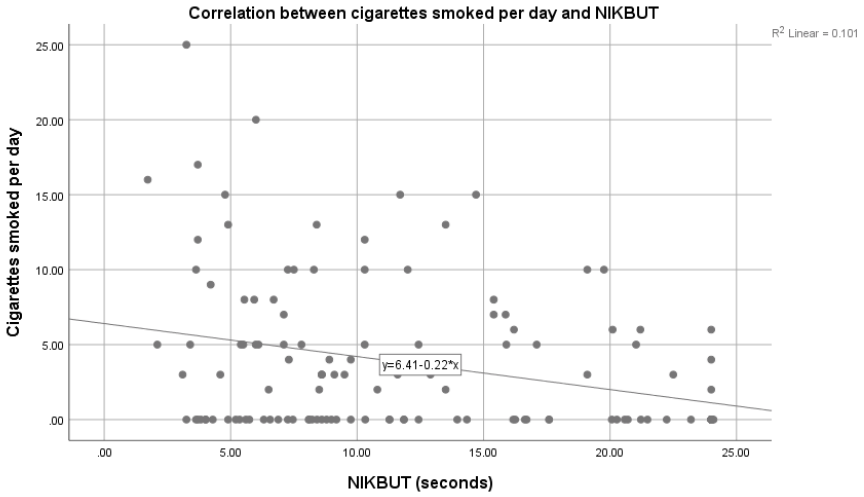


Figure 2.14 (a): correlation between cigarettes smoked per day and non-invasive Keratograph break-up time

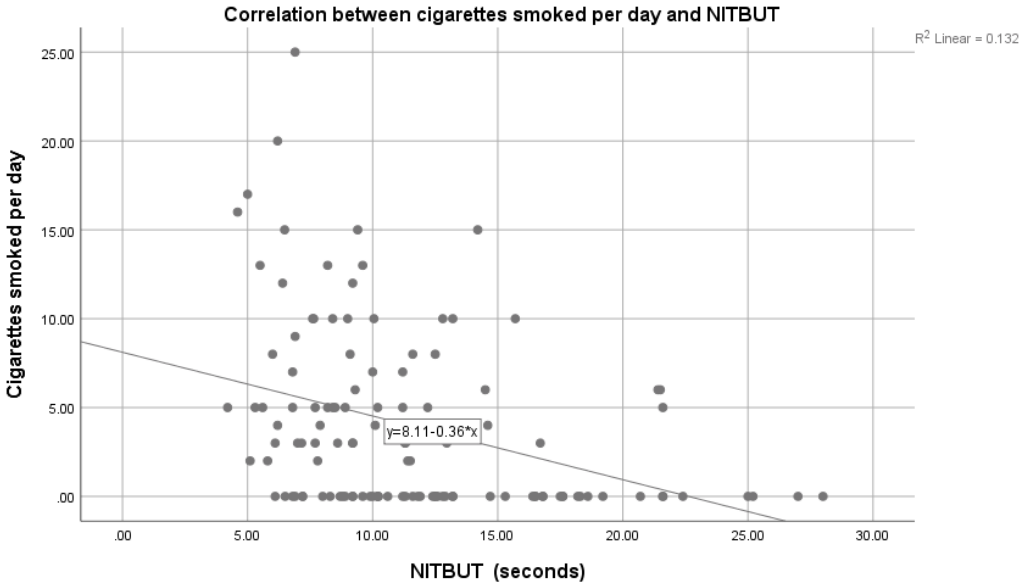


Figure 2.14 (b): correlation between cigarettes smoked per day and non-invasive tearscope break-up time

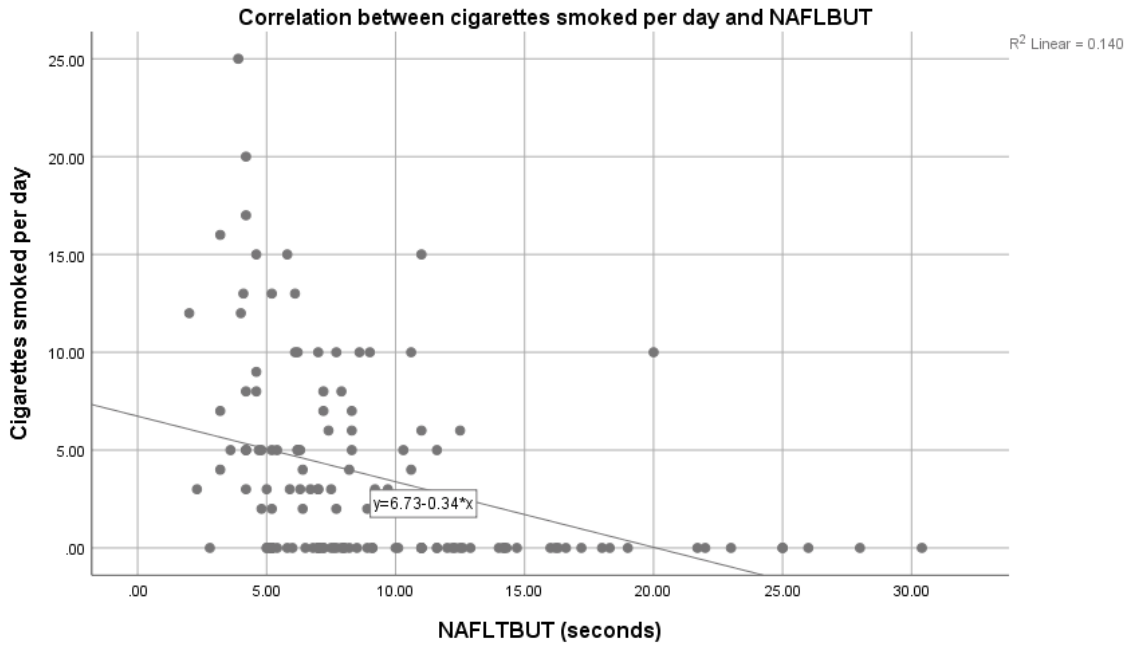


Figure 2.14 (c): correlation between cigarettes smoked per day and invasive fluorescein tear break-up time

2.4.1.10 Different levels of smoking exposure versus TBUT

The mean TBUT for all groups with three different methods are shown in table 2.2 below:

	Passive smoking												P-value
	No exposure				Frequent exposure to passive smoking				Infrequent exposure to passive smoking				
	Mean	S.D	Median	N	Mean	S.D	Median	N	Mean	S.D	Median	N	
NIKBUT in seconds	13.5	8.0	10.5	48	10.8	5.8	9.3	66	16.8	7.9	20.0	17	.02*
NITBUT in seconds	13.4	6.0	11.9	48	9.8	3.7	9.2	66	13.6	5.2	12.8	17	.001*
NALTBUT in seconds	11.0	6.2	10.0	48	6.9	3.3	6.4	66	13.0	6.9	11.0	17	.001*

Table 2.2: mean tear break-up time for all three passive exposure to smoking groups

A Kruskal–Wallis H test was used (Shapiro-Wilk test, $p < 0.05$). The test indicated that there was a significant difference present in all three different methods. For NIKBUT, $X^2(2) = 7.4$, $p = 0.02$, with the mean ranks for no-exposure group = 68.6, infrequent passive exposure = 88.6 and frequent exposure = 58.8 respectively. For NITBUT, $X^2(2) = 20.4$, $p < 0.001$, with the mean ranks for no-exposure group = 71.5, infrequent passive exposure = 76.3 and frequent

exposure = 46.9 respectively. For NAFLTBUT, $X^2(2) = 27.9$, $p < 0.001$, with the mean ranks for no-exposure group = 78.6, infrequent passive exposure = 91.0 and frequent exposure = 50.4 respectively.

The test provided strong evidence of a difference ($p < 0.05$ for all groups) between mean ranks of at least one pair of groups, Dunn's pairwise tests were carried out for the three pairs of the mentioned groups of different smoking exposure. For NIKBUT, there was a strong evidence ($p = 0.02$, adjusted Bonferroni correction) of a difference between frequent exposures to smoke and those who had infrequent exposure to passive smoke. The median NIKBUT of for infrequent exposure to passive smoke was 20.1 (s) compared to frequent exposure to smoke which was 9.3 (s). There was no evidence of a difference between the other pairs.

For NITBUT, there was strong evidence of difference ($p = 0.04$, adjusted Bonferroni correction) between frequent exposure to smoke (median NITBUT = 9.2 s) and those who had infrequent exposure to passive smoke (median NITBUT = 12.8 s). There was also strong evidence ($p = 0.004$, adjusted Bonferroni correction) between frequent exposure to passive smoke (median NITBUT = 9.2 s) and those who had no exposure to smoke (median NITBUT = 12.0 s). There was no evidence of a difference between the other pairs.

For NAFLTBUT, there was strong evidence of difference ($p < 0.001$, adjusted Bonferroni correction) between frequent exposure to passive smoke (median NAFLTBUT = 6.4 s) and those who had Infrequent passive exposure to smoke (median NAFLTBUT = 11.0 s). There was also a strong evidence ($p < 0.001$, adjusted Bonferroni correction) between frequent exposure to smoke (median NAFLTBUT = 6.5 s) and those who had no exposure to smoke (median NAFLTBUT = 10.0 s). There was no evidence of a difference between the other pairs.

2.4.1.11 Cigarette smoked per day versus TBUT

The mean TBUT for all three categories (i.e. non-smokers, light smokers and heavy smokers) with all three different methods are mentioned in table 2.3 below:

	Cigarette smoked per day gradations												
	0.0				1.0				2.0				
	Mean	S.D	Median	N	Mean	S.D	Median	N	Mean	S.D	Median	N	p-value
NIKBUT In seconds	14.6	7.7	14.1	66	11.2	6.1	9.1	53	7.2	4.3	5.4	12	.002*
NITBUT in seconds	13.8	5.5	12.5	66	10.0	4.0	9.1	53	7.6	2.6	6.7	12	.001*
NAFLBUT in seconds	11.8	6.3	10.5	66	7.0	2.9	6.7	53	4.8	2.2	4.2	12	.001*

**Gradations: grade zero = non-smokers, grade one = represents light/mild smokers, and grade two = heavy smokers group*

Table 2.3: mean tear break-up time for all three different cigarette smoking per day categories.

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) was used to determine any statistical difference in TBUT between different smoking exposure levels. The test indicated that there was a significant difference present in all three different methods. For NIKBUT, $X^2(2) = 12.2$, $p = 0.002$, with the mean ranks for non-smokers group = 70.0, light smokers' group = 69.0 and heavy smokers' group = 30.5 respectively. For NITBUT, $X^2(2) = 25.8$, $p < 0.001$, with the mean ranks for non-smokers' group = 76.8, light smokers' group = 50.6 and heavy smokers' group = 29.6 respectively. For NAFLTBUT, $X^2(2) = 36.2$, $p < 0.001$, with the mean ranks for non-smokers' group = 84.1, light smokers' group = 53.0 and heavy smokers' group = 23.6 respectively.

The test provided strong evidence of a difference ($p < 0.05$ for all groups) between mean ranks of at least one pair of groups, Dunn's pairwise tests were carried out for three pairs of the mentioned groups of different smoking exposure. For NIKBUT, there was strong evidence ($p = 0.03$, adjusted Bonferroni correction) of a difference between heavy smokers 'group to those who were non-smokers. The median NIKBUT of for non-smokers' group was 14.6 (s) compared to heavy smokers' group which was 5.4 (s). There was no evidence of a difference between the other pairs. For NITBUT, there was a strong evidence of difference ($p < 0.001$, adjusted Bonferroni correction) between heavy smokers (median NITBUT = 6.7 s) and non-smokers' group (median NITBUT = 12.5 s). There was also strong evidence ($p < 0.001$, adjusted Bonferroni correction) between light smokers' group (median NITBUT = 9.1 s) with non-smokers' group (median NITBUT = 12.5 s). There was no evidence of a difference between the other pairs.

For NAFLTBUT, there was a strong evidence of difference ($p = 0.04$, adjusted Bonferroni correction) between heavy smokers' group (median NAFLTBUT = 4.2 (s)) with light smokers' group (median NAFLTBUT = 6.7 s). There was also a strong evidence ($p < 0.001$, adjusted Bonferroni correction) between light smokers' group (median NAFLTBUT = 6.7 s) with non-smoking group (median NAFLTBUT = 10.5 s). Finally, there was a strong evidence of a difference ($p < 0.001$, adjusted Bonferroni correction) between heavy smokers' group (median NAFLTBUT = 4.2 s) with non-smokers' group (median NAFLTBUT = 10.5 s).

2.4.1.12 Smoking pack years versus TBUT

The mean TBUT for all three categories (mentioned in section 2.3.9 above) with all three different methods are mentioned in table 2.4 below:

	Cigarette smoked per day gradations												p-value
	.00				1.00				2.00				
	Mean	S.D	Median	N	Mean	S.D	Median	N	Mean	S.D	Median	N	
NIKTBUT in seconds	14.6	7.7	14.1	66	11.2	6.1	9.1	53	7.2	4.3	5.4	12	.01*
NITBUT in seconds	13.8	5.5	12.5	66	10.0	3.9	9.1	53	7.6	2.6	6.7	12	.006*
NAFLBUT in seconds	11.8	6.3	10.5	66	7.0	2.9	6.7	53	4.8	2.2	4.2	12	.001*

**Gradations: grade zero = zero smoking pack-years, grade one = smokers smoked less than ten packs, and grade two = smokers smoked more than ten packs.*
Table 2.4: mean tear break-up time for all three smoking pack-years gradations

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) indicated that there was a significant difference present in all three different methods. For NIKBUT, $X^2(2) = 8.1$, $p = 0.01$, with the mean ranks for non-smokers group = 72.1, smokers smoked less than ten pack years' group = 57.5 and smokers smoked more than ten pack years' group = 23.5 respectively. For NITBUT, $X^2(2) = 10.4$, $p = 0.006$, with the mean ranks for non-smokers' group = 68.6, smokers smoked less than ten pack years' group = 49.0 and smokers smoked more than ten pack years' group = 35.8 respectively. For NAFLTBUT, $X^2(2) = 15.4$, $p < 0.001$, with the mean ranks for non-smokers' group = 75.5, smokers smoked less than ten pack years' group = 51.0 and smokers smoked more than 10 pack years' group = 27.3 respectively.

As, the test provided strong evidence of a difference ($p < 0.05$ for all groups) between mean ranks of at least one pair of groups, Dunn's pairwise tests were carried out for the three pairs of the mentioned groups of different smoking exposure. For NIKBUT, The test for mean rank difference was statistically significant but none of the pairwise tests of mean rank difference was statistically significant after controlling for multiple testing. These results indicate that the global test was a false positive finding.

For NITBUT, there was strong evidence of difference ($p = 0.009$, adjusted Bonferroni correction) between the group of smoking years ≤ ten packs (median NITBUT = 10.0 s) and non-smokers' group (median NITBUT = 12.7 s). There was no evidence of a difference between the other pairs. For NAFLTBUT, there was strong evidence of difference ($p < 0.001$, adjusted Bonferroni correction) between group of smoking years ≤ ten packs (median NAFLTBUT = 7.0 s) with non-smokers' group (median NAFLTBUT = 10.6 s). There was no evidence of a difference between the other pairs.

2.4.2 Accommodative ability analysis

The mean AoA for non-smokers was 9.6 ± 2.6 Dioptres (D) which were slightly lower than the mean AoA for smokers 9.9 ± 2.1 D. A Mann–Whitney U test (Shapiro–Wilk test, p > 0.05) was used to test for the hypothesis, and the test indicated that there was no significant difference found in AoA for smokers (median = 10.1) and non-smokers (median = 9.4). *U* = 1846.6, *p* = 0.17.

2.4.2.1 Analysis of AoA in different age groups

Participants were divided into different age groups for a group-wise comparison of AoA. There was no statistical difference of AoA found in the different age groups. The table 2.5 shows mean AoA in different age groups.

	Smoking status							
	non-smoker				Smokers			
	AoA in Dioptres				AoA in Dioptres			
Age groups (years)	Mean	Standard Deviation	N	Median	Mean	Standard Deviation	N	Median
Age 18 -24	10.91	2.32	37	10.25	10.64	1.42	46	10.50
Age 25 -30	9.19	2.06	15	9.00	9.44	1.56	12	9.25
Age 31 - 35	7.70	0.63	7	7.50	8.19	0.09	2	8.19
Age 36- 40	6.34	1.23	4	6.63	7.25		1	7.25
Age 41 -50	5.42	1.61	3	4.75	4.38	1.32	4	4.44

Table 2.5: showing means, standard deviation, and median of the amplitude of accommodation for participants divided into different age groups.

2.4.2.2 Age versus AoA correlation

Age was found to be the strongest predictor of AoA. There was an inverse relationship between Age and AoA. Pearson correlation (r) was used to derive any correlation between AoA and age after a normality check by Shapiro–Wilk test (p >0.05). Outliers were winsorized (replaced by next normal value which was not an outlier) to get normally distributed data. There was a strong but negative correlation found between AoA and age, r (129) = -0.7, p = 0.001. Figure 2.15 showing a negative correlation between AoA and age.

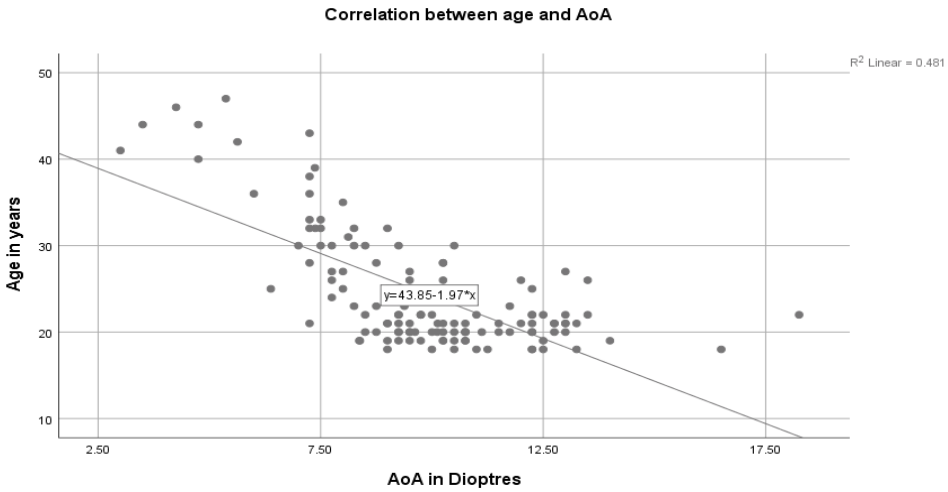


Figure: 2.15: showing a negative correlation between age and amplitude of accommodation

2.4.2.3 Ethnicity versus AoA

The mean AoA for each ethnic group is mentioned in table 2.6 as below:

Ethnic origin	AoA in Dioptres				
	Mean	Standard Deviation	N	Median	P value
Asian (Indo-Pak origin)	9.80	2.50	63	10.00	0.7
White	9.58	2.44	38	9.50	
Others*	9.98	2.11	30	9.75	

**Others category included participants from Chinese, Arab, Kurdish, Persian, and Mixed race backgrounds*

Table 2.6: showing mean amplitude of accommodation of participants with different ethnic backgrounds

There was no significant age difference found between the three ethnic groups, $X^2(2) = 3.5$, $p = 0.1$, with the mean ranks for Asians = 67.4, White = 57.4, Others = 74.2 respectively. A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a non-statistical significant difference in AoA among three ethnic groups, $X^2(2) = 0.7$, $p = 0.7$, with the mean ranks for Asian category = 67.6, White category = 61.5, Others' category = 68.3 respectively.

2.4.2.4 Gender versus AoA

The mean AoA for female participants was numerically higher (10.3 ± 2.4 D) than male counterparts (9.5 ± 2.3 D). A One-Way Analysis of Variance (Shapiro–Wilk test, $p > 0.05$) showed that there was no significant difference presented among mean AoA of male and female participants (F (1,129) = 3.4, p = 0.06).

2.4.2.5 Pupil size versus smoking status

The mean pupil size for non-smoker participants was 5.5 ± 0.9 mm while the mean pupil size for smoker participants was 5.6 ± 1.0 mm. An ANOVA was performed (Shapiro–Wilk test, $p > 0.05$), and the analysis showed a non-significant difference in mean pupil sizes of smokers and non-smokers (F (1,129) = .04, p = 0.8).

2.4.2.6 Analysis of AoA versus smoking status

The mean AoA for non-smokers was 9.6 ± 2.6 Dioptres (D) which were slightly lower than the mean AoA for smokers 9.9 ± 2.1 D. An ANOVA test was used (Shapiro–Wilk test, $p > 0.05$) to determine any significant difference and the test indicated that there was no significant difference found in AoA for smokers and non-smokers (F (1,129) = .03, p = 0.5). A One–Way Analysis of Covariance (ANCOVA) was conducted to determine any significant difference between the different level of smoking status on AoA controlling for gender, age, ethnicity, and drinking status. There was no statistical significant effect of smoking on AoA values (F (1, 125) = 0.1, p = 0.7).

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	367.290[a]	5	73.458	24.620	.000	.496
Intercept	495.275	1	495.275	165.997	.000	.570
Age	320.226	1	320.226	107.327	.000	.462
Gender	3.640	1	3.640	1.220	.271	.010
Drinking	5.083	1	5.083	1.704	.194	.013
pupillometry	.347	1	.347	.116	.734	.001
Smoking status	.284	1	.284	.095	.758	.001
Error	372.955	125	2.984			
Total	13269.012	131				
Corrected Total	740.245	130				

a. R Squared = .496 (Adjusted R Squared = .476)

Table 2.7: showing results for One–Way Analysis of Covariance (ANCOVA) conducted to analyse mean difference of amplitude of accommodation in smokers and non-smoker

2.4.2.7 Correlation between smoking pack years and AoA

Pearson correlation was used to determine any significant correlation between AoA and smoking years, after checking normality with the Shapiro–Wilk test. The normality test showed a normal distribution ($P>0.05$) after adjusting for outliers (with the winsorizing method). There was a weak but negative correlation found between smoking years and AoA r (129) 0.17, p= 0.04. Figure 2.16 showing a negative correlation between AoA and smoking pack-years.

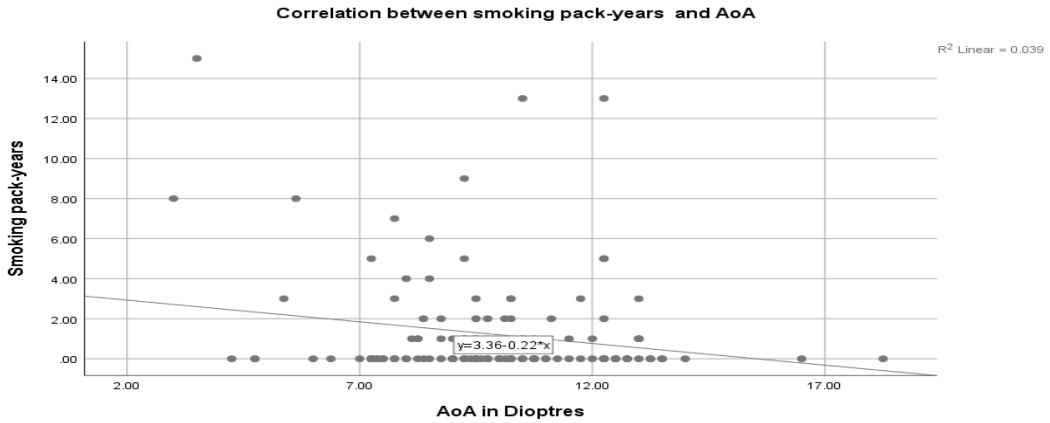


Figure 2.16: negative correlation between pack-years and the amplitude of accommodation

2.4.2.7 Smoking pack-years versus AoA

The mean AoA for all three categories is mentioned in table 2.8, in which non-smokers are represented by grade zero, less than ten smoking years participants are represented by grade one, and grade two represents participants with more than ten smoking years.

	Grading of smoking pack years											
	.00				1.00				2.00			
	Mean	N	S.D	Median	Mean	N	S.D	Median	Mean	N	S.D	Median
AoA in Diopters	9.9	83	2.4	9.7	9.6	45	2.1	9.5	8.7	3	4.6	10.5
Age (years)	24.5	83	6.6	22.0	24.1	45	6.6	22	32.0	3	11.1	30.0

**Grade zero = zero smoking pack-years, grade one = less than ten smoking pack years and grade two = smoking pack years for more than ten years*

Table 2.8: showing descriptive data for the amplitude of accommodation and age for three smoking pack-years groups

2.4.3: Analysis of defocus curves according to the smoking status

Defocus lens power ranging from +1.50 DS (Dioptres sphere) to -5.00 DS was used (with 0.50 DS steps for increase or decrease lens power) to find out any difference of subjective clear vision range between smokers and non-smokers. Defocus lenses and the letter presentation were randomised to wave out any memory effect as suggested by Gupta *et al.* (2008). A Kruskal–Wallis H test (Shapiro-Wilk test, $p > 0.05$) was used to determine any statistical difference of defocusing ability between smoker and non-smoker participants. The mean LogMAR visual acuity (VA) against each defocus lens for smokers, and non-smokers participants are mentioned in table 2.9 below:

Defocus lens power (DS)	smoking status								
	Smoker				non-smoker				
	Mean	Standard Deviation	N	Median	Mean	Standard Deviation	N	Median	P-value
+1.5	0.46	0.17	65	0.42	0.48	0.21	66	0.50	> 0.05
+1.0	0.25	0.14	65	0.22	0.25	0.16	66	0.20	> 0.05
+ 0.5	0.05	0.08	65	0.06	0.02	0.08	66	0.00	< 0.05*
0.0	-0.06	0.05	65	-0.10	-0.06	0.07	66	-0.10	> 0.05
- 0.5	-0.06	0.05	65	-0.10	-0.06	0.06	66	-0.10	> 0.05
- 1.0	-0.05	0.05	65	-0.08	-0.05	0.06	66	-0.10	> 0.05
- 1.5	-0.05	0.06	65	-0.08	-0.04	0.08	66	-0.09	> 0.05
- 2.0	-0.04	0.06	65	0.00	-0.01	0.15	66	-0.06	> 0.05
- 2.5	-0.03	0.08	65	-0.04	0.02	0.16	66	0.00	< 0.05*
- 3.0	0.00	0.11	65	0.00	0.06	0.20	66	0.00	> 0.05
- 3.5	0.03	0.15	65	0.00	0.09	0.25	66	0.00	> 0.05
- 4.0	0.08	0.22	65	0.00	0.17	0.32	66	0.00	> 0.05
- 4.5	0.13	0.25	65	0.00	0.21	0.35	66	0.01	> 0.05
- 5.0	0.19	0.30	65	0.06	0.24	0.37	66	0.04	> 0.05

**P value significant*

Table 2.9: showing mean LogMAR visual acuity from +1.50 DS to 0.0 DS of smokers and non-smokers' participants

The test showed that at +0.50 DS defocus power, LogMAR VA for non-smoker participants was significantly higher (Median = 0.00) when compared to smoker participants (median 0.06), $X^2(1) = 6.4$, p = 0.01. At -2.50 DS defocus power, smoker participants had a significant LogMAR VA (Median = -0.04) when compared to non-smokers (median0.00), $X^2(1) = 4.5$, p = 0.03. At other defocus lens powers the difference of LogMAR VA between smokers and non-smokers were not significant. Figure 2.17 is showing mean LogMAR VA for smoker and non-smoker participants as below:

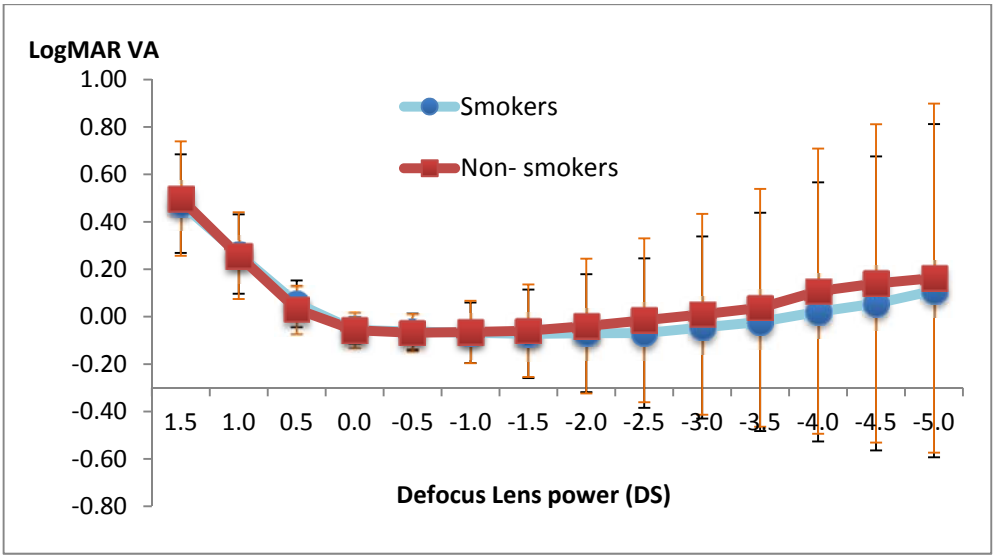


Figure 2.17: Subjective clear vision range attained from defocus curves for smoker and non-smokers participants

2.4.4 Analysis of MPOD

2.4.4.1 Age versus MPOD analysis

There was no significant correlation found between age and MPOD scores. Pearson correlation (r) was used to derive any correlation between MPOD and age after doing a normality check by Shapiro–Wilk test ($p > 0.05$). The result showed a non-significant correlation $r(129) = -0.04$, $p = 0.62$.

2.4.4.2 MPOD analysis versus Gender

There was no numerical difference found between MPOD scores of males and females. The average MPOD score for men was 0.46 ± 0.13 whereas; the mean MPOD score for women was 0.46 ± 0.14. After adjusting for outliers by the winsorizing method, an ANOVA (Shapiro-Wilk test $p > 0.05$) was performed. The analysis showed a non-significant difference in mean MPOD scores of male and females ($F(1,129) = 0.04$, $p = 0.83$).

2.4.4.3 MPOD analysis versus Ethnicity

Numerically, mean MPOD scores for "Others" which included participants from different ethnic background apart from Asian (Indo-Pak origin) and White was higher (i.e. 0.47 ± 0.13) than mean MPOD scores for Asian (i.e. 0.46 ± 0.13) or White participants (i.e. 0.43 ± 0.13). An ANOVA was performed (Shapiro–Wilk test, $p > 0.05$) after adjusting the outliers with the winsorizing method. The analysis showed no significant difference in mean MPOD scores between three ethnic groups ($F(2,128) = 0.8$, $p = 0.4$). Table 2.10 shows the mean MPOD of participants of different ethnicities.

		MPOD value				P-value
		Mean	Standard Deviation	N	Median	
Ethnicity	Asian (Indo-Pak origin)	0.46	0.14	63	0.45	0.4
	White	0.44	0.13	38	0.42	
	Others*	0.48	0.14	30	0.46	

**Others category included participants from Chinese, Arab, Kurdish, Persian, and Mixed race backgrounds*

Table 2.10: showing mean, standard deviation and median of MPOD scores for three ethnic categories

2.4.4.5 MPOD analysis versus smoking status

The mean MPOD scores for non-smokers was slightly higher numerically 0.46 ± 0.14 than mean MPOD scores for smoker participants 0.45 ± 0.13. An ANOVA was performed (Shapiro–Wilk test, p >0.05) after adjusting the outliers with the winsorizing method. The analysis showed non-significant difference in smoker and non-smoker participants (F (1, 129) = 0.11, p = 0.74). A One–Way Analysis of Covariance (ANCOVA) was conducted to determine any significant difference between the different level of smoking status on MPOD scores controlling for gender, age, ethnicity, and drinking status. There was no statistical significant effect of smoking on MPOD score (F (1, 125) = 0.10, p = 0.9).

Tests of Between-Subjects Effects

Dependent Variable: MPOD

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	.075[a]	5	.015	.777	.568
Intercept	1.856	1	1.856	95.886	.000
Age	.019	1	.019	.961	.329
Ethnicity	.005	1	.005	.234	.629
Gender	.000	1	.000	.015	.902
Drinking	.067	1	.067	3.443	.066
Smoking status	.000	1	.000	.010	.921
Error	2.420	125	.019		
Total	30.085	131			
Corrected Total	2.495	130			

a. R Squared = .030 (Adjusted R Squared = -.009)

Table 2.11: showing results for One–Way Analysis of Covariance (ANCOVA) conducted to analyse mean difference of MPOD in smokers and non-smoker

2.4.4.6 MPOD analysis versus drinking status

Participants were divided into six drinking status categories. The mean MPOD scores for each category are shown in table 2.12 as below:

		MPOD value				
		Mean	N	Standard Deviation	Median	P-value
Drinking status	non- drinker	.46	80	.14	.44	0.1
	1-2 unit per week	.51	15	.12	.49	
	3-4 units per week	.51	10	.12	.51	
	5-6 units per week	.46	6	.18	.39	
	more than 7 units per week	.40	20	.14	.38	

Table 2.12: showing descriptive data of MPOD values according to five gradations of drinking status

A Kruskal Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a non-significant difference, $X^2(4) = 6.8$, $p = 0.1$, with the mean ranks form non-drinkers = 65.6, 1-2 units per week = 80.7, 3-4 units per week = 79.7, 5-6 units per week = 60.8, and more than 7 units = 51.0 respectively.

2.5 Discussion

2.5.1 Effect of smoking on tear film

OSDI questionnaire has been in use since 1997, and it is a rapid form of the assessment for dry eye (DE) symptoms. It is a standardized instrument with high reliability and validity (Ozcura *et al.*, 2007, Bakkar *et al.*, 2016a, Schiffman *et al.*, 2000). This study found that smokers had high OSDI scores than non-smokers and that relationship was significant. These results are consistent with the findings of Aktaş *et al.* (2017) and Erginturk Acar *et al.* (2017). Contrarily, Wang *et al.* (2016) did not find any significant difference in OSDI scores of smokers and non-smokers in their study. The possible reason why Wang *et al.* (2017) were unable to find a significant difference could be having a small number of smokers participants (n = 322) when comparing them with a large number of non-smokers (n =2067).

It is understood from the literature (Hua *et al.*, 2014) that apart from smoking status, there are other variables that can affect the outcome of OSDI scores/DE symptoms such as geographical region and gender, race and use of systematic medication (Kastelan *et al.*, 2013). Many studies have used custom-made symptom scores to evaluate DE related symptoms, e.g. (Altinors *et al.*, 2006, Sayin *et al.*, 2014). These studies also found that DE related symptoms scores were higher in smokers when compared to non-smokers. On the contrary, many studies (Hua *et al.*, 2014, Yoon *et al.*, 2005a) did not find any significant difference in symptoms scores between smoking participants and non-smokers.

Cigarette smoking has found to be related with decreased tears stability. It is also related with other effects like low corneal and conjunctival sensitivities, reduction of goblet cells, increased conjunctival squamous metaplasia, and alternation of tears proteins etc. Many studies have been published so far which have accounted above said adverse effects of smoking on the pre-corneal tear film, e.g. (Altinors *et al.*, 2006, Masmali *et al.*, 2016, Sayin *et al.*, 2014, Matsumoto *et al.*, 2008, Yoon *et al.*, 2005b).

In terms of TBUT, this study results' are consistent with the previous studies results present in the literature so far suggesting that smokers have a decreased TBUT compared to non-smokers. Lipid peroxidation and ocular epithelial damage are two of the many possible mechanisms on how smoking can affect the pre-corneal tear film. Thomas *et al.* (2012) suggested that chemical substances in the cigarette smoke could enter through the airway

barrier epithelial barrier, enter the systemic circulation via the pulmonary circulation, and increase the systemic oxidative damage, leading to the development of cigarette smoking-related diseases.

Alternatively, smoking can cause ocular epithelial damage by its direct contact with the ocular surface as suggested by Satici *et al.* (2003), increasing conjunctival squamous cell metaplasia which could be caused by toxic and irritant materials of cigarette smoke. This study has included three different methods for measuring TBUT by both non-invasive, e.g. Keratograph K5M (also captures the video of tears breaking on the pre-corneal tear film) and Easy view+ tearscope and invasive by fluorescein TBUT. Previous studies had measured TBUT invasively. However, this study has included two non-invasive methods as a new addition to the literature. This study shows that smokers have decreased non-invasive TBUT when compared to non-smokers.

In terms of lipid layer thickness, this study used two non-invasive techniques to assess the thickness of lipid layer and although the results showed a non-significant difference in non-smokers and smokers average lipid layer thickness but still numerically average lipid thickness of smokers was at least 10 nm thinner compared to non-smokers. Some of the previous studies used a DR 1–Lipid layer interferometry technique (Altinors *et al.*, 2006, Matsumoto *et al.*, 2008) which is a kinetic way of analysis of lipid layer interference images. Both Altinors et al. (2006) and Matsumoto et al. (2008) showed that lipid spread time for smokers was higher than non-smokers.

This study was unable to perform DR1- Lipid layer interferometry and used the old version of interferometry (static interferometry–analysis of lipid layer pattern for thickness etc.). Hence, it may be possible that this study results could be different if the study used DR1-Lipid interferometry technique. Interestingly, the current study did not find any significant difference in TMH between smokers and non-smokers. In the literature, no study has done TMH test to date so far when investigating the effect of smoking on the tear film but decreased TMH is usually related as a sign of dry eye (Shen *et al.*, 2009).

In terms of cause and effect relationship, only few studies managed to look any cause and effect relationship of smoking with TBUT or tried to find any correlation between smoking and tear film parameters. Thomas *et al.* (2012) did not found a causative relationship between smoking and TBUT. Masmali *et al.* (2016) found a negative correlation between duration of smoking and TBUT. In contrast to Thomas *et al*. (2012), the current study found causation between smoking and TBUT. This study found a causation and correlation between smoking pack years with TBUT and with cigarettes smoked per day with TBUT. More longitudinal studies are required to find out more over cause and effect relationship between smoking and TBUT.

In terms of passive smoking, to the best of knowledge, only El-Shazly *et al.* (2012) studied the impact of passive exposure of smoking and found that passive exposure to smoking in children was significantly related with DE symptoms in children. The current study also found that passive exposure to cigarette smoke was significantly but inversely related to all three methods of TBUT.

2.5.2 Effect of smoking on MPOD

Cigarette smoking is considered as a risk factor for low MPOD scores, and many studies have shown an inverse relationship of smoking with MPOD scores (Raman *et al.*, 2012a, Nolan *et al.*, 2007). In the current study, there was no significant difference shown in MPOD values for smokers and non-smokers. One of the possible reason could be the 'age factor'. Studies have shown a negative association of smoking with MPOD values normally had study participants with an average age of 41.5 ± 19.7 (average age of participants in Hammond and Caruso, 2002) apart from one study (Hammond *et al.* 1996) which had an average age of 32 years for its participants.

In this current study, the average age of study participants was 24.6 ± 6.7 years, which was significantly lower than the average age of other studies. As most of the diseases associated with smoking shows its signs lately, so it could be plausible that at the younger age the effect of smoking on MPOD scores is difficult to evaluate. The other possible cause of non-significant difference of smokers and non-smokers MPOD scores could be due to the level/intensity of smoking, many participants in this study were new to smoking and were light smokers (e.g. less than five cigarettes per day). In terms of pack smoking years, many current smokers (n = 17) fell into the "zero" pack year category. Whereas, only three participants were smoking greater than ten pack-years. Previous literature had (Hammond, 2002, Hammond *et al.*, 1996) demonstrated that smoking has an inverse dose-response relationship with MPOD.

Many studies have shown that MPOD concentration declines with an increase in the age (Hammond, 2002, Kirby *et al.*, 2010, Nolan *et al.*, 2007, Yu *et al.*, 2012, Ji *et al.*, 2015, Abell *et al.*, 2014). Whereas, there are some studies (Raman *et al.*, 2012b, Murray *et al.*, 2013) which have shown no significant association of age with MPOD scores. This study did not find any significant relationship of age with MPOD. One of the possible reason could be because of younger age study participants compared to other studies (already mentioned above).

Gender association with MPOD values are not yet clear, and there are mix results shown from the previous studies done. The current study did not find any significant difference in the MPOD scores between male and female gender. This result is consistent with many previous studies which have shown no significant association of gender with MPOD score (Obana *et al.*, 2014, Ji *et al.*, 2015, Abell *et al.*, 2014, Raman *et al.*, 2012b). There are some studies in the literature, which have shown that female gender is associated with lower MPOD scores

compared to male gender (Hammond, 2002, Yu *et al.*, 2012). On the contrary, a recent study (Alassane *et al.*, 2016) have shown that women had higher MPOD scores compared to men.

2.5.3 Effect of smoking on AoA

Cigarette smoking is associated with cataract formation (Ye *et al.*, 2012), and cessation of cigarette smoking can reduce the risk but will take longer time (Lindblad *et al.*, 2014) to show its effect. Early cataract signs are usually seen after 40 years of age, but the loss of flexibility of lens or increase in the stiffness of the lens can be seen earlier, such as AoA (Weeber and van der Heijde, 2007).

There are many factors, which can affect AoA; out of that, age is the most influential factor. It is well documented that with the increase in age, AoA will decline (Duane, 1912, Mordi and Ciuffreda, 1998, Ovenseri-Ogbomo *et al.*, 2012). This study observed the same relationship of age with AoA and showed that AoA decreased with an increase in age. Ethnic and geographical factors (e.g., areas with high average temperature, tropical regions could affect AoA (Miranda, 1979, Chattopadhyay and Seal, 1984, Ovenseri-Ogbomo *et al.*, 2012, Edwards *et al.*, 1993). It is documented that Indian population or Chinese population have lower AoA and usually have the earlier onset of presbyopia (Miranda, 1979, Jain *et al.*, 1982, Chattopadhyay and Seal, 1984, Edwards *et al.*, 1993) compared to the white and Caucasian population.

Recently, few studies which were conducted on the effects of smoking on presbyopia found that smokers had an earlier onset of presbyopia (Khalaj *et al.*, 2014, Parkesh Kavita, 2017). However, in terms of the effect of smoking on AoA, only one study conducted to find any relationship between smoking and AoA. Ide *et al.* (2012) found that smokers had significantly less AoA compared to non-smokers.

The current study did not find any significant difference in AoA among smokers and non-smokers. This might be because of age factor of the study's participant which was quite young as the average age for both smoker's, and non-smoker's participant was approximately 25 years old, and the majority of the participants were less than 30 years of age (85 per cent of the total data). It could be a point of interest to measure AoA in older subjects (pre-presbyopic population, e.g. 35 or above) according to their smoking status for the further research to evaluate the role of smoking as an allostatic load on lens health.

The previous literature review had demonstrated that in adults female gender had an earlier onset of presbyopia and their AoA was lower than age-matched male counterparts (Mehdi *et al.*, 2013, Nirmalan *et al.*, 2006) This could be because of the hormonal changes, e.g. menopause (Hashemi *et al.*, 2017b). However, in younger age, Hashemi *et al.* (2017b) observed that females had higher AoA compared to males. The current study did not find any significant difference of AoA among male and female participants but females had a

numerically higher AoA compared to male counterparts. Regarding optical factors, which can affect AoA, the current study, measured the mean pupillary diameter (PD) for smokers and non-smokers and did not find any significant difference between them. The study found a weak and negative but significant correlation between smoking pack years and AoA. The result of the correlation suggested that higher smoking addiction was related with low AoA.

Apart from geographical difference, previous evidence suggested that lack of balanced diet (Jain *et al.*, 1982) and high exposure to sunlight/UV radiations could play a role in the early onset of presbyopia (Miranda, 1979, Jain *et al.*, 1982) in Indian population. As Presbyopia is linked with AoA, these factors could also have some impact on AoA. In the current study, these factors were not prominent as Asian participants, White participants had an almost similar intake of dietary elements, and they had a similar exposure to sunlight.

This study did not find any significant difference in AoA between Asians (Indo-PAK origin) and White people. One of the possible reasons for such an outcome could be because almost all Asian participants involved in this study were either born and bred here or were living here for the quite long time. Therefore, Asian participants adopted themselves well in the current environment, and there was no geographical difference presented among Asians and White participants.

The next chapter will explore the relationship between smoking and the tear film and AoA in an Asian population living in their native land.

Chapter 3

Effect of smoking on the tear film, and accommodative ability in a Pakistani cohort

The previous chapter investigated the relationship between smoking and the tear film, smoking and AoA, and smoking and MPOD in a UK study cohort. The subjects recruited in the previous chapter were mainly British-white or British-Asians. This chapter investigates the same relationships, between smoking and the tears and with AoA, but in an Asian cohort living in Pakistan. The MPOD was not measured as it was not available in Pakistan.

3.1 Introduction

In Pakistan, more than 23.9 million smokers are consuming 90,000 tons of tobacco annually (GATS, 2014). Smoking is more prevalent in male gender compared to female (WHO, 2017, Masud and Oyebode, 2018)According to the World Health Organisation (WHO), the prevalence of tobacco smoking is approximately 40% in men and 3% in women in Pakistan (WHO, 2017). High restrictions for producing and buying cigarette products in developed countries have resulted in a shift of the tobacco industry to under developed countries (Gilmore *et al.*, 2015). In Pakistan, most smokers are from low socio-economic groups (Hiscock *et al.*, 2012) and are generally less educated (Ahmad *et al.*, 2005, Masud and Oyebode, 2018)..

Although smoking is a leading cause of mortality and morbidity in Pakistan (Shah and Siddiqui, 2015), most of the Pakistani studies linked with smoking effects are limited to the areas of pulmonary and cardiac research. There is a gap of literature when it comes to research work conducted in Pakistan on adverse effects of smoking on ocular health. There are no published studies that have shown any effect of smoking on the tear film among Pakistani participants. However, few recent studies conducted on the prevalence of dry eye disease (DED) in Pakistan found smoking as a risk factor for DED (Shua Azam, 2016, Abdullah *et al.*, 2017).

Pakistan and India have a similar lifestyle and environmental conditions. A study conducted in northern India has shown the prevalence of DED (Titiyal *et al.*, 2018) has shown smoking was one of the main reasons for DED. In context to effects of smoking on the tear film, a study conducted by Thomas *et al.* (2012) in a South Indian community has found smoking to be responsible for decreased tear break-up time (TBUT), low corneal and conjunctival sensitivities and with increased patient related symptoms of dry eyes. Agrawal *et al.* (2018) also found low TBUT, low Schirmer test scores and high conjunctival squamous metaplasia in Indian smokers compared to non-smokers.

To date, there are no studies on the effect of smoking on the accommodative ability of the crystalline lens in the Pakistani population. However, a recent Indian study found that addiction to smoking was positively correlated with the earlier presbyopia (Parkesh Kavita, 2017).

This study was conducted to evaluate the effects of smoking on tear break-up time (TBUT), on the amplitude of accommodation and on defocus curves in a Pakistani population.

3.2 Study aim

The purpose of this study was to assess the effects of smoking on the TBUT, AoA, on defocus curves in the Pakistani cohort

3.3 Methods

The study design was a prospective cross-sectional study. Smoker and non–smoker participants were recruited from Amer Eye Hospital (AEH) Rawalpindi, Pakistan. The study participants were hospital staff, patients and their relatives reported in the outdoor patient's department (OPD). The Aston University ethics committee approved the study. A local ethical approval from AEH ethical board was also taken and the research followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants after explaining the nature of the study.

3.3.1 Selection criteria

The subjects were selected on the same criteria that were mentioned for the UK cohort of participants in chapter 2.

3.3.2 Study Instruments

- Slit lamp (Model: BP 900 Haag Streit, Clement Clarke Ltd, Essex, UK) for measuring TBUT invasively.
- RAF near point rule (Clement Clarke Ltd, Essex United Kingdom) for measuring amplitudes of accommodation (AoA).
- Fluorescence dye (1 mg fluorescein sodium)
- Trial frame and trial lens box set
- Huvitz Auto Ref/Keratometer CRK-7000 (Huvitz, Anyang, Republic of Korea).

3.3.3 Sample size

The required sample size was 128 participants (64 smokers and 64 non-smokers). The sample size calculation was done with the help of G*Power 3.1(Faul *et al.*, 2007) using a two way paired t-test to show a medium effect size with 80% power and an alpha level of 0.05. In total, 140 participants were enrolled in this study (71 smokers, 69 non-smokers).

3.3.4 Experimental procedure

The procedure followed in the study was the same as that followed in the UK cohort, as detailed in chapter two, section 2.3.5; the only difference from the experimental technique was that in Pakistan, the MPOD device was not available and macular pigment density was not evaluated.

3.3.5 Baseline questionnaire

The baseline questionnaire was done similarly as it was conducted for the UK cohort of the study (mentioned in chapter number two).

3.3.6 Statistical analysis

A similar way to analyse the collected data was used for Pakistani cohort as used in the UK cohort (e.g. SPSS 23.0 statistical package program for Windows (SPSS Inc., Chicago, IL, USA). A Shapiro–Wilk test value $p > 0.05$ was used to check the normality of the data. According to the normality of the data, appropriate statistical tests were used such as (Mann-Whitney U test, Kruskal Wallis H test, ANOVA and ANCOVA) to find any statistically significant difference. A p–value of less than 0.05 was considered statistically significant.

3.4 Results

A total (N) 140 participants (71 smokers and 69 non-smokers) were enrolled in this study. Female participants were 36 (25.7 % of N), and male participants were 104 (74.3% of N). The average age for the male participant was 34.6 ± 9.6 years (range 18 to 50, median 34.5 years) and the average age for females was 34.7 ± 11.4 years (range 18 to 50, median 35.0 years). There was no statistically significant difference observed between male and female ages (U = 1866.5, p = .97).

All smoker participants were male. The study did not find any female smoker participant due to the geographical location of the study and cultural taboos/beliefs associated with smoking. The study will do smoking status analysis with **three subgroups, i.e. female non-smokers, male non-smokers, and male smokers.**

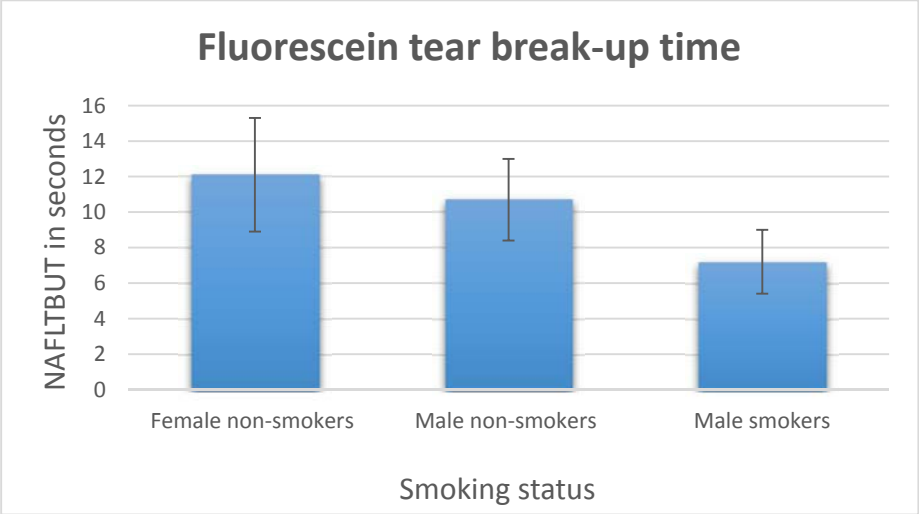
	Smoking status												
	non-smoker				smoker				female non-smoker				
	Mean	N	S.D	Median	Mean	N	S.D	Median	Mean	N	S.D	Median	P-value
Age (years)	35.3	33	10.5	36	34.3	71	9.2	34	34.7	36	11.3	35	0.9
NAFLBUT (seconds)	10.8	33	2.4	11.0	7.3	71	1.9	8.0	12.1	36	3.3	12.0	.001*
OSDI scores	11.1	33	12.0	8.33	9.24	71	7.7	8.3	18.1	36	13.6	14.1	.002*
AoA (Dioptres)	6.8	33	3.0	6.5	6.8	71	1.8	6.8	7.3	36	3.7	6.4	0.9

Table 3.1: Showing baseline of participants with three different smoking statuses

3.4.1 Smoking status versus TBUT

The mean fluorescein tear break-up time (NAFLTBUT) for female non-smokers was 12.1 ± 3.2 seconds (s) which was numerically higher than the mean NAFLTBUT for non-smokers' male participants 10.7 ± 2.3 s and mean NAFLTBUT for male smokers participants 7.2 ± 1.8 s. A Kruskal-Wallis H test was (Shapiro-Wilk test, $p < 0.05$) indicated a significant difference present in all three groups $X^2(2) = 65.0$, $p < 0.001$. The mean rank for female non-smokers group was 103.5, for male non-smokers group 92.1, and male smokers group 43.7.

The test provided strong evidence of a difference ($p < 0.05$) present between the mean ranks of at least one pair of groups. Dunn's pairwise tests were carried out for the three pairs of the mentioned group. There was strong evidence ($p = 0.001$, adjusted for Bonferroni correction) of a difference between male non-smokers group and male smokers group. The test found strong evidence ($p = 0.001$, adjusted Bonferroni correction) of a difference between female non-smokers group and male smokers group. A graphical representation of means and 95 % confidence intervals are laid out in figure 3.1 as below:



** P < 0.001*

Figure 3.1: mean fluorescein tear break-up time measured in seconds for three different smoking statuses

3.4.2 Smoking pack years and TBUT

A smoking pack-years calculation was done to convert a lifetime exposure to smoke into a numerical number (mentioned in section 2.3.9).

Spearman ranked correlations (r_s) was used to derive any correlation between TBUT and smoking years after doing normality check by Shapiro–Wilk test ($p < 0.05$). There was a strong but negative correlation found between NAFLTBUT and smoking years, rs (138) = -.61, p = 0.001.

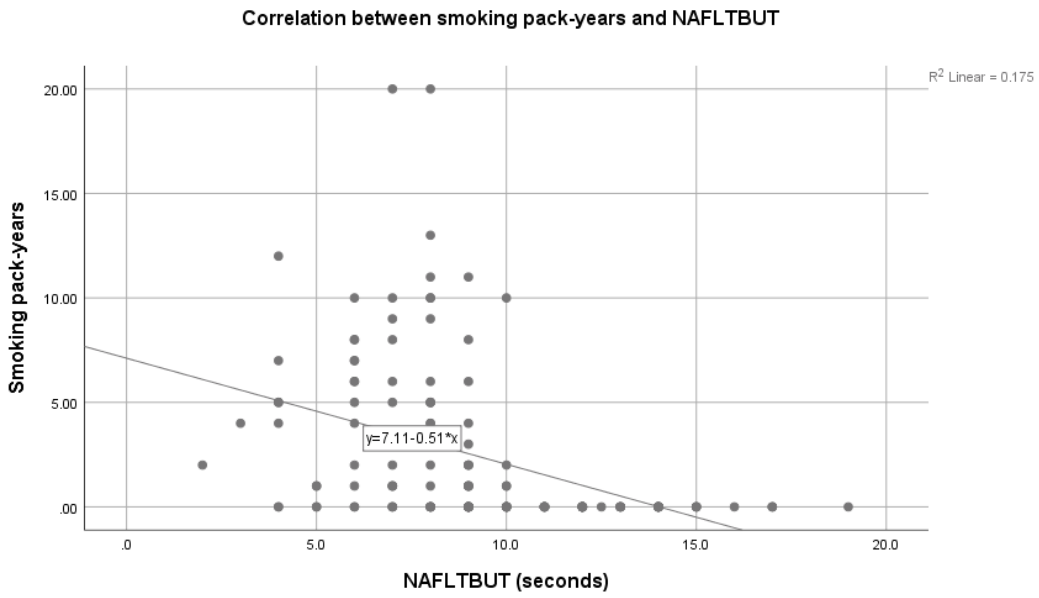


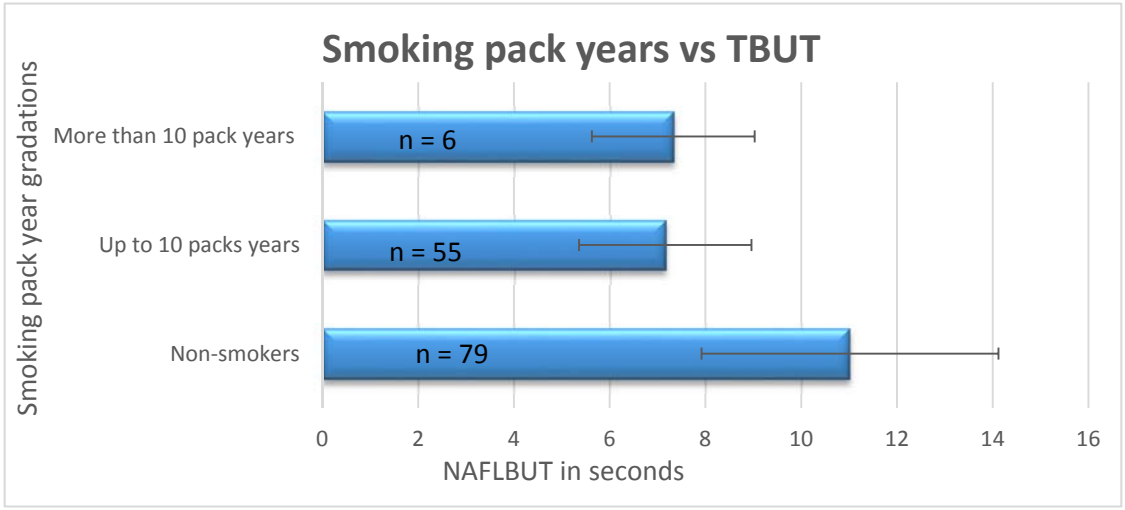
Figure 3.2: correlation between fluorescein tear break-up time measured in seconds and smoking pack-years

Participants were divided into three categories, i.e. non-smokers, smokers smoked less than ten pack years, and smokers smoked more than ten pack years in their lifetime. Table 3.2 shows the descriptive statistics for mean TBUT against three different grades of smoking pack-years as below:

	Smoking Pack years gradings												
	non-smokers				Up to 10 smoking pack years				More than 10 smoking pack years				
	Mean	N	S.D	Median	Mean	N	S.D	Median	Mean	N	S.D	Median	P-value
NAFLBUT (seconds)	11.0	79	3.1	11.0	7.1	55	1.8	7.0	7.3	6	1.7	8.0	.001*

Table 3.2: mean fluorescein tear break-up time measured in seconds for the participants according to smoking pack-years grading.

A Kruskal Wallis H test was used after checking for normality of the data from a Shapiro–Wilk test ($p < 0.05$), which showed a significant difference present in three groups, $X^2(2) = 53.2$, $p = 0.001$. The mean rank for non-smoker participants was 92.3, up to 10 smoking years 42.0, and for more than 10 smoking pack-years 43.8. The test provided strong evidence of a difference ($p < 0.05$) present between the mean ranks of at least one pair of groups. Dunn's pairwise tests were carried out for the three different grades of smoking year groups. There was strong evidence ($p = 0.001$, adjusted for Bonferroni correction) of a difference between non-smokers group and up to 10 smoking pack years. The test also found strong evidence ($p = 0.01$, adjusted Bonferroni correction) of a difference between non-smokers group and more than ten pack years group. A graphical representation of means and 95 % confidence intervals are laid out in figure 3.3 as below:



p < 0.05

Figure 3.3: mean fluorescein tear break-up time (NAFLBUT) for three different grades of smoking pack-years

3.4.3 Cigarettes smoked per day and TBUT

Spearman ranked correlations (r_s) was used to derive any correlation between TBUT and cigarettes smoked per day after doing normality check by Shapiro–Wilk test ($p < 0.05$). There was a strong but a negative correlation found between NAFLTBUT and cigarettes smoked per day, r_s (138) = -.65, p = 0.001.

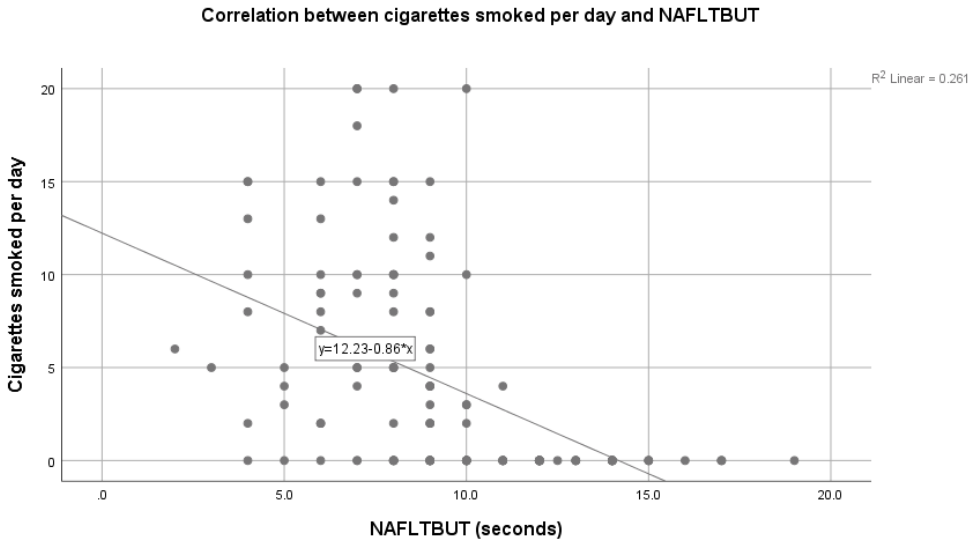


Figure 3.4: correlation between cigarettes smoked per day and fluorescein tear break-up time

Participants were divided into three categories according to their daily smoking habit. Participants who do not smoke were in the category of "non-smokers." Participants who smoked one to ten cigarettes per day were in "light smokers" category. Participants who

smoked more than ten cigarettes per day were in "heavy smokers" category. Table 3.3 shows the descriptive statistics for TBUT against three different daily smoking categories:

	Smoking intensity												
	Non-smokers				Light smokers				Heavy smokers				
	Mean	N	S.D	Median	Mean	N	S.D	Median	Mean	N	S.D	Median	P-value
NAFLBUT (seconds)	11.5	69	2.9	12.0	7.3	53	1.9	8.0	7.1	18	1.8	7.5	.001

**Non-smokers – participants who do not smoke any cigarette*
**Light-smokers – participants who smoked cigarettes ranging from one to ten in a day*
**Heavy-smokers – participants who smoked more than ten cigarettes in a day*
Table 3.3: mean fluorescein tear break-up time of participants according to their daily smoking intensity status

A Kruskal Wallis H test was used after checking for normality ($p < 0.05$) which showed a significant difference present in three groups, $X^2(2) = 63.6$, $p = 0.001$. The mean rank for non-smokers was 98.1, for light smokers 44.3, and heavy smokers 41.7. The test provided strong evidence of a difference ($p < 0.05$) present between the mean ranks of at least one pair of groups. Dunn's pairwise tests were carried out for the three pairs of the daily smoking intensity group. There was strong evidence ($p = 0.001$, adjusted for Bonferroni correction) of a difference between non-smokers group and light smokers group. The test found strong evidence ($p = 0.001$, adjusted Bonferroni correction) of a difference between non-smokers group and heavy-smokers group. A graphical representation of means and 95 % confidence intervals are laid out in figure 3.5:

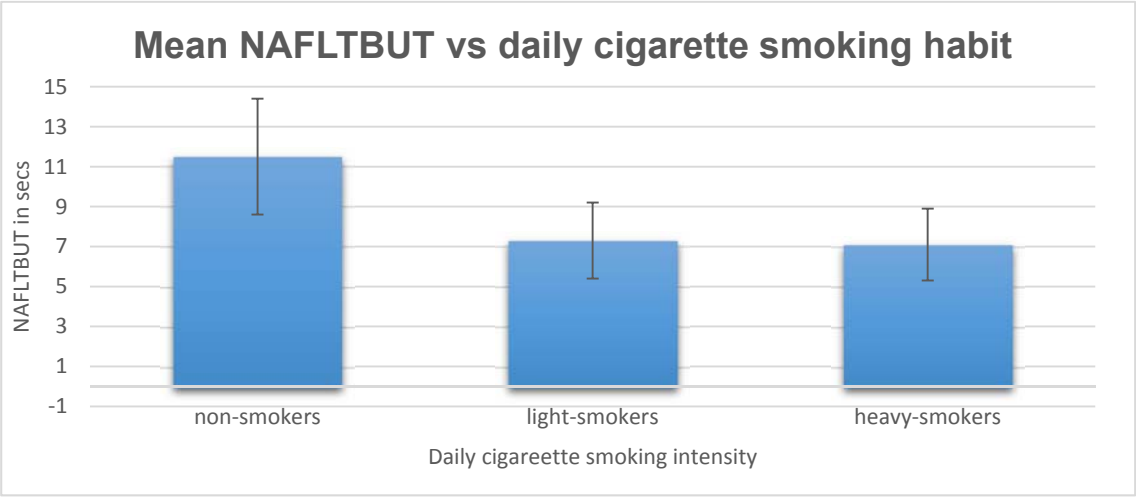


Figure 3.5: showing mean fluorescein tear break-up time according to daily cigarette smoking habits

3.4.4 Ocular surface disease index (OSDI) scores and Gender

The mean OSDI scores for men was 9.8 ± 13.6 which was numerically lower than the mean OSDI scores for women 18.1 ± 9.3. A Shapiro–Wilk test was used to test for normality on the

main dependent variable (OSDI scores) that showed a non-normal distribution ($p < 0.05$). Table 3.4 lays out the descriptive statistics of gender and OSDI scores result.

Gender	Mean	N	Std. Deviation	Median	P-value
Female	18.1	36	13.6	14.1	
Male	9.8	104	9.3	8.3	0.001*

Table 3.4: descriptive statistics for Ocular surface disease index (OSDI) scores and gender

A Mann-Whitney U test was performed to derive any statistically significant difference between OSDI scores of men and women. The mean OSDI scores rank for women (mean rank 91.3) were statistically significantly higher than for men (mean rank 63.2), $U = 1121.5$, $p = 0.001$.

3.4.5 OSDI scores and smoking status

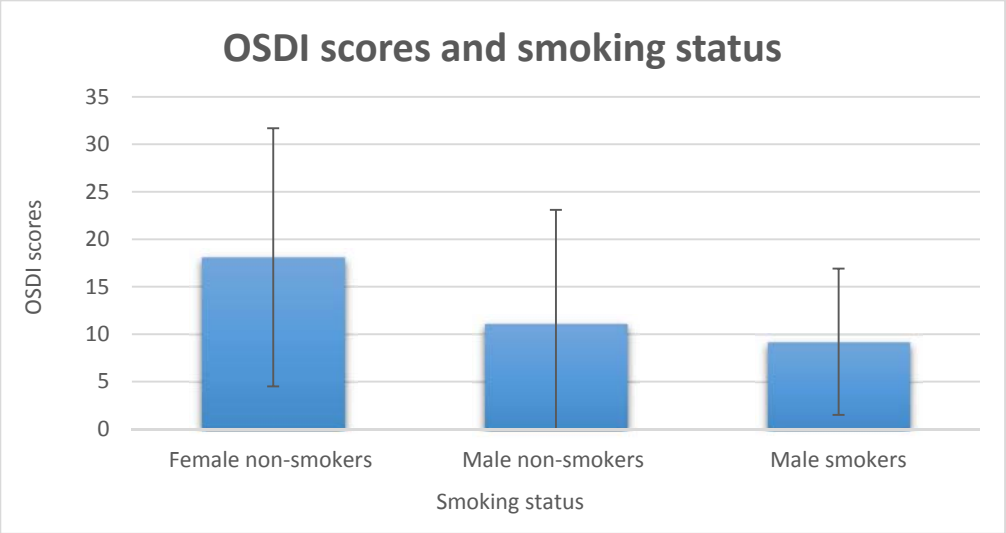
The mean OSDI score for female non-smokers was 18.1 ± 13.6; the mean OSDI score for non-smokers male was 11.1 ± 12.0, and the mean OSDI score for male smokers was 9.2 ± 7.7 as shown in table 3.5 as below:

	Smoking status											
	Non-smokers male				Male smokers				Female non-smokers			
	Mean	N	S.D	Median	Mean	N	S.D	Median	Mean	N	S.D	Median
NAFLBUT (seconds)	11.1	33	12.0	8.3	9.2	71	7.7	8.3	18.1	36	13.6	14.1
P-value	0.002*											

Table 3.5: Descriptive statistics for Ocular Surface Disease Index (OSDI) scores and Smoking status

A Kruskal Wallis H test was performed (Shapiro-Wilk test, $p < 0.05$) showed that there was a significant difference in the mean ranks of three sub-groups of smoking status, $X^2(2) = 12.9$, $p = 0.002$. The mean rank for non-smokers male participants was 64.3, the mean rank for male smokers was 62.7, and the mean rank for non-smokers female was 91.3.

The test provided strong evidence of a difference ($p < 0.05$) present between the mean ranks of at least one pair of groups. Dunn's pairwise tests were carried out for the three pairs of the mentioned group. There was strong evidence ($p = 0.002$, adjusted for Bonferroni correction) of a difference between mean ranks of OSDI scores of male smokers group and female non-smokers group. There was strong evidence ($p = 0.017$, adjusted for Bonferroni correction) of a difference between mean ranks of non-smoker male participants and female non-smokers. A graphical representation of means and 95 % confidence intervals are laid out in figure 3.6 as below:



**p >0.05*

Figure 3.6: Ocular Surface Disease Index (OSDI) scores for three different smoking status

3.4.6 Passive exposure to smoke versus TBUT

The Study divided its participants into three different groups as mentioned in section 2.3.9. The mean TBUT for all groups with three different methods are shown in table 3.6 below:

	Passive smoking											
	No exposure				Frequent exposure to passive smoking				Infrequent exposure to passive smoking			
	Mean	S.D	Median	N	Mean	S.D	Median	N	Mean	S.D	Median	N
NAFLBUT seconds	11.5	3.1	12.0	56	7.2	1.8	8.0	71	11.0	2.0	12.0	13
P-value	0.001*											

Table 3.6: mean fluorescein tear break-up time for three subgroups of passive exposure to smoking

A Kruskal-Wallis H test (Shapiro–Wilk test, $p < 0.05$) indicated a statistical significant difference in mean NAFLTBUT of three sub-groups, $X^2(2) = 63.6$, $p = 0.001$. The mean ranks for no-exposure group = 98.6, Infrequent exposure = 95.5 and frequent exposure = 43.7 respectively.

The test provided strong evidence of a difference ($p < 0.05$ for all groups) between mean ranks of at least one pair of groups, Dunn's pairwise tests were carried out for the three pairs of the mentioned groups of different passive smoking exposure. There was strong evidence of a difference ($p < 0.001$, adjusted Bonferroni correction) between frequent exposure to smoke and those who had infrequent exposure to passive smoke. There was also strong evidence ($p < 0.001$, adjusted Bonferroni correction) between frequent exposure to smoke and those who had no exposure to smoke. There was no evidence of a difference between the other pairs.

3.5 Analysis of the accommodative ability of the lens

3.5.1 AoA versus gender

The mean AoA of female participants was 7.2 ± 3.7 D, which was marginally higher than the mean AoA for male participants 6.8 ± 2.2 D. A Mann–Whitney U test was performed (Shapiro–Wilk test, $p < 0.05$). The test showed a non-significant result, $U = 1849.0$, $p = 0.9$.

3.5.2 Age versus AoA

The study found age as the strongest predictor of AoA. Spearman correlation (r_s) was used to derive any correlation between AoA and age after doing a normality check by Shapiro–Wilk test ($p < 0.05$). There was a strong but negative correlation found between AoA and age, r_s (138) = -0.76, p= 0.001. Figure 3.7 showing a strong but a negative correlation between AoA and age.

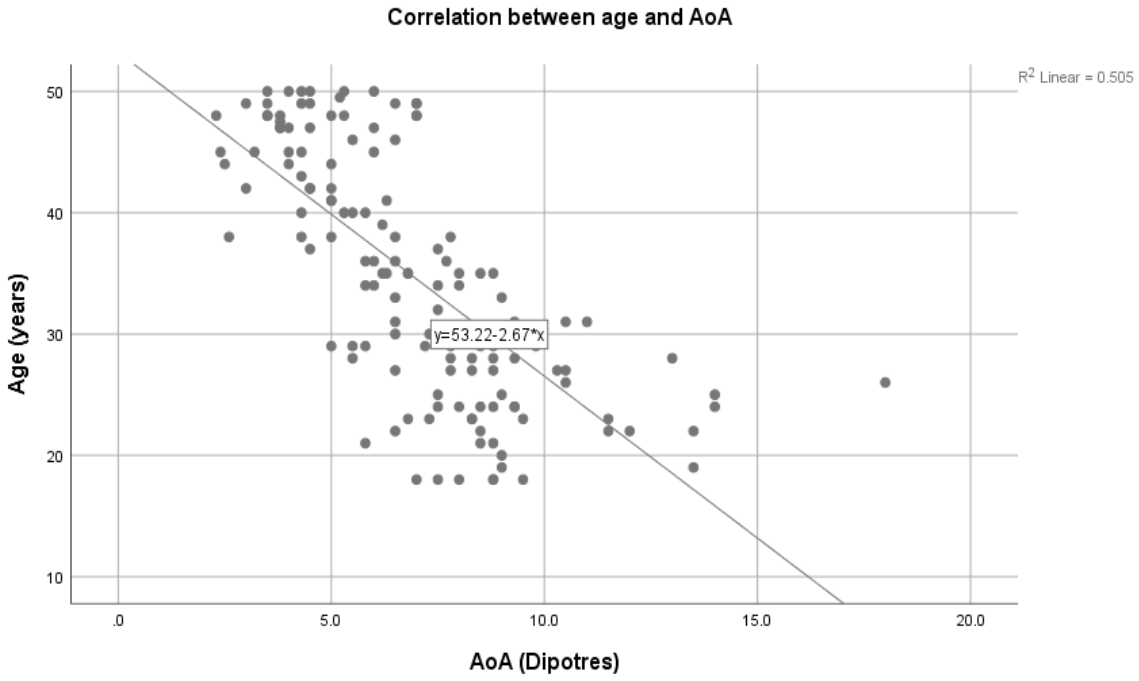


Figure 3.7: showing an inverse relationship between age and amplitude of accommodation in Dioptres

3.5.3 Analysis of amplitude of accommodation (AoA) versus smoking status

The average AoA of female non-smokers was 7.2 ± 3.7 Dioptres (D). The average AoA of male non-smokers was 6.8 ± 3.6 D, and for male smokers' participants, it was 6.8 ± 1.8 D. A Kruskal Wallis H test was used (Shapiro-Wilk test, $p < 0.05$) showed a non-significant results, $X^2(2) = 0.036$, $p = 0.9$ with the mean ranks for female non-smokers = 70.3, male non-smokers =71.6, and smokers male participants = 70.0.

3.5.4 Analysis of AoA in different age groups

Participants were divided into different age groups for a group-wise comparison of AoA as mentioned below in table 3.7:

Age groups (years)	AoA (Dioptres) in different smoking statuses											
	non-smoker				smoker				female non-smoker			
	Mean	St. D	N	Median	Mean	St. D	N	Median	Mean	St. D	N	Median
18 to 24	9.6	2.3	9	8.5	7.9	0.8	10	7.8	9.8	2.3	11	9.3
25 to 30	9.6	2.4	4	8.9	7.8	1.6	21	7.8	12.8	4.1	4	12.2
31 to 35	7.3	0.8	3	7.5	7.7	1.7	12	7.2	8.3	1.5	3	9.0
36 to 40	5.3	2.5	3	5.8	6.0	1.2	10	6.1	5.0	0.5	3	5.0
41 to 50	4.5	1.3	14	4.3	5.0	1.3	18	5.1	4.2	1.1	15	4.0

Table 3.7: showing a mean amplitude of accommodation in different age groups for different smoking status

A Shapiro–Wilk test was performed to check group wise normality. For 18-24 years of age, the data were normally distributed ($p > 0.05$). An ANOVA test was performed to check any statistical significance. The result was non-significant, F (2, 27) = 2.7, $p = 0.08$.

For 25 to 30 years age group, an ANOVA was performed (Shapiro–Wilk test, $p < 0.05$) to check any statistical significance difference in AoA. The test showed a significant result, F (2, 26) = 9.6, $p = 0.001$. Due to unequal sizes, the assumption of homogeneity of variance was violated (Levene test, $p = 0.01$). A Welch F test was conducted, and the result showed a non-significant difference, *Welch's* F (2, 4.4) = 3.3, $p = 0.13$.

Similarly, AoA was normally distributed (Shapiro–Wilk test, $p > 0.05$) among three smoking groups for 31 to 35 years age group, 36 to 40 years age group, and for 41 to 50 years of age group respectively. An ANOVA was performed for each age subgroups; there was no statistically significant difference in the mean AoA found between three subgroups of age. For 31 to 35 years age group, F (2, 15) = 0.26, $p = 0.7$. For 36 to 40 years age group, F (2, 13) = 0.8, $p = 0.48$. Finally, for 41 to 50 years age subgroup, F (2, 44) = 2.4, $p = 0.1$.

3.5.5 Analysis of Defocus curves

To find a subjective clear vision range for the study participants, defocus lenses (power ranging from +1.5 Dioptres sphere (DS) to -5.0 DS with 0.5 DS steps for increase or decrease lens power) were used. Defocus lenses and the letter presentation were randomised to wave out any memory effect as suggested by Gupta *et al.* (2008). The mean LogMAR VA of three different smoking statues is mentioned in the table number 3.8 below:

Defocus lens power (DS)	Smoking Status												
	non-smoker females				non-smoker's male				smoker's male				
	Mean VA	S.D	N	Median	Mean VA	S.D	N	Median	Mean VA	S.D	N	Median	P value
+1.5	0.69	0.22	36	0.73	0.80	0.19	33	0.80	0.82	0.18	71	0.86	0.008*
+1.0	0.41	0.18	36	0.36	0.51	0.17	33	0.50	0.46	0.14	71	0.48	0.09
+0.5	0.13	0.08	36	0.10	0.15	0.06	33	0.16	0.13	0.06	71	0.12	0.2
0.0	-0.01	0.04	36	0.00	-0.01	0.02	33	0.00	0.00	0.02	71	0.00	0.3
-0.5	0.02	0.08	36	0.00	0.02	0.07	33	0.00	0.00	0.02	71	0.00	0.09
-1,0	0.08	0.16	36	0.00	0.10	0.17	33	0.00	0.01	0.05	71	0.00	0.01*
-1.5	0.14	0.23	36	0.01	0.17	0.27	33	0.00	0.04	0.12	71	0.00	0.02*
-2.0	0.27	0.31	36	0.20	0.25	0.32	33	0.08	0.12	0.20	71	0.06	0.054
-2.5	0.40	0.34	36	0.40	0.35	0.34	33	0.30	0.23	0.27	71	0.12	0.051
-3.0	0.56	0.40	36	0.65	0.45	0.38	33	0.40	0.37	0.33	71	0.30	0.08
-3.5	0.66	0.43	36	0.91	0.59	0.41	33	0.76	0.51	0.38	71	0.42	0.17
-4.0	0.75	0.43	36	1.00	0.72	0.41	33	1.00	0.65	0.39	71	0.72	0.3
-4.5	0.81	0.43	36	1.07	0.82	0.39	33	1.00	0.74	0.38	71	0.90	0.2
-5.0	0.84	0.42	36	1.10	0.85	0.37	33	1.10	0.81	0.36	71	1.00	0.2

Table 3.8: descriptive data for LogMAR visual acuity attained from three different smoking statues.

A Kruskal–Wallis H test was used (Shapiro–Wilk test, $p < 0.05$) to determine any statistical difference of defocusing ability between female non-smokers, male non-smokers, and male smokers.

For +1.5 DS defocus lens, the test indicated a statistically significant difference in mean LogMAR VA of three sub-groups, $X^2(2) = 9.6$, $p = 0.008$. The mean ranks for female non-smokers group were 52.6, male non-smokers group = 74.7 and male smokers group = 77.6 respectively. The test provided strong evidence of a difference ($p < 0.05$ for all groups) between mean ranks of at least one pair of groups, Dunn's pairwise tests were carried out for the three pairs of the smoking statuses. There was strong evidence of a difference ($p = 0.007$, adjusted Bonferroni correction) between female non-smokers group and male smokers group, indicating that female non-smokers group had better LogMAR VA compared to male smokers group. There was no evidence of a difference between the other pairs.

There was no statistical significant difference observed in LogMAR VA attained from +1.0 DS defocus lens, $X^2(2) = 4.8$, $p = 0.09$. The mean ranks for female non-smokers group was 58.7, male non-smokers group = 79.7 and male smokers group = 72.1 respectively. There was no statistical significant difference found in LogMAR VA attained from +0.5 DS defocus lens power, $X^2(2) = 3.4$, $p = 0.2$ (mean ranks for female non-smokers =62.4, non-smokers male = 80.4, and for smokers male = 70.0). No statistical significant difference observed in LogMAR VA from 0.0 DS defocus lens power, $X^2(2) = 2.1$, $p = 0.3$ (mean ranks for female non-smokers =66.2, non-smokers male = 71.7, and for smokers male = 72.1).

Male smokers' participants had numerically better LogMAR VA attained from - 0.5 DS compared to female non-smokers and male non-smokers but that difference turned into a non-statistical significant difference after a Kruskal Wallis H test was performed to determine any statistical significance, $X^2(2) = 4.8$, $p = 0.09$. The mean ranks for female non-smokers group were 73.8, male non-smokers group = 78.1 and male smokers group = 65.2 respectively.

For - 1.0 DS defocus lens, the test indicated a statistically significant difference presented in the mean LogMAR VA of three sub-groups, $X^2(2) = 8.6$, $p = 0.01$. The mean ranks for female non-smokers group were 76.2, male non-smokers group = 81.0 and male smokers group = 62.7 respectively. The test provided strong evidence of a difference ($p < 0.05$ for all groups) between mean ranks of at least one pair of groups, Dunn's pairwise tests were carried out for the three pairs of the smoking statuses. There was strong evidence of a difference ($p = 0.023$, adjusted Bonferroni correction) between male smokers group and male non-smokers group, indicating that male smokers group had better LogMAR VA compared to non-smokers male group. There was no evidence of a difference between the other pairs.

For -1.5 DS defocus lens, there was a significant difference presented in the mean LogMAR VA of three sub-groups, $X^2(2) = 7.7$, $p = 0.02$. The mean ranks for female non-smokers group were 79.6, male non-smokers group = 78.2 and male smokers group = 62.3 respectively. The test for the mean rank difference was statistically significant, but none of the pairwise tests of mean rank difference was statistically significant after controlling for multiple testing. These results indicate one of two possibilities: either the global test was a false positive finding or the post hoc tests lack power.

In numerical expression, mean LogMAR VAs attained from -2.0 and -2.5 DS defocus lenses were better in smokers' male as compared to non-smokers male and female non-smokers. That difference was turned not be significant after a Kruskal Wallis H test was performed to determine any statistical significance. For -2.0 DS, $X^2(2) = 5.8$, $p = 0.054$ (mean ranks for female non-smokers =82.0, non-smokers male = 74.1, and for smokers male = 63.0) and for -2.5 DS, $X^2(2) = 5.9$, $p = 0.051$ (mean ranks for female non-smokers =81.4, non-smokers male = 75.7, and for smokers male = 62.5).

There was no significant difference observed in mean LogMAR VAs attained from defocus lenses ranging from -3.0 DS to -5.0 DS among different smoking groups. For -3.0 DS, $X^2(2) = 5.0$, $p = 0.08$ (mean ranks for female non-smokers = 81.5, non-smokers male = 71.7, and for smokers male = 63.2). For -3.5 DS, $X^2(2) = 3.5$, $p = 0.17$ (mean ranks for female non-smokers = 79.0, non-smokers male = 72.6, and for smokers male = 64.0). For -4.0 DS, $X^2(2) = 2.4$, $p = 0.3$ (mean ranks for female non-smokers = 77.0, non-smokers male = 73.1, and for smokers male = 65.0). For -4.5 DS, $X^2(2) = 3.2$, $p = 0.2$ (mean ranks for female non-smokers = 76.31, non-smokers male = 75.6, and for smokers male = 64.1) and for -5.0 DS, $X^2(2) = 1.7$, $p = 0.2$

(mean ranks for female non-smokers = 76.0, non-smokers male = 71.7, and for smokers male = 66.1) respectively.

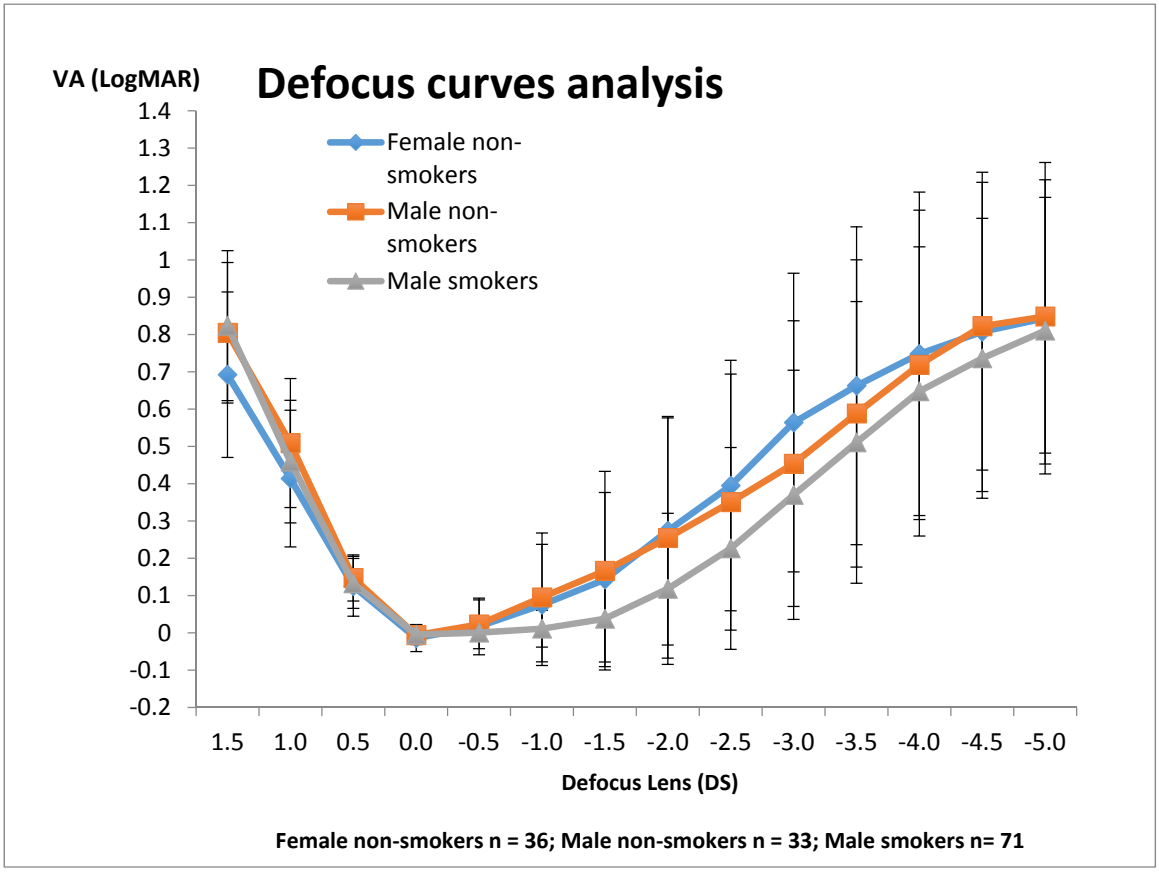


Figure 3.8: Pakistani defocus curves for three different smoking statuses

3.6 Discussion

3.6.1 Effects of smoking on tear film

According to United Nations (UN) country classification, Pakistan is a developing economic country (UN, 2019), and it is facing many financial challenges which adversely impact on the wellbeing of the society (Murtaza *et al.*, 2015). It is also evident that social and financial constraints are one of the main barriers for better healthcare and a healthy lifestyle (Shiell, 1991). Pakistan is one of the biggest consumer of cigarettes and tobacco-related products in South Asia (Masud and Oyebode, 2018). In return, Pakistan is facing a heavy burden of smoking-related diseases and Pakistan is included among the top 15 countries, which are worse effected by smoking-related ill-health (WHO, 2017).

The OSDI questionnaire is a rapid form of assessment for dry eye-related symptoms. This study found that smokers had significantly less OSDI scores compared to non-smokers. This result is in contrast with some previous studies results (Aktaş *et al.*, 2017, Agrawal *et al.*, 2018) that have mentioned that smokers had high OSDI scores compared to non-smokers. One of

the possible reason for finding smokers OSDI scores lower than non-smokers is due to the inclusion of female non-smoker participants in "non-smokers" category while there were no female smokers in the "smoker" category. It is believed that gender, race and geographical factors can affect the outcome of OSDI scores (Hua *et al.*, 2014).

The current study found that OSDI scores for women were significantly higher than men participants of data gathered in Pakistan. This result was also consistent with some previous studies (Garza-León *et al.*, 2016, Hua *et al.*, 2014, Bakkar *et al.*, 2016b). As there were no female smoker participants participated in the study, so this study just compared smoker and non-smoker men OSDI score. There was no significant difference observed in OSDI scores between non-smokers and smokers men. This result is consistent with some previous studies that found no significant difference in OSDI scores between smoking and non-smoking participants (Hua *et al.*, 2014, Yoon *et al.*, 2005a). In contrast, many studies have reported that smoker participants had higher OSDI scores compared to non-smokers (Aktaş *et al.*, 2017, Erginturk Acar *et al.*, 2017).

There are no published studies based on the Pakistani data that shows the effects of smoking on the tear film so far. However, a recent study (Abdullah *et al.*, 2017) investigated the prevalence of Dry eye syndrome (DED) in Pakistan and found smoking habit as one of the main risk factors for DED. In terms of tear break-up time (TBUT), this study results are consistent of previous studies results that showed that smokers had decreased TBUT as compared to non-smokers (Agrawal *et al.*, 2018, Masmali *et al.*, 2016, Sayin *et al.*, 2014, Thomas *et al.*, 2012). The present study found that passive exposure to smoking was inversely related to TBUT in Pakistani participants. This result was consistent of El-Shazly *et al.* (2012) findings that found decreased TBUT in children exposed to passive smoke.

3.6.2 Effects of smoking on accommodative ability

Smoking association with cataract formation is widely reported (Ye *et al.*, 2012). There is a little information available, however, on the effect of smoking on the lens prior to cataract formation. Few studies have shown an association of smoking with earlier presbyopia (Khalaj *et al.*, 2014, Parkesh Kavita, 2017). A study showed smoking association with AoA (Ide *et al.*, 2012). In addition to presbyopia and AoA, evidence (Hammond *et al.*, 1999a) suggests that smoking is associated with higher lens optical density and there was a significant dose-response relationship between smoking frequency and lens optical density. Hammond *et al.* (1999a) observed in their age matched study that smoker participants had significantly higher lens optical density compared to non-smokers (for smokers 1.63 ± 0.23 optical density, and for non-smokers 1.51 ± 0.17, $p = 0.005$). Based on previous formulae relating increase in lens optical density with age (Hammond *et al.*, 1997) that showed a linear increase of 0.01 optical

density units per year, their study concluded that smokers had almost 12 years more lens aging compared to non-smokers.

In the current study, there was no significant difference observed in the mean ages of all three smoking statuses (i.e. smoker's men, non-smoker men, and female non-smokers) in Pakistani data. There was no significant difference observed in mean AoA between different smoking statuses either as a whole group or in different age group wise analysis. This result is different from Ide *et al.* (2012) findings that showed that smokers had less AoA compared to non-smokers. Apart from Ide *et al.* (2012), there are no studies present in the literature, which have shown any relationship of smoking with AoA. One of the possible reason could be the age factor; average age for smokers and non-smokers participants of Ide et al. (2012) study was around 39.0 years (presbyopic age range) whereas, participants of the current study had average age of 34.0 (for smoker men and non-smoker men) and 35.0 (for non-smoker females) and were pre-presbyopic in age. Smoking can show its adverse effects on the crystalline lens in the form of cataract formation lately, and it is highly dose-responsive (Hammond *et al.*, 1999a). Ide et al. (2012) study did not measure smoking intensity (in terms of smoking years and cigarette smoking per day) so there could be a possibility in a difference of results.

As a next step, it would be useful to compare data collected from the UK cohort (as described in chapter 2) and the cohort gathered in Pakistan. Chapter 7 compares the UK subjects with the Pakistani subjects to investigate if dietary and environmental factors play a role when assessing the tears and AoA of the two groups. Chapter 8 takes the sub-group of British-Asians to compare those with the Pakistani subjects, as this will remove the ethnic differences.

Chapter 4

Effect of diet on the tear film, accommodative ability and on macular pigment optical density in the UK cohort participants

Chapter 2 investigated the effect of smoking on the tears, AoA and MPOD. This chapter looks at the ocular effects of diet within a UK cohort.

4.1 Introduction

Studies have shown that intake of specific nutritional elements, i.e. vitamin A (vit A), omega 3 & omega 6 fatty acids are suitable for the protection of the tear film specifically (Roncone *et al.*, 2010, Kawakita *et al.*, 2013, Galor *et al.*, 2014) and their deficiency can cause dry eye syndrome (Foulks *et al.*, 2007). There are inconsistent results about vitamin D (vit D) and its effects on the tear film. Some studies suggest it does not affect managing dry eye condition (Jee *et al.*, 2016, Jeon *et al.*, 2017). In contrast, some studies have shown a positive effect of vit D on the tear film (Demirci *et al.*, 2018, Yang *et al.*, 2018).

To date, there are no studies that have shown any effect of diet on the accommodative ability of the lens. In the literature, however, inconsistent have been seen about multivitamin supplements on cataract formation or its prevention. Many studies have shown a positive association of diet and dietary supplements with cataract prevention, such as a meta-analysis conducted by Zhang *et al.* (2015b). This study reported that dietary and supplementary intake of vitamin E and a high level of serum tocopherol may be associated with a lower risk of age-related cataract. High serum level of vitamin D is also reported to have a protective effect against age-related cataract (Jee *et al.*, 2016, Park and Choi, 2017). Similarly, a decreased risk of cataract formation was observed by Appleby *et al.* (2011) from high meat eaters to low meat eaters, fish-eating participants, vegetarians, and vegans.

Zhang *et al.* (2015a) found a similar trend, they reported that the intake of vegetables and fruits were associated with a lower risk of age-related cataract development. In contrast, other studies have contradicted a positive role of antioxidant supplementations on prevention or slowdown of cataract formation (AREDS, 2001b, Mathew *et al.*, 2012). However, it is evident from previous studies Sperduto *et al.* (1993b) that the use of multivitamin and mineral supplements can be beneficial for malnourished communities who are at risk of cataract, especially in developing countries (McCusker *et al.*, 2015).

A high-quality diet (fruits and vegetables) is usually associated with a lower risk of non-communicable diseases (WHO, 2003). Dietary intake of fruits, vegetables, and eggs is considered protective and beneficial for macular health. Many studies have shown a protective effect of a diet rich in lutein and zeaxanthin (L and Z) on macular pigment (Raman *et al.*, 2012b,

Alassane *et al.*, 2016, Estévez-Santiago *et al.*, 2016). Other studies have reported the beneficial effects of nutritional supplements mostly related to intake of L and Z (Dawczynski *et al.*, 2013, Olmedilla-Alonso *et al.*, 2018, Richer *et al.*, 2011b). There is some new evidence suggesting that omega–3 and polyunsaturated fatty acids have a beneficial effect (Merle *et al.*, 2017) on macular pigment optical density (MPOD) values as well.

4.2 Study aim

The purpose of this study was to assess the effect of diet on the tear film, accommodative ability, and MPOD in the UK cohort.

4.3 Methods

The methods detailed in chapter number two were also employed in this study. .

4.3.1 Dietary Intake and analysis software

A 24–hour dietary recall method was used in the study. Participants were asked to write down what they had eaten on the previous day. There are several methods to assess the dietary intake of an individual i.e. food diaries, food frequency questionnaire (FFQ), and 24–hour dietary recalls. The most common problem is the reliability of results because of misreporting of dietary intake/information (Hebert *et al.*, 1995). When estimating mean intakes of nutritional elements, food diaries and 24-hour recall methods have an advantage over FFQ. It has also an advantage over taking a detailed dietary history. The latter methods provide means of foods eaten and not the long-term food eating habits.

Another important reason for selecting a 24-hour diet recall method over FFQ or detailed dietary history was due to the possibility of recording an overestimation of foods eaten and possible participant related bias for reporting a more healthy diet (fruits and vegetables) than the actual foods consumed (Johansson *et al.*, 1992).

An overestimation of food can also be a problem in a 24-hour recall diary method. In this study, this was minimised by encouraging participants to quantify their food portions with specific details, i.e. instead of participants reporting they consumed '*slices of bread*' participants were taught by the researcher to write in a specific format, e.g. 2 x slices of thin brown bread. Reported food portions can also vary and for this reason, the Zimbabwe Hand Jive method (Kinshuck, 2014, Stevens *et al.*, 2015) was used to quantify foods by the principal investigator (NL). The Zimbabwe Hand Jive method uses individual's hands as a measurement tool to gauge appropriate portion sizes of carbohydrates, fats, proteins and vegetables such as one cupped hand of nuts this would equate to half a cup of nuts.

The nutritional software *A la Calc* was used to extract nutritional information from the participants' food diaries. For this study, extraction of nutritional information was customised and only desired nutritional elements, e.g. vitamin A (IU and RAE values), vitamin D (IU and mcg values), polyunsaturated trans-fatty acids (in grams), and lutein zeaxanthin (mcg) values were recorded. In terms of the accuracy and validity of results, this software used the information from three well-recognised nutritional databases. These are:

- A UK ingredient database maintained by McCance and Widdowson. It is officially approved by UK government organisations as UK nutrient bank.
- A US database in which data is provided by the US Department of Agriculture (USDA), and it is the official database in the US to use.
- A database maintained by *A la Calc* (Red Hot Rails LLP, Doncaster, UK) that is used for specific ingredients such as stabilisers, flavour enhancers. The manufacturers of those products provide this data.

These databases contain nutritional information of thousands of different food ingredients in various states, e.g. cooked, frozen, grilled, or raw. Although, this nutritional software is being updated regularly some food items, there are some discrepancies in the ingredients. For example in chicken pizza, the nutritional value was zero, probably due to no recipe saved in its system. In such instances, the principal investigator (NL) manually input the ingredients in order to obtain the dietary values of the nutritional elements. The method of 24-hour recalls was successfully employed in a previous study (Stevens *et al.*, 2015) analysing the nutritional behaviour in people with and without age-related Macular disease.

4.3.2 Dietary unit conversions

Vitamin A (IU) values were converted into vitamin A as beta–carotene in milligram values (mg), for calculation purposes whereby, 1 IU was equal to 0.6 mcg (NIH, 2016). To convert the IU values into mg values, my pharma tools calculator was used which was available to use freely online (link: https://mypharmatools.com/othertools/iu).

4.4 Results

One hundred and twenty-eight (128) participants were enrolled in this study. The intake of vitamin A (vit A), vitamin D (vit D), lutein and zeaxanthin (L/Z) and polyunsaturated trans-fatty acids (PUFA) were considered for this research study. Intake of vit A was measured in both international units (IU) and retinol activity equivalent (RAE). Vit A (RAE) readings were recorded in micrograms (mcg or µg). Intake of vit D measurement was taken in mcg. L/Z intake was measured in mcg. Intake of PUFA was measured in grams (g).

4.4.1 Dietary intake analysis versus ethnicity

Table 4.1 shows the mean, median, numbers of participants and standard deviation (S.D) of all three ethnic groups (mentioned in section 2.3.10) as below:

Dietary elements	Ethnicity												
	Asian (Indo-Pak origin)				White				Others				
	Mean	N	S.D	Median	Mean	N	S.D	Median	Mean	N	S.D	Median	P-value
Vitamin A IU intake	1720.9	63	1490.4	1258.2	2475.3	38	4333.7	1472.1	1854.4	30	1942.5	983.2	0.7
Vitamin A RAE intake (µg)	333.4	63	295.5	279.5	453.8	38	486.7	294.4	334.8	30	363.9	179.0	0.3
Vitamin D intake (µg)	2.6	63	3.99	1.1	2.5	38	2.8	1.4	2.5	30	3.51	0.9	0.5
Lutein Zeaxanthin intake (µg)	412.6	63	764.2	237.4	572.7	38	1137.3	210.7	538.3	30	1690.9	152.1	0.8
Polyunsaturated trans-fatty acids (g)	12.6	63	10.1	9.6	20.5	38	69.5	7.9	9.8	30	5.9	8.8	0.3

Table 4.1: Descriptive data on dietary analysis and ethnicity

A Kruskal Wallis H test was conducted (Shapiro-Wilk test, $p < 0.05$), which showed non-significant results.

For vitamin A (IU) intake, $X^2(2) = 0.7$, $p = 0.7$, with the mean ranks for Asian (Indo-Pak) = 65.7, White = 69.7, Others = 61.8 respectively. For vitamin A (RAE) intake, $X^2(2) = 2.1$, $p = 0.3$, with the mean ranks for Asian (Indo-Pak) = 65.5, White = 72.4, Others = 58.7 respectively.

For vitamin D (mcg) intake, $X^2(2) = 1.1$, $p = 0.5$, with the mean ranks for Asian (Indo-Pak) = 65.2, White = 71.0, Others = 61.2 respectively. For L/Z (mcg) intake, $X^2(2) = 0.4$, $p = 0.8$, with the mean ranks for Asian (Indo-Pak) = 66.1, White = 68.4, Others = 62.6 respectively. Finally, for PUFA, $X^2(2) = 2.4$, $p = 0.3$, with the mean ranks for Asian (Indo-Pak) = 71.3, White = 59.7, Others = 62.8 respectively.

4.4.2 Gender versus dietary intake

Table 4.2 describes the mean, standard deviation, and median, of dietary intake elements for male and female participants' below:

Dietary elements	Gender								P-value
	Male				Female				
	Mean	N	Standard Deviation	Median	Mean	N	Standard Deviation	Median	
Vitamin A IU intake	1946.5	91	3042.9	1149.4	2024.6	40	1773.0	1550.7	[a] 0.3
Vitamin A RAE intake (µg)	358.8	91	386.9	249.0	391.1	40	354.8	294.5	[a] 0.4
Vitamin D intake (µg)	2.8	91	3.8	1.3	1.9	40	2.8	1.2	[a] 0.1
Lutein Zeaxanthin intake (µg)	378.3	91	648.1	219.0	737.2	40	1799.6	208.7	[a] 0.3
Polyunsaturated Trans Fatty acids in (g)	12.4	91	8.8	10.2	18.6	40	68.1	6.3	[a] 0.001*

**p value significant, [a] Mann-Whitney U test*
Table 4.2: Daily average amount of dietary elements taken by male and female participants.

4.4.3 Smoking status versus dietary intake

The mean dietary intake values of vit A (IU), vit A (RAE) and PUFA were numerically higher for non-smokers than smoker participants as shown in table 4.3. A Man-Whitney U test was used (Shapiro-Wilk test, $p < 0.05$) to evaluate any significance. There were no significant differences found between mean intakes of vit A (IU and RAE) and PUFA between non-smoker and smokers. For vit A, $U = 1990.0$, $p = 0.4$, for vit A (RAE), $U = 2078.0$, $p = 0.7$ and for PUFA, $U = 2036.5$, $p = 0.6$ respectively.

In contrast, the mean intake of vit D was marginally higher in smoker participants compared to non-smoker participants. The mean intake of L/Z was numerically higher in smokers compared to non-smokers as shown in table number 4.3. A Man-Whitney U test was used (Shapiro-Wilk test, $p < 0.05$) to evaluate any significant difference. These numerically significant values for smoker participants were not significant compared to the non-smoker participants values for vit D intake, $U = 1930.0$, $p = 0.3$, and for L/Z intake $U = 1869.5$, $p = 0.2$.

Table 4.3 describes the mean, standard deviation, and median of dietary intake elements for smokers and non-smoker participants' below:

Dietary elements	Smoking status								P-value
	non-smoker				smokers				
	Mean	N	Standard Deviation	Median	Mean	N	Standard Deviation	Median	
Vitamin A IU intake	2169.0	66	3423.5	1453.6	1768.6	65	1718.6	1128.0	0.4
Vitamin A RAE intake (µg)	386.5	66	419.8	273.3	350.6	65	328.6	279.5	0.7
Vitamin D intake (µg)	2.6	66	4.1	1.1	2.6	65	3.0	1.6	0.3
Lutein Zeaxanthin intake (µg)	286.4	66	350.2	182.8	692.4	65	1552.8	246.5	0.2
Polyunsaturated Trans Fatty acids in (g)	17.9	66	53.1	9.4	10.7	65	7.6	8.7	0.6

Table 4.3: descriptive data for the average intake of dietary elements taken by smoker and non-smoker participants.

4.4.4 Correlation between dietary intake elements and TBUT

Spearman ranked correlations (r_s) was used (Shapiro–Wilk test, $p < 0.05$) to derive any correlation between different methods of TBUT with intake of dietary elements. For all three methods (i.e. NIKBUT, NITBUT, NAFLTBUT), there was no significant correlation found between vit A (IU) intakes with all three different methods of TBUT (for NIKBUT $r_s = 0.078$, $p = 0.37$; for NITBUT, $r_s = 0.015$, $p = 0.86$; for NAFLTBUT $r_s = 0.037$, $p = 0.67$).

There was no significant correlation found between vit A (RAE) intake and all three different methods of TBUT (for NIKBUT $r_s = 0.070$, $p = 0.42$; for NITBUT, $r_s = -0.023$, $p = 0.80$; for NAFLTBUT $r_s = 0.003$, $p = 0.97$).

There was no significant correlation found between vit D intake and the three different methods of TBUT. For NIKBUT, $r_s = 0.03$, $p = 0.7$, for NITBUT, $r_s = -0.46$, $p = 0.6$ and for NAFLTBUT, $r_s = -0.5$, $p = 0.5$ respectively. There was no significant correlation found between L/Z intake and the three different methods of TBUT. For NIKBUT, $r_s = -0.02$, $p = 0.7$, for NITBUT, $r_s = -0.07$, $p = 0.4$ and for NAFLTBUT, $r_s = -0.08$, $p = 0.3$ respectively.

Similarly, there was no significant correlation found between NITBUT and NAFLTBUT with PUFA intake (r_s for NITBUT = 0.091, $p = 0.32$; r_s for NAFLTBUT = 0.038, $p = 0.66$). There was however a weak significant correlation found between PUFA intake and NIKBUT ($r_s = 0.18$, $p = 0.04$).

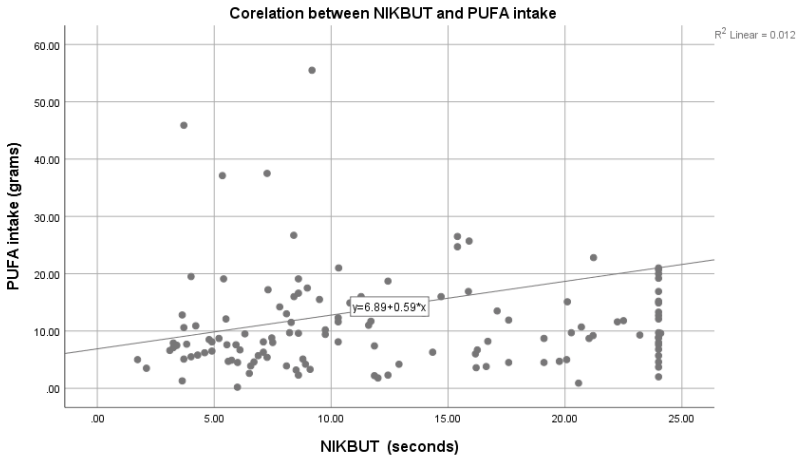


Figure 4.1: a weak correlation between poly-unsaturated trans-fatty acids intake (grams) and non-invasive Keratograph break-up time (seconds)

4.4.5 Analysis of TBUT based on PUFA intake

PUFA values were divided into four grades (i.e. one = under 10.0 g, two = 10.1 to 20.0 g, three = 20.1 to 30.0 g and four = above 30.0 g). Table 4.4 displays mean TBUT for each gradations attained by three different methods as below:

Break-up time (seconds)		PUFA gradations (grams)				
		under 10	10.1 to 20.0	20.1 to 30.0	30.10 and above	P-value
NIKBUT	Mean	11.77	13.61	16.83	9.39	0.06
	Total N	75	43	8	5	
	Standard Deviation	7.44	6.85	5.86	7.06	
	Median	8.60	11.60	15.65	7.26	
NITBUT	Mean	11.54	11.27	13.24	11.50	0.6
	Total N	75	43	8	5	
	Standard Deviation	5.55	4.49	5.03	6.28	
	Median	9.60	10.60	12.50	10.00	
NAFLTBUT	Mean	9.27	9.05	12.34	5.90	0.2
	Total N	75	43	8	5	
	Standard Deviation	5.79	4.84	8.00	1.51	
	Median	7.40	7.70	8.10	5.40	

Table 4.4: showing descriptive data of tear break-up time from three different methods measured in seconds against poly-unsaturated trans-fatty acids gradations measured in grams.

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) was used to determine any statistical difference in TBUT between different PUFA intake categories. The test showed a non-significant result for all three different TBUT methods, $X^2(3) = 7.43$, $p = 0.06$ for NIKBUT with the mean rank for grade one = 60.5, grade two = 73.1, grade three = 89.8 and grade four = 47.7.

For NITBUT, $X^2(3) = 1.52$, $p = 0.67$ with the mean rank for grade one = 58.5, grade two = 61.0, grade three = 74.5 and grade four = 58.7. For NAFLTBUT, $X^2(3) = 3.91$, $p = 0.27$ with the mean rank for grade one = 65.5, grade two = 66.8, grade three = 82.6 and grade four = 40.0.

4.4.6 Analysis of TBUT based on vit A (IU) intake

Vitamin A (IU) values were converted into vitamin A as beta–carotene in milligram values (mg) as mentioned in section 4.3.2. Table 4.5 displays the mean break-up time measured in seconds from three different measuring methods as below:

Break-up time (seconds)		Vitamin A gradations (mg)							
		under 0.50	0.51 to 1.00	1.01 to 1.50	1.51 to 2.0	2.01 to 2.50	2.51 to 3.50	above 3.50	P value
NIKBUT	Mean	12.5	11.6	15.1	9.8	14.9	17.2	11.7	0.4
	Total N	45.0	38.0	15.0	11.0	9.0	5.0	8.0	
	Standard Deviation	7.4	6.7	7.8	5.1	8.2	6.2	8.8	
	Median	9.8	9.3	16.2	9.0	15.9	20.1	7.2	
NITBUT	Mean	11.7	10.6	15.3	10.1	11.9	10.6	11.7	0.6
	Total N	45.0	38.0	15.0	11.0	9.0	5.0	8.0	
	Standard Deviation	5.3	4.1	7.6	2.9	5.4	3.6	5.7	
	Median	10.2	9.6	14.4	9.3	10.9	9.6	9.9	
NAFLBUT	Mean	8.9	8.3	13.9	7.7	8.7	10.6	8.6	0.8
	Total N	45.0	38.0	15.0	11.0	9.0	5.0	8.0	
	Standard Deviation	4.8	3.6	10.3	3.7	6.0	5.4	4.1	
	Median	7.5	7.5	7.9	6.8	7.6	9.1	8.2	

Table 4.5: showing descriptive data of tear break-up time from three different methods measured in seconds against vitamin A gradations measured in milligrams.

After converting from IU to mg, values were graded in to seven categories/grades according to the level of intake by study participants. Grade one = up to 0.50 mg, grade two = 0.51 to 1.0 mg, grade three = 1.01 to 1.50 mg, grade four = 1.51 to 2.0 mg, grade five = 2.01 to 2.5 mg, grade six = 2.51 to 3.50 mg and Grade seven = 3.51 or above.

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) was used to determine if there was any statistical difference in TBUT between different vitamin A intake categories.

The test showed non-significant results for all three different methods of TBUT. For NIKBUT, $X^2(6) = 6.1$, $p = 0.4$ with the mean ranks for grade one = 65.8, grade two = 61.0, grade three = 77.8, grade four = 53.6, grade five = 76.1, grade six = 89.4 and grade seven = 59.3 respectively.

For NITBUT, $X^2(6) = 4.1$, $p = 0.6$ with mean ranks for grade one = 62.0, grade two = 54.9, grade three = 76.9, grade four = 54.4, grade five = 64.0, grade six = 57.8 and grade seven = 59.0 respectively.

For NAFLTBUT, $X^2(6) = 2.9$, p = 0.8 with mean ranks for grade one = 65.2, grade two = 64.7, grade three = 77.2, grade four = 56.3, grade five = 60.4, grade six = 79.9 and grade seven = 66.0 respectively.

4.4.7 Analysis of TBUT based on vit A (RAE) intake

Vitamin A (RAE) values were measured in micrograms (µg) and these values were then divided into three categories (i.e. one = up to 400.0, two = 400.1 to 700.0 & three = 700.1 and above) according to participants' daily intake.

Break-up time (seconds)		Vitamin A RAE gradations			
		1.00	2.00	3.00	P-value
NIKBUT	Mean	12.10	13.27	14.58	
	Total N	94	21	16	
	Standard Deviation	7.05	7.91	7.43	0.4
	Median	9.47	10.80	14.68	
NITBUT	Mean	11.53	11.42	11.92	
	Total N	94	21	16	
	Standard Deviation	5.13	5.66	5.05	0.9
	Median	10.08	10.20	10.60	
NAFLTBUT	Mean	9.05	10.55	8.76	
	Total N	94	21	16	
	Standard Deviation	5.36	6.95	5.01	0.8
	Median	7.45	8.20	7.30	

** Grades of vitamin A (retinol activity equivalent): grade one = up to 400.0, two = 400.1 to 700.0 & three = 700.1 and above*

Table 4.6: showing descriptive data of tear break-up time from three different methods measured in seconds against vitamin A (retinol activity equivalent) gradations measured in micrograms.

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed non-significant results. For NIKBUT, $X^2(2) = 1.6$, p = 0.4, with the mean ranks for category one = 63.6, category two = 69.2 and category three = 75.9 respectively. For NITBUT, $X^2(2) = 0.2$, p= 0.9, with the mean ranks for category one = 60.7, category two = 57.6 and category three = 63.1 respectively. For NAFLTBUT, $X^2(2) = 0.4$, p = 0.8, with the mean ranks for category one = 65.5, category two = 70.7 and category three = 62.8 respectively.

4.4.8 Analysis of TBUT based on vit D intake

Vitamin D values obtained from participants' daily average diet was further divided into seven grades (i.e. grade one = up to 1.0 mcg, grade two = 1.01 to 2.0 mcg, grade three =2.01 to 3.0

mcg, grade four = 3.01 to 4.0 mcg, grade five = 4.01 to 5.0 mcg, grade six = 5.01 to 10.0 mcg and grade seven = above 10.01 mcg).

Break-up time seconds		Vitamin D gradations							
		Under 1.0	1.01 to 2.0	2.01 to 3.00	3.01 to 4.00	4.01 to 5.00	5.01 to 10.00	10.01 and above	P-value
NIKBUT	Mean	13.1	11.7	12.4	9.5	10.6	13.4	16.9	0.5
	N	57	29	12	10	6	9	8	
	Standard Deviation	7.8	7.3	6.9	4.4	7.0	4.8	8.4	
	Median	11.6	8.6	11.0	10.5	8.9	13.5	21.5	
NITBUT	Mean	11.8	11.7	10.9	10.6	8.0	10.4	14.9	0.4
	N	57	29	12	10	6	9	8	
	Standard Deviation	5.2	5.6	4.5	3.9	2.1	4.9	6.7	
	Median	11.2	9.2	10.2	11.0	7.6	9.1	14.5	
NAFLBUT	Mean	9.5	9.0	8.0	8.7	8.7	7.7	13.0	0.4
	N	57	29	12	10	6	9	8	
	Standard Deviation	5.5	5.9	3.6	4.0	9.7	2.9	7.5	
	Median	8.2	6.7	7.7	8.6	5.6	6.4	11.1	

Table 4.7: showing descriptive data of tear break-up time from three different methods measured in seconds against vitamin D gradations measured in micrograms.

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed non-significant results for all three different methods of TBUT. For NIKBUT, $X^2(6) = 4.7$, $p = 0.5$, with the mean ranks for grade one = 67.2, grade two = 60.9, grade three = 67.5, grade four = 52.7, grade five = 58.1, grade six = 75.3 and grade seven = 85.1 respectively.

For NITBUT, $X^2(6) = 5.6$, $p = 0.4$, with the mean ranks for grade one = 70.1, grade two = 61.0, grade three = 60.8, grade four = 66.1, grade five = 45.5, grade six = 59.0 and grade seven = 89.0 respectively.

For NAFLTBUT, $X^2(6) = 6.1$, $p = 0.4$, with the mean ranks for grade one = 63.1, grade two = 59.5, grade three = 59.4, grade four = 57.7, grade five = 33.1, grade six = 51.2 and grade seven = 78.4 respectively.

4.4.9 Analysis of TBUT based on L/Z intake

L/Z intake values obtained from participants' daily average diet was further divided into seven grades (one = up to 50, two = 50.1 to 100, three = 100.1 to 200, four = 200.1 to 350.0, five = 350.1 to 500.0, six = 500.1 to 1000.0 and seven = 1000.1or above) according to participants' average daily intake.

Break-up time seconds		L/Z intake gradations							
		Up to 50.0	51.0 to 100	101.0 to 200.0	201.0 to 350.0	351.0 to 500.0	501.0 to 1000.0	Above 1000.0	P-value
NIKBUT	Mean	12.9	11.1	14.3	13.3	10.9	12.9	10.7	0.7
	Total N	35	11	18	26	16	13	12	
	Standard Deviation	7.9	6.7	7.3	7.2	6.9	7.3	6.4	
	Median	9.1	10.3	14.1	13.2	8.6	10.8	9.4	
NITBUT	Mean	12.2	11.0	12.8	10.8	10.6	12.3	10.6	0.7
	Total N	35	11	18	26	16	13	12	
	Standard Deviation	5.6	4.9	5.3	4.8	5.2	5.7	4.5	
	Median	10.4	9.4	12.4	9.0	9.2	11.4	10.2	
NAFLBUT	Mean	9.4	9.5	11.5	8.3	8.7	8.8	8.4	0.6
	Total N	35	11	18	26	16	13	12	
	Standard Deviation	5.5	6.5	6.4	5.0	5.0	7.1	3.7	
	Median	7.6	6.5	11.0	6.7	7.0	7.2	8.0	

Table 4.8: showing descriptive data of tear break-up time from three different methods measured in seconds against lutein and zeaxanthin intake gradations measured in micrograms

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed non-significant results for all three different methods of TBUT. For NIKBUT, $X^2(6) = 3.3$, $p = 0.7$, with the mean ranks for grade one = 66.5, grade two = 59.1, grade three = 76.2, grade four = 68.4, grade five =57.8, grade six = 70.1 and grade seven = 56.8 respectively.

For NITBUT, $X^2(6) = 3.6$, $p = 0.7$, with the mean ranks for grade one = 64.3, grade two = 57.6, grade three = 69.8, grade four = 54.5, grade five =52.5, grade six = 65.8 and grade seven = 55.7 respectively.

For NAFLTBUT, $X^2(6) = 4.5$, $p = 0.6$, with the mean ranks for grade one = 69.2, grade two = 63.0, grade three = 80.7, grade four = 58.6, grade five =62.2, grade six = 59.5 and grade seven = 65.4 respectively.

4.4.10 Correlations between OSDI scores and dietary elements

Spearman ranked correlations (Shapiro-Wilk test, $p < 0.05$) was used to derive any correlation between dietary elements and OSDI score. There was no significant correlation found between PUFA intake and OSDI scores, r_s (129) = -.16, p = 0.06.

There was a weak but significant negative correlation found between OSDI scores and Vitamin A (mg) values, r_s (129) = -.20, p = 0.02.

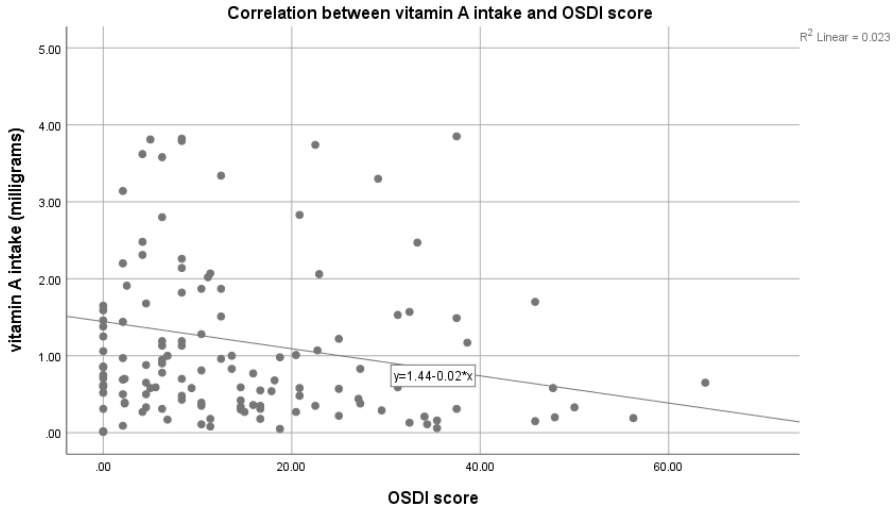


Figure 4.2: correlation between vitamin A measured in milligrams and Ocular Surface Disease Index scores

There was a significant negative correlation found between OSDI scores and Vitamin A (RAE) values, r_s (129) = -.25, p = 0.005.

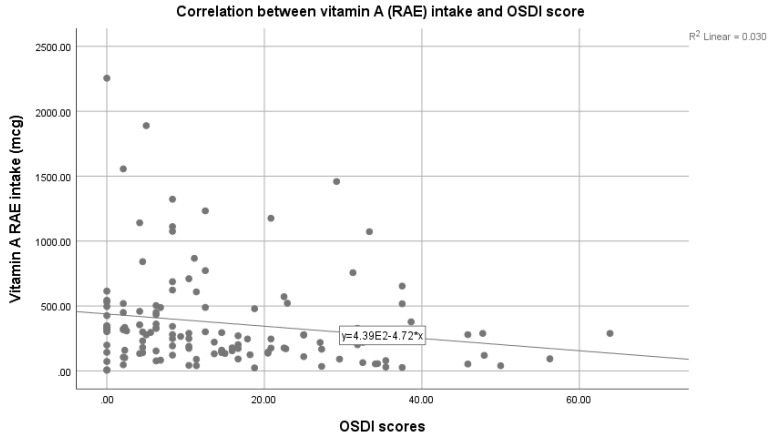


Figure 4.3: correlation between vitamin A (retinol activity equivalent) measured in micrograms and Ocular Surface Disease Index scores

There was a no significant correlation found between OSDI scores and Vitamin D (µg) values, r_s (129) = -.16, p = 0.06. There was a negative but non-significant correlation found between OSDI scores and L/Z (µg) values, r_s (129) = -.17, p = 0.054.

4.4.11 Correlations of dietary elements with AoA

Spearman ranked correlations was used to derive any correlation between AoA and with dietary elements intake (vit A (IU and RAE), vit D, L/Z and PUFA) Shapiro–Wilk test (p< 0.05

for all). There was no significant correlation found between these dietary elements and AoA. For vit A (IU), r_s (129) = -.12, p= 0.18, for vit A (RAE) r_s (129) = -.12, p = 0.17, for vit D mcg r_s (129) = -.12, p =0.17, for L/Z r_s (129) = -.11, p = 0.20 and for PUFA r_s (129) = -0.38, p = 0.6 respectively.

4.4.12 Analysis of AoA based on vit A (IU) intake

Vitamin A (IU) measurements were converted into milligrams (mg) and graded into seven grades (mentioned above in 4.3.2). Table 4.9 shows mean AoA for each category of vitamin A as below:

		Vitamin A gradations in milligrams						
		Under 0.50	0.51 to 1.00	1.01 to 1.50	1.51 to 2.0	2.01 to 2.50	2.51 to 3.50	Above 3.50
AoA (Dioptres)	Mean	10.2	9.1	8.8	11.7	10.1	10.4	8.7
	Total N	45	38	15	11	9	5	8
	Standard Deviation	2.0	2.6	1.7	3.3	2.1	1.8	1.2
	Median	10.0	9.7	8.5	10.7	10.2	10.7	9.0
P-value		0.03*						

**p value < 0.05*

Table 4.9: showing descriptive data of amplitude of accommodation measured in Dioptres against each category of vitamin A measured in milligrams

A Kruskal Wallis H test (Shapiro-Wilk test, p < 0.05) showed a significant difference of AoA among different categories of vitamin A (mg) intake, $X^2(6) = 13.5$, p= 0.036 with the mean ranks for grade one (i.e. under 0.50 mg) = 73.3, grade two = 61.2, grade three = 45.0, grade four = 88.0, grade five = 70.8, grade six =75.8, grade seven = 45.2 respectively.

The test for the mean rank difference was significant but none of the pairwise tests of mean rank difference was significant after controlling for multiple testing. These results indicate one of two possibilities: either the global test was a false positive finding or the post hoc tests lacked power due to insufficient numbers. A new power calculation suggested that the number was adequate so it would appear that the former is true.

4.4.13 Analysis of AoA based on vit A (RAE) intake

Vitamin A (RAE) values were divided into three categories according to the participants' daily intakes mentioned above. The mean AoA for each grading of Vit A (RAE) is shown in table 4.10 as below:

		Vitamin A RAE gradations		
		1.00	2.00	3.00
AoA (Dioptres)	Mean	9.7	9.9	9.8
	Total N	94	21	16
	Standard Deviation	2.2	2.6	2.8
	Median	9.7	9.5	9.8
	P-value	0.9		

** Grades of vitamin A (retinol activity equivalent): grade one = up to 400.0, two = 400.1 to 700.0 & three = 700.1 and above*

Table 4.10: Mean amplitude of accommodation measured in Dioptres across three groups of vitamin A (RAE) intake

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) was used to determine any statistical difference in AoA between different vitamin A (RAE) intake categories. The test showed a non-significant result, $X^2(2) = 0.2$, $p= 0.9$ with the mean ranks for grade one = 66.5, grade two = 66.4, and for grade three = 62.0 respectively.

4.4.14 Analysis of AoA based on vit D intake

The mean AoA for each of the gradations of vit D intake (mcg) is shown in table 4.11 as below:

		Vitamin D gradations (micrograms)						
		Under 1.0	1.01 to 2.0	2.01 to 3.00	3.01 to 4.00	4.01 to 5.00	5.01 to 10.00	10.01 and above
AoA (Dioptres)	Mean	10.0	9.3	10.4	9.8	8.5	10.4	9.6
	Total N	57	29	12	10	6	9	8
	Standard Deviation	2.6	2.4	2.6	1.8	1.2	2.3	1.3
	Median	10.0	9.3	10.5	9.4	8.0	10.8	9.4
	P-value	0.6						

Table 4.11: mean amplitude of accommodation measured in Dioptres across seven grades of vitamin D intake measured in micrograms.

After adjusting for outliers, an ANOVA test (Shapiro-Wilk test, $p > 0.05$) was performed to check if there was any significant difference of AoA between the groups which showed a non-statistical and non-significant difference (F (6,124) = 0.77, p = 0.60).

4.4.15 Analysis of AoA based on L/Z intake

L/Z intake values obtained from participants' daily average diet was divided into seven grades mentioned above in this chapter (4.4.9).

The mean AoA for each category is shown in table 4.12 as below:

		L/Z intake gradations in micrograms						
		Up to 50	51.0 to 100	101.0 to 200.0	201.0 to 350.0	351.0 to 500.0	501.0 to 1000.0	Above 1000.0
AoA (Dioptres)	Mean	10.0	10.4	10.2	9.3	10.2	9.0	9.4
	Total N	35	11	18	26	16	13	12
	Standard Deviation	2.9	2.1	2.0	2.4	1.4	1.2	3.4
	Median	9.8	10.5	10.3	9.3	10.4	9.3	8.8
P-value		0.5						

Table 4.12: Mean amplitude of accommodation measured in Dioptres across seven grades of lutein and zeaxanthin intake measured in micrograms.

An ANOVA test (Shapiro-Wilk test, $p > 0.05$) was performed to check any significant difference of AoA between the groups which showed a non-statistical and non-significant difference ($F(6,124) = 0.81$, $p = 0.56$).

4.4.16 Analysis of AoA based on PUFA intake

PUFA values were divided into four grades mentioned above in this chapter. The mean AoA for each category is mentioned in table 4.13 as below:

		PUFA gradations (grams)			
		Under 10	10.1 to 20.0	20.1 to 30.0	30.10 and above
AoA (Dioptres)	Mean	9.8	9.5	10.5	10.4
	Total N	75	43	8	5
	Standard Deviation	2.4	2.5	1.0	2.2
	Median	9.5	9.8	10.5	10.3
P-value		0.5			

Table 4.13: Mean amplitude of accommodation measured in Dioptres across four grades of polyunsaturated trans-fatty acids intake (grams)

An ANOVA test (Shapiro-Wilk test, $p > 0.05$) was performed to check any significant difference of AoA between the groups which showed a non-statistical and non-significant difference ($F(3,127) = 0.74$, $p = 0.52$).

4.4.17 Correlations of dietary elements versus MPOD scores

Pearson correlation was used to derive any correlation between the MPOD scores and the dietary elements intake (Shapiro–Wilk test, $p > 0.05$ for all).

There was no significant correlation found between dietary elements and MPOD scores. For vit A (IU) r (129) = 0.06, p =0.32, for vit A (RAE) r (129) = 0.006, p= 0.94, for vit D, r (129) = 0.095, p= 0.28, for L/Z, r (129) = 0.12, p = 0.16 and for PUFA r (129) = -0.87, p = 0.32.

4.4.18 Analysis MPOD scores versus vitamin A (IU) intake

Vitamin A (IU) values were converted in to vit A (mg) measurements were graded into seven grades (mentioned above in the 4.4.6).

		Vitamin A gradations						
		Under 0.50	0.51 to 1.00	1.01 to 1.50	1.51 to 2.0	2.01 to 2.50	2.51 to 3.50	Above 3.50
MPOD value	Mean	0.45	0.45	0.47	0.44	0.46	0.49	0.51
	Total N	45	38	15	11	9	5	8
	Standard Deviation	0.14	0.14	0.17	0.15	0.14	0.04	0.10
	Median	0.43	0.46	0.49	0.39	0.43	0.50	0.52
P-value		0.7						

Table 4.14: Mean macular pigment optical density scores across seven grades of vitamin A measured in milligrams.

A Kruskal–Wallis H (Shapiro–Wilk test, $p < 0.05$) showed a non-significant difference of MPOD scores among different categories of vitamin A (mg) intake, $X^2(6) = 3.6$, p= 0.7 with the mean ranks for grade one = 61.6, grade two = 66.5, grade three = 68.8, grade four = 60.4, grade five = 64.6, grade six = 82.6 and grade seven = 83.2.

4.4.19 Analysis of MPOD scores based on vit A (RAE) intake

The mean MPOD score for each grades of vit A (RAE) is shown in table 4.15 below:

		Vitamin A (RAE) gradations		
		1.00	2.00	3.00
MPOD value	Mean	0.46	0.46	0.44
	Total N	94	21	16
	Standard Deviation	0.13	0.17	0.12
	Median	0.44	0.45	0.43
P-value		0.9		

** Grading of vitamin A (retinol activity equivalent): grade one = up to 400.0, two = 400.1 to 700.0 & three = 700.1 and above*

Table 4.15: Mean macular pigment optical density scores across three grades of vitamin A (retinol activity equivalent) intake measured in micrograms.

A Kruskal–Wallis H test (Shapiro–Wilk test, $p < 0.05$) showed a non-significant difference of MPOD scores among different categories of vitamin A (RAE) $X^2(2) = 0.8$, p= 0.9 with the mean ranks for grade one = 66.4, grade two = 65.4, grade three = 63.4 respectively.

4.4.20 Analysis of MPOD scores based on vit D intake

The mean MPOD score for each vit D grade is shown in table 4.16 below:

		Vitamin D gradations						
		Under 1.0	1.01 to 2.0	2.01 to 3.00	3.01 to 4.00	4.01 to 5.00	5.01 to 10.00	10.01 and above
MPOD value	Mean	0.46	0.45	0.44	0.44	0.55	0.39	0.54
	Total N	57	29	12	10	6	9	8
	Standard Deviation	0.14	0.15	0.12	0.09	0.19	0.10	0.15
	Median	0.44	0.43	0.40	0.43	0.51	0.38	0.55
P-value								

Table 4.16: mean macular pigment optical density scores across seven grades of vitamin D intake measured in micrograms.

An Analysis of Variance (ANOVA) The test showed a non-significant result (F (6, 124) = 1.8, p = 0.1).

4.4.21 Analysis of MPOD scores based on L/Z intake

The mean MPOD scores for seven gradations of L/Z intake is displayed in table 4.16 as below:

		L/Z gradations						
		Up to 50	51.0 to 100.0	101.0 to 200.0	201.0 to 350.0	351.0 to 500.0	501.0 to 1000.0	Above 1000.0
MPOD value	Mean	0.47	0.43	0.41	0.43	0.49	0.52	0.47
	Total N	35	11	18	26	16	13	12
	Standard Deviation	0.15	0.12	0.15	0.11	0.13	0.16	0.10
	Median	0.43	0.43	0.41	0.43	0.48	0.51	0.46
	P-value	0.3						

Table 4.17: Mean macular pigment optical density scores across seven grades of lutein and zeaxanthin intake measured in micrograms.

An ANOVA test (Shapiro-Wilk, p >0.05) showed a non-significant result (F (6, 124) = 1.1, p = 0.3).

4.4.22 Analysis of MPOD scores based on PUFA intake

The mean MPOD values for four gradations of PUFA intake is displayed in table 4.18 as below:

		PUFA gradations			
		Under 10.0	10.1 to 20.0	20.1 to 30.0	30.10 and above
MPOD value	Mean	0.48	0.45	0.34	0.46
	Total N	75	43	8	5
	Standard Deviation	0.13	0.14	0.15	0.16
	Median	0.45	0.44	0.33	0.41
	P-value	0.1			

Table 4.18: mean macular pigment optical density scores across four grades of polyunsaturated trans-fatty acids intake measured in grams.

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a non-significant difference of MPOD scores among different categories of PUFA's intake $X^2(3) = 5.6$, $p= 0.1$ with the mean ranks for grade one = 70.0, grade two = 65.0, grade three = 36.8 and for grade four = 61.5 respectively.

4.5 Discussion

4.5.1 Effect of dietary elements on tear film

This study did not find any significant correlation between vitamin A, vitamin D, lutein, and zeaxanthin intakes with TBUT. A weak positive correlation of PUFA's with NIKBUT but not with NITBUT or with NAFLTBUT was revealed.

In terms of subjective symptoms expressed by participants in the form of OSDI scores, this study found did not find any significant correlation between PUFA intake and OSDI score although the correlation was near to significance ($p= 0.06$). These findings are consistent with Larmo *et al.* (2010) and Olenik *et al.* (2013) who also reported no significant changes in OSDI scores. In contrast, results found by Kangari *et al.* (2013) and Sheppard *et al.* (2013) showed an improvement of OSDI scores after receiving dietary supplements of omega-3 and omega-6 fatty acids.

This study found a strong negative correlation between OSDI scores and vitamin A levels. This suggests that high OSDI scores are related to low vitamin A levels. These results are consistent with the findings of Pinazo-Durán *et al.* (2013).. Pinazo-Duran *et al.* (2013) however found this effect on a dietary supplement, which contained antioxidants (including 133.3 µg of vitamin A), and omega–3 fatty acids.

This study observed a weak negative correlation of vitamin D with OSDI score. The current study results are consistent with the results of Galor *et al.* (2014) and Yang *et al.* (2018) that conveyed higher levels of vitamin D were associated with decreased DE syndrome symptoms. Another study (Demirci *et al.*, 2018) also noted a significant difference in mean OSDI scores between vitamin D deficient participants and healthy participants.

It is believed that vitamin A is an essential element in maintaining epithelial cell health and it can contribute to the evaporative form of dry eye (Foulks *et al.*, 2007). The current study did not find any association between vitamin A with TBUT either in the beta–carotene form or in the form of retinol activity equivalent (RAE).

There are currently very few studies in the literature, which have shown any effect of vitamin A on the tear film or dry eye. Patel *et al.* (1993) found that dietary supplement of multivitamins (A, B1, B2, B6, E) and trace elements (calcium, iron and magnesium) had a protective role in tears stability when compared with a control group. Similarly, Khurana *et al.* (1991) found low vitamin A dietary intake in patients suffering from DE while assessing the epidemiological aspect of dry eye by Blades *et al.* (2001) reported that oral antioxidant supplements (including vitamin A in the form of β-Carotene) had a beneficial effect on tear film stability and in increasing goblet cell density.

This present study did not find any significant association between vitamin D levels and TBUT. These findings correlate with the results reported by Galor *et al.* (2014), who also found no significant association between vitamin D intake and TBUT. In contrast, some studies had shown a positive effect of vitamin D on TBUT (Kurtul *et al.*, 2015a, Yildirim *et al.*, 2016, Yang *et al.*, 2018, Demirci *et al.*, 2018). However, these studies focussed on participants that were either vitamin D deficient or not (e.g. Kurtul et al. 2015, Yildirim et al. 2016, and Demirici et al. 2018). One study provided vitamin D supplementation to participants (Yang et al. 2018).

This study did not find any correlation or association between L/Z intake and TBUT. In the literature, the effects of L/Z intake has been associated with cataract formation or age-related macular degeneration (Hobbs and Bernstein, 2014).

Many randomised controlled trials (RCT) studies have found a beneficial effect of omega 3 and omega 6 (Creuzot *et al.*, 2006, Brignole-Baudouin *et al.*, 2011, Kangari *et al.*, 2013, Pinazo-Duran *et al.*, 2013, Bhargava *et al.*, 2016) on DE syndrome. in particular it has been reported omega-3 and omega-6 fatty acids are capable of reducing conjunctival epithelium expression of the inflammatory marker human leukocyte antigen-DR (HLA-DR) Brignole-Baudouin *et al.* (2011) Pinazo-Duran *et al.* (2013). Other researchers Kangari *et al.* (2013) and Wojtowicz *et al.* (2011) concluded that dietary supplements of omega-3 fatty acids are beneficial in terms of increase in tears production and tears volume. In comparison , a cross-sectional study done by Galor *et al.* (2014) did not find any positive effect of omega-3 fatty

acid on DE symptoms/TBUT. This current study results are consistent with Galor *et al.* (2014) no significant association between PUFA intakes with NIBUT and NAFLTBUT was found. The current study, however, observed a weak, positive correlation between PUFA intake and NIKBUT.

The difference in observations found by RCT studies and this study may be because of the differences in study design. RCT designs are more scientifically proven to observe associations and the RCT study design allows the observer to compare the baseline results with the endpoint results. This difference may have also occurred because all the above-mentioned RCT studies made a comparison based on dietary supplements given to individuals for a limited time. In contrast, this study made a comparison of the actual nutritional intake of participants similar to (Galor *et al.* 2014). This study was also unable to divide PUFA intake into omega 3 and omega-6 essential fatty acids, which may have an impact on the results.

4.5.2 Effect of dietary intake on accommodative ability

There is no clear consensus about the effects of dietary/multivitamin intake on cataract progression or prevention. Studies have shown that dietary supplements, in particular, L/Z, vitamin E and B have a protective role against age-related cataract progression (Christen *et al.*, 2008, Christen *et al.*, 2014, Glaser *et al.*, 2015a). High blood concentration of L/Z is associated with decreased risk of nuclear cataract (Liu *et al.*, 2014). High levels of vitamin D is also associated with a lower risk of posterior sub-capsular cataract (Brown and Akaichi, 2015). However many studies have not confirmed any protective effect of dietary supplements or multimineral on the cataract progression (AREDS, 2001b, Chew *et al.*, 2013, Christen *et al.*, 2016).

To date no studies have been conducted on finding the effect of dietary elements on AoA. This study did not find any association or correlation of vitamin A, vitamin D, lutein & zeaxanthin and polyunsaturated trans-fatty acids with AoA. An explanation for this could be respondent bias, participants were asked to self-report the dietary intake; this may have influenced the results. Another possible factor could be the age of participants; almost 85 per cent of the participants were less than 30's the year of age. There is a possibility that diet-related ocular allostatic load is difficult to assess in those under 40 and the effects may be more prominent in later years of life (Crimmins *et al.*, 2003).

4.5.3 Effect of dietary elements on MPOD

Studies have reported a positive or protective effect of dietary supplements on MPOD (Merle *et al.*, 2017, Kelly *et al.*, 2014). The effects of L/Z supplementation on MPOD is widely investigated, and studies have reported its beneficial results (Dawczynski *et al.*, 2013,

Trieschmann *et al.*, 2007, Dietzel *et al.*, 2011, Richer *et al.*, 2011b, Hammond *et al.*, 2014, Huang *et al.*, 2015).

Dietary intake of β-carotene and L/Z intake is usually associated as beneficial for MPOD values. There are studies, which have shown some positive effect of eating a diet enriched with L/Z on MPOD values (Abell *et al.*, 2014, Curran-Celentano *et al.*, 2001, Raman *et al.*, 2012a, Estévez-Santiago *et al.*, 2016). The current study did not find any significant association of dietary intake of L/Z, PUFA, and vit A and D with MPOD scores.

In contrast, there are few studies, which have observed no significant association of nutritional supplements with MPOD scores (Obana *et al.*, 2015, Sasamoto *et al.*, 2011, Korobelnik *et al.*, 2017). An explanation for the non-significant association found in this current study could be due to the inclusion of younger age participants and the fact that at their current ages, allostatic load produced by the cumulative effects of different lifestyles is not strong enough to create any significant changes in their macular pigments. Another explanation is that at a young age the macular is already saturated with retinal carotenoids and reached a plateau. Even though plasma concentration of retinal carotenoids, PUFA, or beta-carotenes could increase or decrease with dietary intake variations but the macular concentration of these nutritional elements remains the same. This condition was observed in "Limpia Study" conducted by Merle *et al.* (2017) where density of macular pigment showed an initial increase with an intake of diet formulation of L/Z and omega 3 in first three months of the trial and then reached a plateau at six months. Later in the trial, the values remained stabled for remaining months of the 12-month trial.

Chapter 3 introduced a study cohort from Pakistan. It would be interesting to investigate the ocular effects of diet from that group. The next chapter looks at this.

Chapter 5

Effect of diet on the tear film and amplitude of accommodation in a Pakistani cohort

Chapter 4 investigated the ocular effects of diet of the UK cohort. The diet of subjects in Pakistan may have very different features to the diet consumed in the UK. This chapter looks at the ocular effects of diet in Pakistan.

5.1 Introduction

The benefits of dietary elements such as vitamin A, vitamin D, lutein, zeaxanthin, and omega 3 and omega 6 fatty acids on eye health have been well documented in the literature (Blades *et al.*, 2001, Huang *et al.*, 2015, Bhargava *et al.*, 2016, Demirci *et al.*, 2018). These studies however were mainly carried out in developed countries with the dietary effects on ocular health for underdeveloped countries being under reported.

With respect to dietary effect on ocular health, few studies have been carried on the Pakistani population that reported the effects of diet on patients with age-related macular degeneration (Nadeem *et al.*, 2003, Qureshi, 2018). Malnutrition, however, is reported widely in the Pakistani population, particularly in children and in females (Khan *et al.*, 2012, Rifat-uz-Zaman and Ali, 2013, Bhutta *et al.*, 2017, Naseer *et al.*, 2018). Despite the prevalence of malnutrition to date, no studies have been carried out in Pakistan that have investigated the associations between diet and tear film, and diet and amplitude of accommodation.

5.2 Study aim

The purpose of this study was to assess the effects of diet on tear film and the accommodative ability of Pakistani participants.

5.3 Methods

The selection criteria, study instruments, and the experimental procedure for this investigation were similar to chapter 3. One hundred and forty participants were enrolled in this study. Of the 140 participants enrolled, three were unable to provide a detailed 24 hours food dairy recall and were therefore excluded. In all, 137 participant's diet diaries were analysed. The sample size of the study was based on the smoking status of the participants and was independent of the dietary status.

5.3.1 Dietary Intake and analysis software

The 24 hours of food recall diaries were used to collect the data, which was similar to data collected for UK cohort participants (chapter 4). Dietary elements intake of vitamin A (Vit A), vitamin D (Vit D), Lutein Zeaxanthin (L/Z), and polyunsaturated trans-fatty acids (PUFA) were measured from the self-reported 24 hours food recall diaries. Information on the dietary intake

measurements and the nutritional analysis software is detailed in chapter four (see section number 4.2.2).

5.4 Results

5.4.1 Gender versus Dietary elements intake

The mean dietary elements intake for females was numerically lower than the mean dietary intake of male participants, as shown below in table 5.1:

Dietary elements intake		Gender		
		Female	Male	P-value
Vitamin A (international units)	Mean	1453.4	2223.1	0.04*
	Total N	36	104	
	Standard Deviation	1477.8	2684.0	
	Median	937.8	1249.3	
Vitamin A RAE (micrograms)	Mean	386.3	613.0	0.02*
	Total N	36	104	
	Standard Deviation	440.4	762.1	
	Median	224.7	341.2	
Vitamin D (micrograms)	Mean	1.0	1.3	0.3
	Total N	36	104	
	Standard Deviation	0.9	1.1	
	Median	0.9	1.0	
Lutein and zeaxanthin (micrograms)	Mean	196.4	322.8	0.2
	Total N	36	104	
	Standard Deviation	372.8	1133.6	
	Median	49.7	186.8	
Polyunsaturated trans-fatty acids (grams)	Mean	7.07	11.58	0.001*
	Total N	36	104	
	Standard Deviation	4.52	5.85	
	Median	6.50	11.20	

**p value < 0.05*

Table 5.1: showing descriptive data on the average intake of dietary elements for women and men of Pakistani cohort

A Mann–Whitney U test (Shapiro-Wilk test, $p < 0.05$) showed a statistically significant difference of dietary elements intake of vitamin A (measured in IU) between men (median = 1249.3) and women (median =937.8), $U = 1348.0$, $p = 0.04$. Intake of the dietary element Vit A (RAE) was significantly different in females (median = 224.6) compared to the males (median = 341.2), $U = 1288.0$, $p = 0.02$.

Similarly, the test found a significant difference in intake of PUFA between males (median = 11.2) and females (median = 6.5), $U = 948.0$, $p = 0.001$. These results indicated that women had a lower dietary intake of Vit A (both IU and RAE) and polyunsaturated trans-fatty acids compared to the male participants. There was no statistical significant difference observed in

the dietary intake of Vit D, *U* = 1558.0, p = 0.3, and in L/Z intake, *U* = 1501.0, p = 0.2 respectively.

5.4.2 Smoking status versus dietary intake

The mean dietary intake of female non-smoker participants was numerically lower than a male non-smoker and male smoker participants, as shown below in table 5.2:

Dietary elements intake		Smoking status			
		Non-smoker	Smoker	Female non-smoker	P-value
Vitamin A (International units)	Mean	1842.2	2402.7	1453.4	0.1
	Total N	33	71	36	
	Standard Deviation	1798.1	3007.9	1477.8	
	Median	1450.0	1243.2	937.8	
Vitamin A RAE (micrograms)	Mean	535.1	649.7	386.3	0.07
	Total N	33	71	36	
	Standard Deviation	540.7	847.8	440.4	
	Median	377.1	330.4	224.7	
Vitamin D (micrograms)	Mean	1.3	1.3	1.0	0.5
	Total N	33	71	36	
	Standard Deviation	1.0	1.1	0.9	
	Median	1.0	1.0	0.9	
Lutein and zeaxanthin (micrograms)	Mean	191.7	384.6	196.4	0.4
	Total N	33	71	36	
	Standard Deviation	191.0	1367.7	372.8	
	Median	205.6	158.3	49.7	
Polyunsaturated trans-fatty acids (grams)	Mean	11.43	11.65	7.07	0.001*
	Total N	33	71	36	
	Standard Deviation	6.35	5.64	4.52	
	Median	9.30	11.35	6.50	

**p value < 0.05*

Table 5.2: shows descriptive data on the average intake of dietary elements with respect to the smoking status of the Pakistani cohort

A Kruskal–Wallis H test (Shapiro-Wilk test, p < 0.05) showed no significant difference observed in mean intake of Vit A (IU) dietary element between smokers (mean rank = 74.2), non-smokers male (mean rank = 70.0) and in non-smoker females (mean rank = 57. 1), $X^2(2)$ = 4.3, p = 0.1. There was no statistical significant difference observed in mean intake of Vit A (RAE) dietary element between male smokers (mean rank = 74.0), non-smokers males (mean rank = 72.4) and in non-smoker females (mean rank = 55. 4), $X^2(2)$ = 5.3, p = 0.07 in Pakistani cohort.

In terms of Vit D (mcg) intake, the test did not find any significant difference between smoker males (mean rank = 70.0), non-smoker males (mean rank = 72.7) and non-smoker female participants (mean rank = 63.3), $X^2(2) = 1.0$, $p = 0.5$. Similarly, there was no significant difference observed in mean intake of L/Z (mcg) between male smokers (mean rank = 71.0), male non-smokers (mean rank = 72.5) and non-smoker female participants (mean rank = 61.6), $X^2(2) = 1.6$, $p = 0.4$.

There was a significant difference observed in the mean intake of PUFA intake between smoker males (mean rank = 78.0), non-smoker males (mean rank = 74.2) and female non-smokers (mean rank = 45.4), $X^2(2) = 16.2$, $p = 0.001$. As, the test provided strong evidence of a difference ($p < 0.05$ for all groups) between mean ranks of at least one pair of groups, Dunn's pairwise test were carried out for the three pairs of the above-mentioned smoking status groups.

A significant difference was found ($p = 0.009$, adjusted Bonferroni correction) between non-smoker females (median PUFA intake = 6.5) and non-smokers male (median PUFA intake = 9.3). A significant difference was found ($p = 0.001$, adjusted Bonferroni correction) between non-smoker females (median PUFA intake = 6.5) and male smoker participants (median PUFA intake = 11.3) respectively.

5.4.3 Dietary element correlations with NAFLTBUT

Spearman ranked correlation (r_s) was used to derive any correlation between NAFLTBUT and intake of dietary elements after doing a normality check by Shapiro–Wilk test ($p < 0.05$). There was a significant weak negative correlation found between intake of Vit A (IU) and NAFLTBUT, $r_s = - 0.2$, $p = 0.02$. There was a significant weak negative correlation found between Vit A (RAE) and NAFLTBUT, $r_s = - 0.2$, $p = 0.02$.There was no significant correlation found between Vit D (mcg) and NAFLTBUT, $r_s = 0.056$, $p = 0.5$. Similarly, there was a non-significant correlation found between intake of L/Z and NAFLTBUT ($r_s = 0.04$, $p = 0.6$) and between PUFA and NAFLTBUT ($r_s = - 0.07$, $p = 0.4$).

5.4.4 Analysis of NAFLTBUT versus dietary intake of vitamin A (IU)

Vit A (IU) values were converted into vitamin A as beta-carotene values in milligram (mg) for ease to use, as mentioned earlier on in chapter number four. After converting from IU to mg, values were then graded into seven grades. Grade one = up to 0.50 mg, grade two = 0.51 to 1.0 mg, grade three = 1.10 to 1.50 mg, grade four = 1.51 to 2.0 mg, grade five = 2.51 to 3.0

mg, grade six = 3.10 to 3.50 mg and grade seven = 3.51 or above. The mean, standard deviation, and median of all seven categories are mentioned in table 5.3 as below:

		Vitamin A gradations (mg)						
		Up to 0.50	0.51 to 1.0	1.01 to 1.50	1.51 to 2.0	2.01 to 2.50	2.51 to 3.50	Above 3.51
NAFLTBUT	Mean	10.5	8.9	8.1	9.6	7.7	9.2	8.6
	Total N	44	45	15	10	7	9	7
	Standard Deviation	3.6	3.3	2.9	1.6	2.8	3.2	2.0
	Median	10.5	8.0	8.0	9.5	7.0	9.0	8.0
	P-value	0.08						

Table 5.3: showing a descriptive data of fluorescein tear break-up time against seven gradations of vitamin A (as beta-carotene) measured in milligrams.

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a non-statistically significant result. $X^2(6) = 11.1$, $p = 0.08$ with mean ranks for grade one = 82.8, grade two = 63.3, grade three = 54.0, grade four = 78.0, grade five = 49.2, grade six = 66.5 and grade seven = 60.6 respectively.

5.4.5 Analysis of NAFLTBUT versus dietary intake of Vit A (RAE)

Vitamin A (RAE) values were measured in micrograms (µg). It was further divided into three grades according to the participants' daily intake, i.e. grade one = up to 400.0 µg, grade two = 400.1 to 700.0 µg and grade three = above 700.1 µg. The mean, standard deviation, and median of all three categories are displayed in a table 5.4 as shown below:

		vitamin A (RAE) gradations		
		1.00	2.00	3.00
NAFLTBUT	Mean	9.7	8.5	8.9
	Total N	84	19	34
	Standard Deviation	3.4	3.2	2.6
	Median	9.0	8.0	9.0
	P-value	0.2		

**Grade one=up to 400.0µg, grade two=400.1 to 700.0µg and grade three=above 700.1µg*
Table 5.4: showing a descriptive data of fluorescein tear break-up time against three grades of vitamin A (retinol activity equivalent) measured in micrograms.

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a non-statistically significant result. $X^2(2) = 2.8$, $p = 0.2$, with mean rank for grade one = 73.0, grade two = 57.2, and for grade three = 65.5 respectively.

5.4.6 Analysis of NAFLTBUT versus dietary intake of Vit D

Vit D values from the participants' diet were measured in microgram (µg / mcg) level. Values obtained from participants' daily average diet was further divided into four grades (i.e. grade one = up to 1.0 µg, grade two = 1.1 to 2.0 µg, grade three =2.1 to 3.0 µg, grade four = above 3.1 µg). These grades were different from the grades used for the UK cohort (as mentioned in chapter four). This is because; none of the Pakistani participants had more than 6.0 µg intake of Vit D. The mean, standard deviation, and median of all seven categories are displayed in a table 5.5 as shown below:

		Vitamin D gradations			
		1.00	2.00	3.00	4.00
NAFLTBUT	Mean	9.2	9.4	9.4	9.9
	Total N	73	37	20	7
	Standard Deviation	3.0	3.7	3.3	3.0
	Median	9.0	9.0	9.5	9.0
	P-value	0.9			

**Grades of vitamin D: one=up to 1.0µg, two=1.1 to 2.0µg, three=2.1 to 3.0µg, four=above 3.1µg*

Table 5.5: showing a descriptive data of fluorescein tear break-up time against four grades of vitamin D measured in micrograms.

A Kruskal–Wallis H test showed (Shapiro-Wilk test, $p < 0.05$) a non-statistically significant result. $X^2(3) = 0.5$, $p = 0.9$, with mean rank for grade 1 = 67.0, grade 2 = 71.2, grade 3 = 70.4, and for grade 4 = 75.3 respectively.

5.4.7 Analysis of NAFLTBUT versus dietary intake of L/Z

L/Z intake value was measured in micrograms. Values obtained from participants' daily average diet was further divided into seven grades (one = up to 50 µg, two = 50.1 to 100 µg, three = 100.1 to 200 µg, four = 200.1 to 350.0 µg, five = 350.1 to 500.0 µg, six = 500.1 to 1000.0 µg and, seven = 1000.1 µg) according to participants' average daily intake.

The mean, standard deviation, and median of all seven grades are displayed in a table 5.6 as below:

		L/Z intake gradations						
		Up to 50.0	50.1 to 100.0	100.1 to 200.0	200.1 to 350.0	350.1 to 500.0	500.1 to 1000.0	1000.1 and above
NAFLTBUT	Mean	9.4	8.8	10.3	8.8	9.3	10.3	9.8
	Total N	58	9	7	38	10	10	5
	Standard Deviation	3.4	5.0	2.7	2.8	2.4	3.4	2.0
	Median	9.0	6.0	9.0	9.0	9.0	9.0	10.0
	P-value	0.8						

Table 5.6: showing a descriptive data of fluorescein tear break-up time against seven gradations of lutein and zeaxanthin intake measured in micrograms.

A Kruskal–Wallis H test (Shapiro-Wilk test, p < 0.05) showed a non-statistically significant result, $X^2(6) = 2.8$, p = 0.8 with mean ranks for grade one = 69.8, grade two = 61.2, grade three = 82.1, grade four = 63.1, grade five = 69.6, grade six = 78.3, and grade seven = 79.0 respectively.

5.4.8 Analysis of NAFLTBUT versus dietary intake of PUFA

PUFA values obtained in grams (g) were further divided into three grades (i.e. *) according to participants daily intake. This grading was different to UK cohort grades. The reason was limited numbers of participants who had more than 30.0 grams of PUFA intake. The mean, standard deviation, and median of all seven categories are displayed in a table 5.7 as below:

		PUFA gradations		
		1.00	2.00	3.00
NAFLTBUT	Mean	9.6	9.0	8.9
	Total N	70	59	8
	Standard Deviation	3.6	2.8	2.2
	Median	9.0	9.0	9.5
	P-value	0.6		

** Grade one=under 10.0 g, grade two=10.1 to 20.0 g, grade three=above 20.1 g*
Table 5.7: showing a descriptive data of fluorescein tear break-up time against three different grades of polyunsaturated trans-fatty acid intake measured in grams.

A Kruskal–Wallis H test showed (Shapiro-Wilk test, p < 0.05) a non-statistically significant result, $X^2(2) = 0.9$, p = 0.6 with mean ranks for grade one = 72.1, grade two = 65.5, grade three = 67.4 respectively.

5.4.9 Correlations between Ocular Surface Disease Index (OSDI) scores and dietary elements

Spearman ranked correlations (r_s) was used (Shapiro-Wilk test, p < 0.05) to derive any correlation between dietary elements and OSDI score. There was a non-significant correlation found between OSDI scores and Vitamin A values, r_s (138) = -0.09, p = 0.3. There was no significant correlation found between OSDI scores and Vitamin A (RAE) values, r_s (138) = -0.08, p = 0.3.

There was a weak negative but significant correlation found between OSDI scores and Vitamin D (µg) values, r_s (138) = - 0.17, p = 0.048.

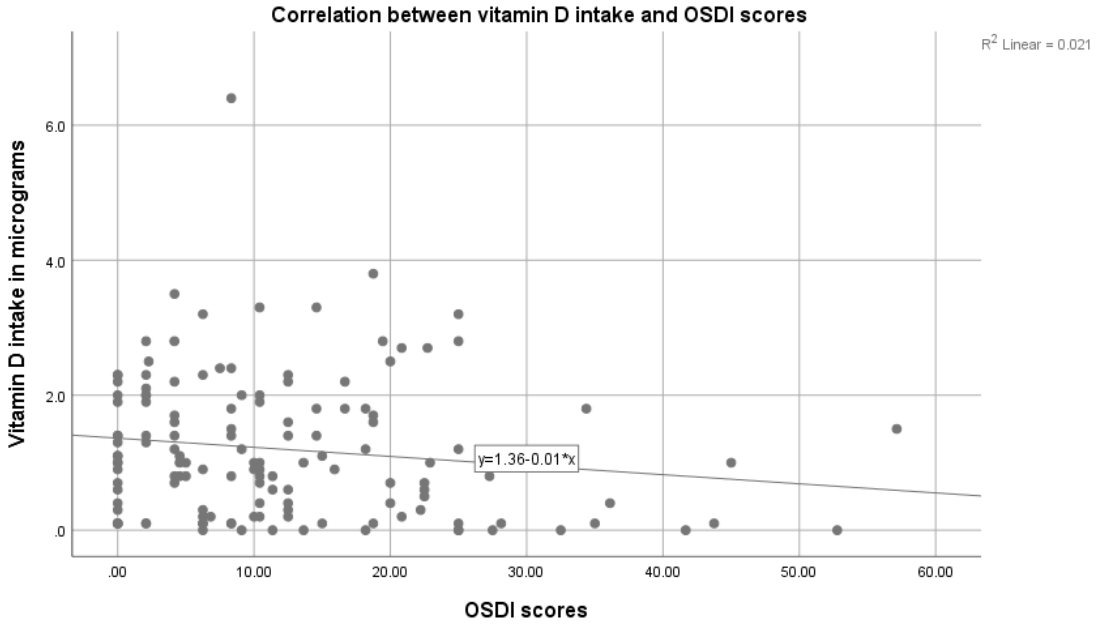


Figure 5.1: showing the correlation between Ocular Surface Disease Index scores and vitamin D intake in micrograms

There was a negative but non-significant correlation found between OSDI scores and L/Z intake values, r_s (138) = - 0.14, p = 0.09. There was a significant moderate negative correlation found between OSDI scores and PUFA values, r_s (138) = - 0.3, p = 0.001.

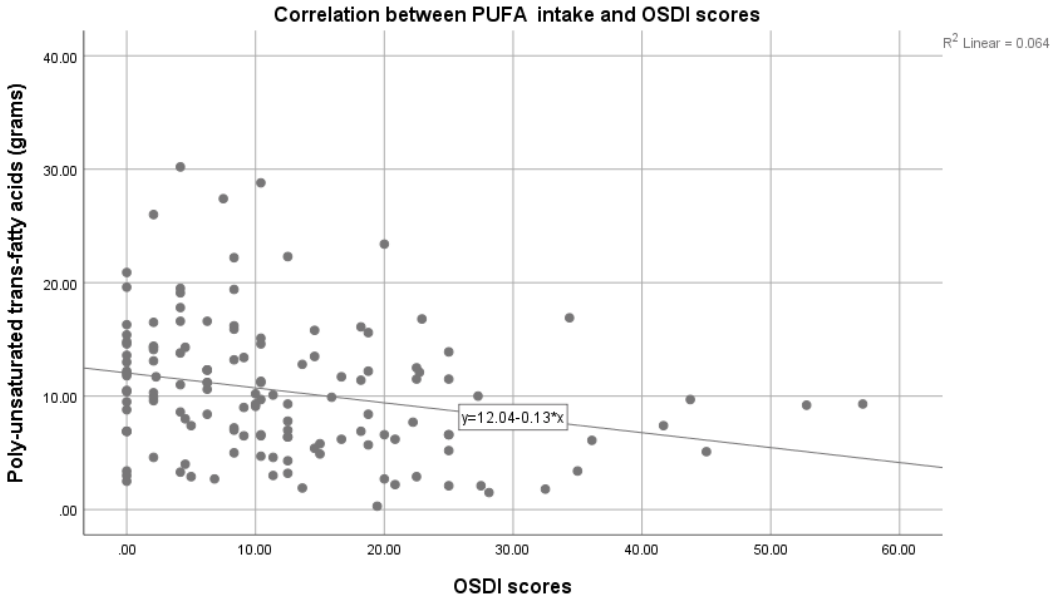


Figure 5.2: showing the correlation between Ocular Surface Disease Index (OSDI) scores and polyunsaturated trans-fatty acids intake in grams

5.4.10 Correlation between AoA and intake of dietary elements

Spearman ranked correlations (r_s) was used (Shapiro–Wilk test ($p < 0.05$) to derive any correlation between AoA and with dietary elements intake. There was no significant correlation found between Vit A and AoA, r_s (138) = -0.01, p = 0.9. There was no significant correlation found between Vit A (RAE) and AoA, r_s (138) = - 0.03, p = 0.7. Similarly, there was no significant correlation found between L/Z and AoA, r_s (138) = 0.09, p = 0.2.

The study found a significant, weak positive correlation between vit D intake and AoA, r_s (138) = 0.2, p =0.009. In the case of PUFA intake, the study also found a significant weak positive correlation between PUFA intake and AoA, r_s (138) = 0.2, p = 0.002. Figure 5.3 and 5.4 are displaying a graphical presentation of a significant correlation between Vit D (mcg) intake and AoA and between PUFA and AoA, respectively as below:

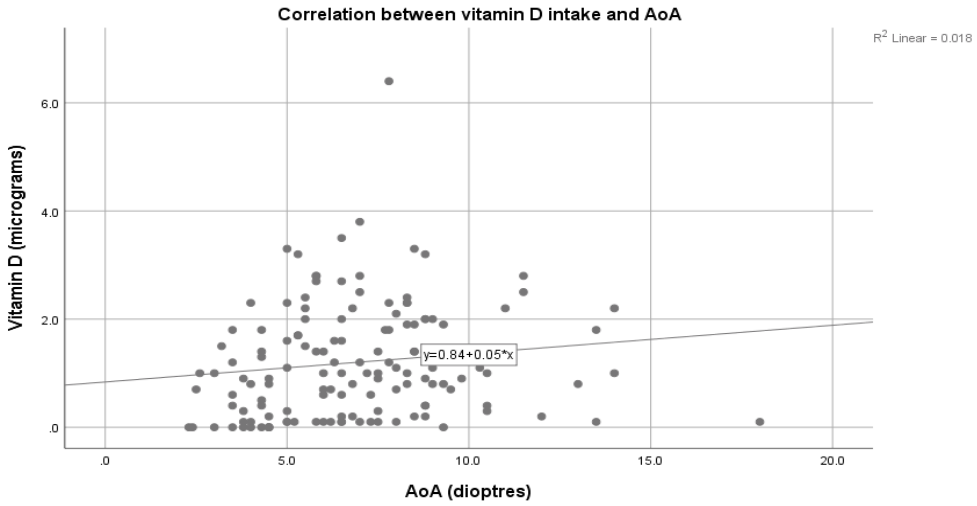


Figure 5.3: shows the correlation between amplitude of accommodation measured in Dioptres and vitamin D intake in micrograms

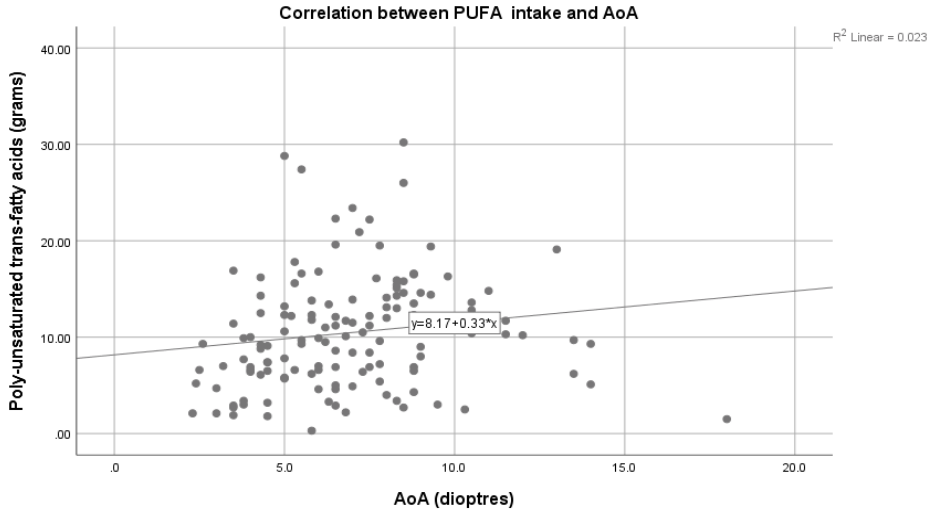


Figure 5.4: shows the correlation between amplitude of accommodation measured in Dioptres and polyunsaturated trans-fatty acids intake in grams

5.4.11 Analysis of AoA versus Vit A (IU) intake

Vit A (IU) readings were converted into milligrams. It was further divided into seven gradations (as mentioned earlier in 5.4.4). The mean AoA is mentioned in table 5.8 as shown below:

		Vitamin A gradations						
		Up to 0.50	0.51 to 1.0	1.01 to 1.50	1.51 to 2.0	2.01 to 2.50	2.51 to 3.50	Above 3.51
AoA (Dioptres)	Mean	7.1	6.7	6.4	6.7	8.3	6.4	7.6
	N	44	45	15	10	7	9	7
	Standard Deviation	3.0	2.4	3.3	1.9	1.2	3.1	2.4
	Median	6.8	6.5	5.8	6.8	8.0	5.0	7.5
	P-value	0.3						

Table 5.8: showing descriptive data for the amplitude of accommodation measured in Dioptres for each category of vitamin A intake measured in milligrams

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a non-significant result, X^2 (6) = 7.0, p = 0.3 with mean ranks for grade one = 71.3, grade two = 67.0, grade three = 56.5, grade four = 69.2, grade five = 96.8, grade six = 56.4, and grade seven = 82.8 respectively.

5.4.12 Analysis of AoA versus Vit A (RAE) intake

Vit A (RAE) readings measured in micrograms were further divided into three gradations (as mentioned earlier in 5.4.5). The mean AoA is mentioned in table 5.9 as shown below:

		Vitamin A (RAE) gradations		
		1.00	2.00	3.00
AoA (Dioptres)	Mean	7.1	5.9	7.0
	Total N	84	19	34
	Standard Deviation	2.9	2.0	2.4
	Median	6.8	6.2	7.0
	P-value	0.2		

** Grade one=up to 400.0 µg, grade two=400.1 to 700.0 µg and grade three=above 700.1 µg*
Table 5.9: showing descriptive data for the amplitude of accommodation measured in Dioptres for each category of vitamin A (retinol activity equivalent) intake measured in micrograms

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a non-statistically significant result. X^2 (2) = 3.0, p = 0.2, with mean rank for grade one = 71.3, grade two = 54.4, and for grade three = 71.3 respectively.

5.4.13 Analysis of AoA versus vitamin D (micrograms) intake

Vit D dietary intake measured in micrograms were further divided into four gradations (as mentioned earlier in 5.4.6). The mean AoA is mentioned in table 5.10 as below:

		Vitamin D gradations			
		1.00	2.00	3.00	4.00
AoA (Dioptres)	Mean	6.7	7.0	7.7	7.0
	Total N	73	37	20	7
	Standard Deviation	3.0	2.2	2.6	1.5
	Median	6.5	7.0	7.0	7.0
	P-value	0.3			

**Grade one = up to 1.0 µg, grade two = 1.1 to 2.0 µg, grade three = 2.1 to 3.0 µg, and grade four = above 3.1 µg*

Table 5.10: showing descriptive data for the amplitude of accommodation (Dioptres) for each category of vitamin D intake measured in micrograms

A Kruskal–Wallis H test showed (Shapiro-Wilk test, $p < 0.05$) a non-statistically significant result. X^2 (3) = 3.1, p = 0.3, with mean rank for grade one = 63.7, grade two = 72.8, for grade three = 79.3, and for grade four = 74.8 respectively.

5.4.14 Analysis of AoA versus L/Z intake

L/Z dietary intakes measured in micrograms were further divided into seven gradations (as mentioned earlier in 5.4.7). The mean AoA for each category is mentioned in table 5.11 as below:

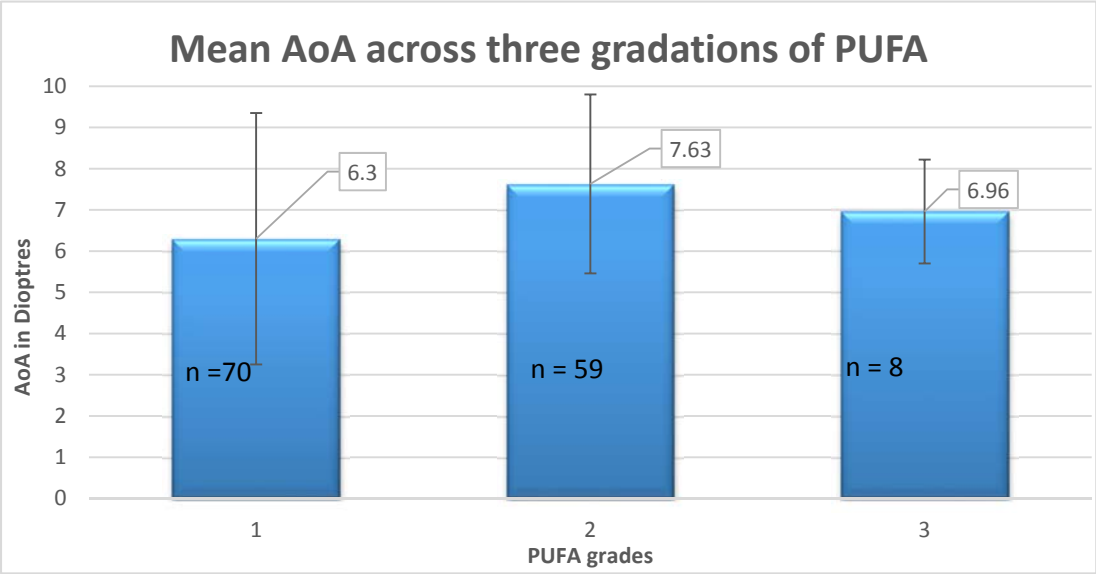
		L/Z intake gradations						
		up to 50.0	50.1 to 100.0	100.1 to 200.0	200.1 to 350.0	350.1 to 500.0	500.1 to 1000.0	1000.1 and above
AoA (Dioptres)	Mean	6.7	6.3	7.9	6.9	8.2	7.8	5.0
	Total N	58	9	7	38	10	10	5
	Standard Deviation	2.9	2.2	1.0	2.3	2.9	3.5	3.2
	Median	6.2	6.3	8.0	6.8	8.4	8.4	4.0
	P-value	0.09						

Table 5.11: shows descriptive data for the amplitude of accommodation measured in Dioptres for each category of lutein and zeaxanthin intake measured in micrograms

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a non-statistically significant result, $X^2(6) = 10.7$ p = 0.09 with mean ranks for grade one = 63.7, grade two = 62.5, grade three = 92.1, grade four = 69.5, grade five = 90.0, grade six = 82.2, and grade seven = 37.4 respectively.

5.4.15 Analysis of AoA versus PUFA intake

PUFA dietary intakes measured in grams were divided into three gradations (as mentioned earlier in 5.4.8).The mean AoA for each grading is mentioned in figure 5.5 as below:



**P value = 0.001*
Grade one = under 10.0 g, grade two = 10.1 to 20.0 g, grade three = above 20.1 g
Figure 5.5: showing descriptive data for the amplitude of accommodation measured in Dioptres for each category of polyunsaturated trans-fatty acids intake measured in grams

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a significant result, $X^2(2) = 14.1$ $p = 0.001$ with mean ranks for grade one = 56.7, grade two = 83.0, grade three = 74.3 respectively.

As the Kruskal–Wallis H test provided strong evidence of a difference ($p < 0.05$ for all groups) between mean ranks of at least one pair of PUFA grades. Dunn's pairwise test was carried out for all three pairs of PUFA gradations. There was a significant difference ($p = 0.001$, adjusted Bonferroni correction) between grade one (e.g. up to 10.0 g intake of PUFA) and grade two (i.e. 10.1 to 20.0 g intake of PUFA). There was no evidence of a difference in other pairs.

5.5 Discussion

5.5.1 Effect of dietary elements on tear film

This study found a difference in dietary intake habits of males and females participants of Pakistan. Overall, Pakistani females were found to have a less nutritious diet compared to the males in this study. This nutritional difference turned to be significantly lower in the dietary intake of Vit A (IU and RAE) compared to the males. The difference between polyunsaturated trans-fatty acids (PUFA) intake was significantly lower in females as well.

The difference in dietary intake could be influenced by many factors, i.e. cultural, health, social, environmental, lifestyle and economical (Khan *et al.*, 2009, Safdar *et al.*, 2014). This study found that smokers had a better dietary intake of Vit A (RAE) and PUFA than compared to non-smokers males and females. The other dietary elements intake, Vit A (IU), L/Z, and Vit D, were almost similar in smoker and non-smokers males. Raman *et al.* (2012a) observed a similar dietary intake pattern in smokers and non-smokers participants while observing the effect of smoking on age-related macular degeneration in Indian participants.

This study found that subjective symptoms expressed by Pakistani participants in the form of OSDI scores were negatively correlated with PUFA intake. This result was consistent with previous randomised control trial studies which showed an improvement in OSDI scores (from high to low OSDI scores) after receiving dietary supplementation of omega 3 and omega 6 (Kangari *et al.*, 2013, Sheppard *et al.*, 2013). This current studies results were consistent with Galor *et al.* (2014) for Vit D intake. This study also showed that higher level of Vit D levels was associated with lower OSDI scores.

5.5.2 Effect of dietary elements on accommodative ability

There was a weak, positive correlation found between AoA and Vit D intake. So far, no studies which have shown any correlation of Vit D on AoA. However, in the literature, some studies have shown Vit D deficiency association with cataract formation (Brown and Akaichi, 2015, Abdellah *et al.*, 2019). Similarly, this study found a weak positive correlation between PUFA and AoA. This study also found that AoA increased with an increase in PUFA intake as well. Again, there are no studies present in the literature, which report the effects of PUFA with AoA. There is no consensus in the literature for the effect of PUFA intake with cataract formation. Some studies report that PUFA intake is positively associated with cataract formation (Lu *et al.*, 2013). There is also some evidence which showed a positive role of PUFA (in the form of omega 3) intake against cataract protection (Sedaghat *et al.*, 2017).

This study did not find any association of Vit A and L/Z intakes with AoA. There are no studies in the literature report the effects of Vit A on AoA. Similarly, none of the studies in the literature has reported any effect of L/Z on AoA. Some studies, However, have shown a protective effect of L/Z intake against cataract formation (Vu *et al.*, 2006, Chasan-Taber *et al.*, 1999) and others report no effect of L/Z intake on cataract progression (Glaser *et al.*, 2015b).

The current study showed the beneficial effect of PUFA and Vit D on AoA in the Pakistani participants. This effect was absent in the UK cohort of participants (chapter four). A possible reason for this contradiction could be due to the average age difference between the two cohorts. The Pakistani cohort (mean age 34.6 ± 10.0 years) was significantly older than the UK cohort (mean age 24.6 ± 6.7 years). If AoA measurement is seen as a surrogate measure

of lens health, then the difference of age could be a possible reason the difference in the reported results.

Chronic damage to the cellular formation of proteins, lipids, and DNA from oxidative stress is one of the leading etiological factors in ageing, and for chronic diseases such as cataract (Thomas, 2006). This damage can be repaired by using multivitamins and antioxidants (Thomas, 2006). As this damage increases with age, vitamin levels decrease (Smotkin-Tangorra *et al.*, 2007, Orwoll *et al.*, 2009, Kim *et al.*, 2013). In older adults in low socioeconomic and undernourished population, higher intake of vitamins could be beneficial and help to prevent the damage caused by oxidative stress (such as cataract formation) Sperduto *et al.* (1993a).

Chapter 4 investigated the ocular effect of diet in the UK cohort and this chapter (chapter 5) looked at the same but in the Pakistani cohort. As a next step it would be useful to compare these two data sets. Chapter 7 compares the UK subjects with the Pakistani subjects to investigate if dietary and environmental factors play a role when assessing the tears and AoA of the two groups. Chapter 8 takes the sub-group of British-Asians to compare those with the Pakistani subjects as this will remove the ethnic differences. As a preliminary step chapter 6 looks at the ocular effects of sunlight (UVR) of the two cohorts.

Chapter 6

Environmental factors affecting tear film, amplitude of accommodation and macular pigment optical density

This chapter looks at the environmental differences, focussing on sunlight (UVR), of the UK based subjects and those in Pakistan.

6.1 Introduction

The effect of ultraviolet radiation (UVR) is associated with many ocular conditions. Multifactorial causes such as climate change (ozone depletion) and increased outdoor leisure activities under intense UVR contribute to the prevalence of such ocular conditions and create a significant public health concern (Yam and Kwok, 2014). There are no studies, which show an adverse effect of sunlight exposure on tear film, but in the literature, many studies have reported a negative association between UVR exposure eyelid carcinomas, corneal and conjunctival diseases (Rosso *et al.*, 1996, Naldi *et al.*, 2000, Gray *et al.*, 1992, Cullen, 2002).

Epidemiological studies have reported an inverse relationship between UVR exposure, and different types of cataract(McCarty and Taylor, 2002). Researchers have reported an association between an increased risk of developing cortical, nuclear, and mixed cataracts with UVR (mostly UV-B) exposure and outdoor activity (Neale *et al.*, 2003, Delcourt *et al.*, 2000, Tang *et al.*, 2015, West *et al.*, 1998).

In contrast research carried out by Pastor-Valero *et al.* (2007) did not find any association between cataract formation and UVR exposure. To date, there have been no studies that have researched the effects of sunlight (UV) exposure on the accommodative ability of the crystalline lens. It has been reported however that sunlight exposure at a younger age (20 – 30 years) is related with an increased risk of cataract formation in later life (Neale *et al.*, 2003).

There are a few studies which report that geographical effects such as extensive exposure to sunlight and high environmental temperatures accelerate the lens-ageing process (Freeman and Fatt, 1973, Al-Ghadyan and Cotlier, 1986). A high prevalence of cataract has been seen in people involved in aviation industry who are exposed to cosmic radiations (Rafnsson *et al.*, 2005). High average temperatures and increased duration to sunlight exposure may lead to an earlier onset of presbyopia (due to a decrease in amplitude of accommodation) as observed in an Indian study conducted by Jain *et al.* (1982) and by Miranda (1979).

The association between sunlight/UVR exposure and AMD is documented by many epidemiological studies but the results drawn from those studies are inconsistent. Many studies did not find any association of sunlight/UVR exposure with age related maculopathies (Cruickshanks *et al.*, 1993, Taylor *et al.*, 1992, Khan *et al.*, 2006). The follow-ups of Beaver

Dam study, however, found a positive association of sunlight/UVR exposure with early AMD (Cruickshanks *et al.*, 2001, Tomany *et al.*, 2004). It is believed that sunlight exposure at a younger age is considered as a risk factor for age-related macular degeneration in later life (Delcourt *et al.*, 2014, Schick *et al.*, 2016). Based on MPOD measurements, some studies have however reported that UV exposure did not have any significant association with MPOD values (Wenzel *et al.*, 2003, Raman *et al.*, 2012b). In contrast, Raman *et al.* (2012a) found an inverse relationship between sunlight/UVR exposure and MPOD values. It was reported that participants with low sunlight/UVR exposure had higher MPOD values compared to those who had high sunlight/UVR exposure.

6.2 Study aim

The purpose of this study was to assess the effects of sunlight exposure on tear film, accommodative ability, and MPOD in UK and Pakistani participants.

6.3 Methods

The selection criteria, study instruments, and the experimental procedure were similar to chapter two. The sample size of the study was based on the smoking status of the participants and was independent to the sunlight exposure status. In the OSDI questionnaire that was completed by the subject an additional question was asked. This question related to sunlight exposure and asked “How many hours you spend outside during daylight hours?” (See Appendix One).

6.4 UK cohort results

A total (N) 131 participants were enrolled in this study. The descriptive of UK cohort participants are mentioned under section 2.4, page 110.

6.4.1 Correlation between sunlight hours and TBUT

An average daylight/sunlight exposure time (in hours) of participants was collected to find any correlation between sunlight exposure hours with TBUT. A Spearman's correlation (r_s) was used to (Shapiro-Wilk test, $p < 0.05$) derive any possible correlation. There was no correlation observed between sunlight hours and TBUT for any of the above three mentioned TBUT methods. For NIKBUT, r_s (129) = -0.7, $p = 0.4$, for NITBUT r_s (129) = -1.2, $p = 0.1$, and for NAFLTBUT, r_s (129) = -0.9, $p = 0.3$.

6.4.2 Analysis of TBUT based on daylight hour's exposure

Average sunlight exposure was measured in hours. Values obtained from participants' daily exposure were further divided into three grades (i.e. grade one = up to 3 hours, grade two = 4 to 6 hours and grade three = > 6 hours).

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a non-statistically significant result for all three different methods of TBUT. For NIKBUT, $X^2(2) = 3.3$, $p = 0.7$, with the mean ranks for grade one = 68.4, grade two = 61.0, grade three = 66.4 respectively. For NITBUT, $X^2(2) = 1.7$, $p = 0.4$, with the mean ranks for grade one = 63.2, grade two = 53.8, grade three = 62.9 respectively. For NAFLTBUT, $X^2(2) = 0.5$, $p = 0.7$, with the mean ranks for grade one = 67.8, grade two = 62.9, grade two = 62.0 respectively.

The mean TBUT against all three gradations of sunlight exposure hours for all three different methods is mentioned in table 6.1 as below:

Tear break-up time (seconds)		Sunlight hours gradations			
		1.00	2.00	3.00	P-value
NIKBUT	Mean	13.1	11.6	12.0	0.7
	Total N	84	39	8	
	Standard Deviation	7.6	6.6	6.2	
	Median	10.6	10.3	10.4	
NITBUT	Mean	12.0	10.6	10.9	0.4
	Total N	84	39	8	
	Standard Deviation	5.5	4.8	2.7	
	Median	10.2	9.4	11.2	
NAFLTBUT	Mean	9.8	8.4	8.1	0.7
	Total N	84	39	8	
	Standard Deviation	6.1	4.5	3.8	
	Median	7.6	7.9	7.0	

**sunlight hours grades: grade one = up to 3 hours exposure, grade two = 4 to 6 hours exposure, and grade three = above 6 hours exposure*

Table 6.1: showing the mean tear break-up time measured in seconds against each grade of sunlight exposure measured in hours from three different methods for the UK cohort

6.4.3 Correlation between sunlight exposed hours and AoA

The average sunlight exposure (in hours) of participants was collected to find any correlation between sunlight exposure hours with AoA. A Spearman's correlation (r_s) was used to derive any possible correlation (Shapiro-Wilk test, $p < 0.05$). A positive weak correlation was found between AoA and sunlight exposed hours, r_s (129) = 0.17, p = 0.048.

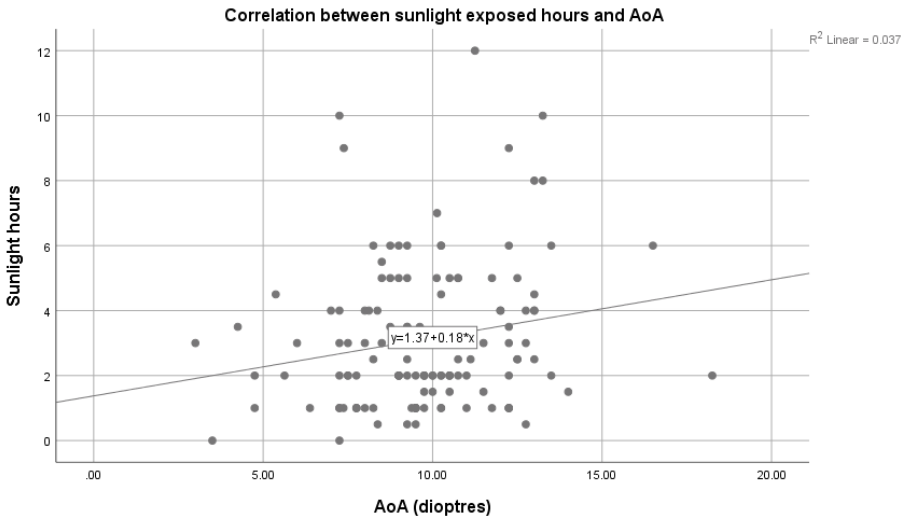


Figure 6.1: showing a weak but positive correlation between the amplitude of accommodation and sunlight exposure hours in the UK cohort

6.4.4 Analysis of AoA in terms of sunlight exposure hours

Daily exposure to sunlight was divided into three grades as mentioned above in section 6.4.2. A Kruskal–Wallis H test showed (Shapiro-Wilk test, $p < 0.05$) a non-statistically significant result, $X^2(2) = 3.1$, p = 0.2 with the mean ranks for grade one = 62.7, grade two = 69.0, and for grade three = 86.3 respectively. The mean AoA for each category of sunlight exposure is mentioned in table 6.2 as below:

		Sunlight hours gradations		
		1.0	2.0	3.0
AoA (Dioptres)	Mean	9.5	10.0	11.0
	Total N	84	39	8
	Standard Deviation	2.4	2.4	2.5
	Median	9.5	9.6	11.8
	P-value	0.2		

*Sunlight hours grades: grade one = up to 3 hours exposure, grade two = 4 to 6 hours exposure, and grade three = above 6 hours exposure

Table 6.2: showing the average amplitude of accommodation measured in Dioptres against each category of sunlight exposure time measured in hours for the UK cohort

6.4.5 Correlation between sunlight exposure and MPOD

A Spearman's correlation (r_s) test (Shapiro-Wilk test, $p < 0.05$) revealed a non-statistically significant correlation between sunlight hours and MPOD scores r_s (129) = 0.047, p = 0.6.

6.4.6 Analysis of MPOD in terms of sunlight exposure hours

Daily exposure to sunlight was further divided into three grades (one = up to 3 hours, two = 4 to 6 hours and three = above 6 hours). A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a non-statistically significant result, $X^2(2) = 0.17$, p = 0.9. The mean MPOD score for each category of sunlight exposure is mentioned in table 6.3 as below:

		Daily sunlight hours gradations		
		1.0	2.0	3.0
MPOD value	Mean	0.46	0.46	0.51
	Total N	84	39	8
	Standard Deviation	0.14	0.12	0.20
	Median	0.45	0.43	0.40
	P-value	0.9		

**sunlight hours gradations: grade one = up to 3 hours exposure, grade two = 4 to 6 hours exposure, and grade three = above 6 hours exposure*

Table 6.3: showing the MPOD score against each grade of sunlight exposure measured in hours for the UK participants

6.5 Presentation of Pakistani data

A total (N) 140 participants were enrolled in this study. The descriptive of Pakistani participants data is mentioned in section 3.5, page 135. Due to unavailability of MPS9000 instrument (for measuring MPOD), only data related to TBUT and AoA was collected for the Pakistani cohort.

6.5.1 Correlation between sunlight hours and TBUT

A Spearman's correlation (r_s) was used (Shapiro-Wilk test, $p < 0.05$) that showed a weak but negative significant correlation was found between sunlight hours and NAFLTBUT, r_s (138) = -2.4, p = 0.003.

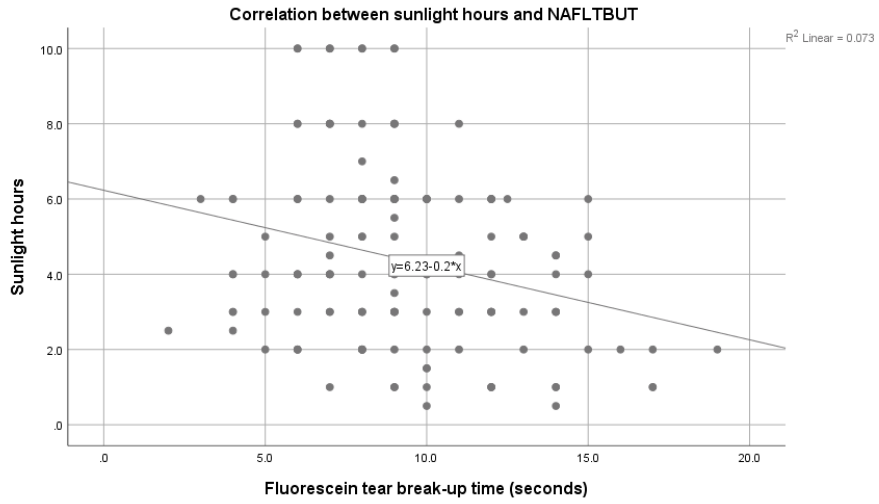


Figure 6.2: showing a weak but negative correlation between sunlight hours and fluorescein tear break-up time in the Pakistani cohort

6.5.2 Analysis of TBUT based on sunlight exposure

Average sunlight exposure was measured in hours. Values obtained from participants' daily exposure were further divided into three grades (mentioned above in section 6.3.2). The table number 6.4 displays the mean NAFLTBUT according to the sunlight gradations as shown below:

		Daily sunlight hours gradations		
		1.00	2.00	3.00
NAFLTBUT (seconds)	Mean	10.1	9.2	7.8
	Total N	58	62	20
	Standard Deviation	3.7	3.0	1.4
	Median	10.0	9.0	8.0
	P-value	< 0.001*		

** P value < 0.05*
Grade one = sunlight exposure up to 3 hours, grade two = sunlight exposure from 3.1 to 6.0 hours and grade three = sunlight exposure above than 6.0 hours
Table 6.4: showing descriptive data for fluorescein tear break-up time against three grades of sunlight exposure in the Pakistani participants

A One-way Analysis of Variance (ANOVA) was performed (Shapiro-Wilk test, $p > 0.05$) after adjusting outliers with winsorizing method. The test for homogeneity of variance (Levene's F test) revealed that the assumption of homogeneity of variance was violated ($p = 0.001$). As such, the Welch's F test was conducted. The test showed a statistically significant difference in TBUT of different gradations, *Welch's F* (2, 81.7) = 8.9, $p < .001$. Post hoc comparisons, using the Games-Howell post hoc procedure showed that participants of grade one (mean = 10.0, S.D = 3.6) had significantly higher mean TBUT than participants from grade three (mean

= 7.8, S.D = 1.3). Participants of grade two had a significantly higher TBUT (mean = 9.1, S.D = 3.0) than participants of grade three (mean = 7.8, S.D = 1.3).

6.5.3 Correlation between sunlight hours and AoA

The mean sunlight exposure (in hours) of participants was collected to find any correlation between sunlight exposure hours and AoA. A Spearman's correlation (r_s) was used (Shapiro-Wilk test, $p < 0.05$) to derive any possible correlation. There was no significant correlation found between sunlight hours and AoA, r_s (138) = 1.5, p = 0.07.

6.5.4 Analysis of AoA based on sunlight exposure

The mean AoA for each sunlight exposure gradation is mentioned in table 6.5 below:

		Daily sunlight hours gradations		
		1.00	2.00	3.00
AoA (Dioptres)	Mean	6.7	7.2	7.1
	Total N	58	62	20
	Standard Deviation	3.3	2.3	1.9
	Median	5.8	7.0	7.4
	P-value	0.1		

**Grade one = sunlight exposure up to 3 hours, grade two = sunlight exposure from 3.1 to 6.0 hours and grade three = sunlight exposure above than 6.0 hours*
Table 6.5: showing descriptive data for the amplitude of accommodation measured in Dioptres against three gradations of sunlight exposure for the Pakistani cohort

A Kruskal–Wallis H test (Shapiro-Wilk test, $p < 0.05$) showed a non-statistically significant result, $X^2(2) = 4.1$ p = 0.1 with mean ranks for grade one = 62.2, grade two = 76.1, grade three = 77.1 respectively.

6.6 Discussion

6.6.1 Tear film results

Based on the UK cohort results, the current study did not find any significant correlation or association of daylight hour's exposure with TBUT. However, in the Pakistani cohort, the current study found that exposure to sunlight was negatively associated with TBUT.

There are many explanations for this finding. Firstly, this study relied only on sunlight exposure as a source of UVR and did not take into the account the use of mobile, television, or other LED video games usage, which may be a source of artificial UVR. Secondly, daily exposure of sunlight was not converted into a UV index value. Time-specific questions were not asked as UVR intensity is different in different times during daylight hours, and it is believed that 80 per cent of UVR reaches the earth between 10 am to 2 pm (Walsh, 2009). Geographical

differences between two countries could contribute to the variation of the TBUT results. The ratio of UVR fallen on earth depends on the solar zenith angle that depends on latitude, season and time of day. The absolute and relative quantity of UVR (mostly UV- B) is maximum when the sun is high in the sky (Young, 2006). Based on latitudinal reference, Pakistan (30.3°North, 69.3°East) is situated closer to equator when compared to United Kingdom (55.3° North, 3.4° West). UVR levels are generally higher in countries closed to the equator (WHO, 2002b).

6.6.2 Accommodative ability results

In the existing literature, there is no consensus about the effects of sunlight exposure and risk of cataract formation. Many studies have observed that sunlight exposure is related to cataract development (West *et al.*, 1998, Neale *et al.*, 2003, Tang *et al.*, 2015) yet other report no effect of sunlight exposure and cataract formation (Pastor-Valero *et al.*, 2007). To date, no studies were found that reported an association between sunlight exposure and AoA. Few studies have reported a damaging effect of ultraviolet radiation by facilitating in the photoxidation process of the lens, which could cause an earlier onset of presbyopia (Miranda, 1979, Jain *et al.*, 1982).

The current study found a weak positive correlation of sunlight exposure hours with AoA in the participants of the UK cohort. However, the current study did not find any significant association of sunlight exposure with AoA in the UK cohort. In the Pakistani cohort, there was no correlation or association found between AoA and sunlight exposure hours. There is a possibility that the current study found a weak positive correlation of sunlight exposure hours with AoA by chance in the UK cohort, as this study did not use any specific empirical model (e.g. Rosenthal exposure model) developed by Rosenthal *et al.* (1988) or any physical dosimeter or UV meter watches to measure sunlight exposure.

6.6.3 MPOD results

This study did not find any correlation between sunlight exposure hours and MPOD scores; neither did it find any association between them. The current study did not measure MPOD values for the Pakistani participants due to unavailability of MP instrument. MPOD results of UK cohort are in antithesis with a previous study (Raman *et al.*, 2012a) that found that high UVR exposure as a significant risk factor for wet AMD. Raman *et al.* (2012a) observed an inverse association of UVR exposure with MPOD scores in South Indian population. Apart from, UV index measurement difference (Melbourne visual impairment project model for Raman et al study and daily sunlight exposure hours in current study), In Raman *et al.* (2012a) study, participants mean age was 69 years (controls) and 71 years, whereas, in the current UK cohort the mean age was 24 years. The difference in geographical location is another important factor. According to WHO (2002a) in India average daily ambient UVR (4500 – 5499 J/m^2), the level was higher than the average UVR level in the UK (< 2500 J/m^2).

This chapter seems to show that the data for TBUT, when compared to sunlight exposure, is not conclusive in the two cohorts, nor are the results for AoA. There may be many reasons behind this, which will be discussed in chapter 10 under the limitations of the thesis. At this point, there seems to be no value in further analysing the environmental data between the two cohorts.

Chapter 7

Comparison of Pakistani results with UK results

As mentioned at the end of chapters 3 and 5, it would be interesting to compare data collected from the UK cohort (as described in chapter 2) and the cohort gathered in Pakistan (chapter 3). This chapter will compare the results gathered from UK and the Pakistani cohorts based on smoking and dietary effects.

7.1 Descriptive data analysis

Total 140 (N) participants from the Pakistani cohort were included in this study. The descriptive statistics of the Pakistani cohort of data is mentioned below in table 7.1 (a) and 7.1 (b):

Smoking status			
	Female non-smokers	Male non-smokers	Male smokers
Mean age (years)	34.7± 11.4	35.3± 10.5	34.3± 9.2
Percentage of total data (%)	25.7	23.6	50.7
Number of participants (n)	36	33	71
P-value	0.9		

Table 7.1a: descriptive statistics based on smoking status of the Pakistani cohort

Gender		
	Male	Female
Mean age (years)	34.6 ± 9.6	34.7 ± 11.4
Percentage of total data (%)	74.3	25.7
Number of participants (n)	104	36
P-value	0.9	

Table 7.1b: descriptive statistics based on gender of the Pakistani cohort

There was no significant difference between the ages of the male and female participants (U = 1866.5, p = 0.97). There was no significant difference found between ages of three smoking statuses $X^2(2) = 0.036$, p = 0.9 with the mean rank for female non-smokers = 70.3, male non-smokers = 71.6 and smokers = 70.0 respectively.

In the United Kingdom (UK) cohort, total study participants were 131 (N =131). The descriptive statistics of the UK cohort of data is mentioned below in table 7.2a and b:

Smoking status		
	Non-smokers	Smokers
Mean age (years)	25.5 ± 7.0	23.6 ± 6.4
Percentage of total data (%)	50.4	49.6
Number of participants (n)	66	65
P-value	0.1	

Table 7.2 a: descriptive statistics based on smoking status of the UK cohort

Gender		
	Male	Female
Mean age (years)	25.1 ± 7.2	23.4 ± 5.4
Percentage of total data (%)	69.5	30.5
Number of participants (n)	91	40
P-value	0.5	

Table 7.2 b: descriptive statistics based on gender of the UK cohort

There was no significant difference presented between male and female ages (U =1710.0, p= 0.56) and between smokers and non-smokers ages of the UK cohort (U = 1799.0, p = 0.1).

The mean age of UK cohort (24.6 ± 6.7 years) was significantly younger than the mean age of Pakistani cohort (34.6 ± 10.0), Z = 7.23, p = 0.001.

7.2 Analysis of Tear break-up time (TBUT) versus smoking status

This study used an invasive method of measuring TBUT only, i.e. fluorescein TBUT (NAFLTBUT). TBUT measured from non-invasive methods (e.g. from Keratograph and tearscope) were not available in Pakistan and therefore not collected for the Pakistani data.

The mean NAFLTBUT of UK smokers was 6.6 ± 2.9 seconds (s) which was not significantly different from the mean NAFLTBUT of Pakistani smokers 7.2 ± 1.9 s, Z = -1.78, p = 0.07. The mean NAFLTBUT of UK non-smokers was 11.8 ± 6.4 secs which was also not significantly different from the mean NAFLTBUT of Pakistani non-smokers 11.5 ± 1.9 s, Z = -0.3, p = 0.7.

7.3 Comparison of TBUT based on gender and smoking status

Pakistani data for females that were smokers was not available; in order to get detailed analysis a gender and smoking status wise comparison was carried out between the UK and Pakistani data.

7.3.1 Comparison of NAFLTBUT between the UK and Pakistani participants

The mean NAFLTBUT for Pakistani and UK participants according to their smoking statuses are mentioned in table 7.3 as below:

	Pakistani female non-smokers	UK female non-smokers	Pakistani male non-smokers	UK male non-smokers	Pakistani male smokers	UK male smokers
Mean NAFLTBUT (seconds)	12.1	11.2	10.8	12	7.2	6.5
Standard deviation	3.2	7.2	2.3	6	1.8	2.5
Number of participants (n)	36	19	33	47	71	44
P value	0.6		0.2		0.1	

Table 7.3: mean fluorescein tear break-up time measured in seconds for Pakistani and United Kingdom participants according to their smoking statuses

There was no significant difference in NAFLTBUT observed between Pakistani and UK female non-smokers. A Wilcoxon Signed Rank test (Shapiro- Wilk, $p < 0.05$) showed a non-significant result, $Z = -1.9$, $p = 0.6$. There was no significant difference in NAFLTBUT observed between Pakistani and UK male non-smokers. A Wilcoxon Signed Rank test (Shapiro- Wilk, $p < 0.05$) showed a non-significant result, $Z = -1.2$, $p = 0.2$. A paired-sample t-test (Shapiro- Wilk, $p > 0.05$), showed there was no significant difference present between NAFLTBUT values of UK and Pakistani males smokers, $t(43) = -1.4$, $p = 0.16$.

7.3.2 Passive exposure to smoke and TBUT comparison

The mean NAFLTBUT for Pakistani and the UK participants against each category of passive smoking are mentioned in table 7.4 as below:

	No exposure to smoke Pakistan	No exposure to smoke UK	Passive exposure to smoke Pakistan	Passive exposure to smoke UK	Frequent exposure to smoke Pakistan	Frequent exposure to smoke UK
Mean	11.6	11.1	11.0	13.1	7.3	7.0
N	56	48	13	17	71	66
Standard Deviation	3.1	6.2	2.0	6.9	1.9	3.4
Median	12.0	10.1	12.0	11.0	8.0	6.4
P value	0.1		0.07		0.09	

Table 7.4: showing mean fluorescein tear break-up time for three different levels of passive smoking exposure for Pakistani and United Kingdom cohorts of the data

A Wilcoxon Signed Rank test (Shapiro-Wilk $p < 0.05$) to showed no significant difference of NAFLTBUT observed between two cohorts of no exposure groups, $Z = -1.5$, $p = 0.12$. A Paired Samples t-test (Shapiro-Wilk $p > 0.05$) showed no significant difference of NAFLTBUT observed between two cohorts of infrequent exposure to passive smoke groups, $t(12) = -2.0$, $p = 0.07$.

A Wilcoxon Signed Rank test A Wilcoxon Signed Rank test (Shapiro-Wilk $p < 0.05$) showed no significant difference of NAFLTBUT observed between two cohorts of frequent exposure to passive smoke groups, $Z = -1.7$, $p = 0.09$.

7.4 Comparison of OSDI scores between the UK and Pakistani participants based on gender

The mean OSDI scores for Pakistani and UK participants are mentioned in table 7.5 as below:

	OSDI scores for female UK	OSDI scores for female Pakistan	OSDI scores for male UK	OSDI scores for male Pakistan
Mean	17.3	18.2	13.8	9.8
N	40	36	91	104
Standard Deviation	15.6	13.7	13.0	9.3
Median	13.5	14.1	10.4	8.3
P value	0.2		0.07	

Table 7.5: showing mean, standard deviation, and median of Ocular Surface Disease Index scores for Pakistani and United Kingdom cohort participants on the basis of gender

A Wilcoxon Signed Rank test (Shapiro-Wilk test, $p < 0.05$) showed no statistically significant difference between OSDI scores of Pakistani and UK female participants, $Z = -1.2$, $p = 0.2$. Similarly, there was not statistically significant difference observed between OSDI scores of Pakistani and UK participants, $Z = -1.8$, $p = 0.07$.

7.4.1 Comparison of OSDI scores between the UK and Pakistani participants based on gender and smoking status

The mean OSDI scores for Pakistani and UK participants according to their smoking statuses are mentioned in table 7.6 below:

	OSDI scores for UK non-smokers female	OSDI scores for Pakistani non-smokers female	OSDI scores for UK non-smokers male	OSDI scores for Pakistani non-smokers male	OSDI scores for UK smokers male	OSDI scores for Pakistani smokers male
Mean	12.3	18.2	9.5	11.1	18.4	9.2
N	19	36	47	33	44	71
Standard Deviation	14.0	13.7	10.0	12.1	14.4	7.8
Median	4.5	14.1	8.3	8.3	14.1	8.3
P value	0.1		0.1		0.001*	

Table 7.6: descriptive data for Ocular Surface Disease Index scores of Pakistani and United Kingdom cohorts according to gender and smoking status.

A Wilcoxon Signed Rank test (Shapiro-Wilk, $p < 0.05$) showed a non-significant result between the mean OSDI scores of Pakistani female non-smokers and UK female non-smokers, $Z = -1.6$, $p = 0.10$. A Wilcoxon Signed Rank test (Shapiro-Wilk, $p < 0.05$) showed a non-significant result between the mean OSDI scores of Pakistani male non-smokers and UK male non-smokers, $Z = -1.4$, $p = 0.16$.

There was a significant difference observed between mean OSDI scores of Pakistani and UK male smokers ($Z = -4.0$, $p = 0.001$) indicating that male smokers of UK cohort had reported higher subjective symptoms of dryness compared to male smokers from Pakistan.

7.5 Comparison of AoA in different age groups

The mean AoA measured in Dioptres (D) in different age groups for Pakistani and UK cohorts is mentioned in table 7.7 (a) and 7.7 (b) respectively as below:

	AoA for 18 to 24 years old UK	AoA for 18 to 24 years old PAK	AoA for 25 to 30 years old UK	AoA for 25 to 30 years old PAK	AoA for 31 to 35 years old UK	AoA for 31 to 35 years old PAK
Mean``	10.7	9.1	9.3	8.7	7.8	7.7
N	83	30	27	29	9	18
Std. Deviation	1.8	2.01	1.8	2.7	0.5	1.5
Median	10.5	8.8	9.2	8.3	7.5	7.5
P value	0.049*		0.1		0.5	

**p value < 0.05*

Table 7.7a: showing descriptive data for amplitude of accommodation measured in Dioptres for three different age groups of United Kingdom and Pakistani cohorts.

A Wilcoxon Signed Rank test (Shapiro-Wilk test, $p < 0.05$) showed a significant difference in AoA between Pakistani and UK cohorts participants of 18-24 years age group, $Z = -2.9$, $p = 0.049$. The result suggested that 18-24 years old UK participants had better AoA compared to Pakistani counterparts.

A Wilcoxon Signed Rank test (Shapiro-Wilk test, $p < 0.05$) showed a non-significant difference in AoA between Pakistani and UK cohorts participants of 25-30 years age group, $Z = - 1.4$, $p = 0.15$. The result suggested that both cohorts had almost similar AoA in 25 to 30 years of age group.

There was no significant difference observed in AoA between Pakistani and UK cohorts participants of age group 31-35 years old by a Paired Samples t-test (Shapiro Wilk, $p > 0.05$ for both cohorts), $t(8) = 0.7$, $p = 0.5$. The result indicated that the mean AoA of Pakistani and UK participants of 31 to 35 years age group was almost similar.

	AoA for 36 to 40 years old UK	AoA for 36 to 40 years old PAK	AoA for 41 to 50 years old UK	AoA for 41 to 50 years old PAK
Mean	6.5	5.7	4.8	4.5
N	5	16	7	47
Std. Deviation	1.1	1.3	1.4	1.4
Median	7.2	5.8	4.7	4.5
P value	0.01*		0.2	

**P value < 0.05*

Table 7.7 b: showing descriptive data for amplitude of accommodation for 36 to 40 years old and 41 to 51 years old age groups of United Kingdom and Pakistani cohorts.

There was a significant difference observed in AoA between Pakistani and UK cohorts participants of age group 36-40 years old by a Paired Samples t-test (Shapiro Wilk, $p > 0.05$ for both cohorts), $t(4) = 10.2$, $p = 0.01$. The test indicated that UK participants of 36 to 40 years had high AoA compared to Pakistani counterparts of similar age group.

There was no significant difference observed in AoA between Pakistani and UK cohorts participants of age group 41-50 years old by a Paired Samples t-test (Shapiro Wilk, $p > 0.05$ for both cohorts), $t(6) = 1.4$, $p = 0.2$. The result indicated that the mean AoA of Pakistani and UK participants of 31 to 35 years age group was almost similar.

7.5.1 Comparison of AoA between the UK and Pakistani data based on gender and smoking status

The mean, S.D and median AoA of Pakistani female non-smokers and UK female non-smokers are laid out in table 7.8 as below:

	AoA for Pakistani female non-smoker 18 to 24 years old	AoA for UK female non-smoker 18 to 24 years old	AoA for Pakistani female non-smoker 25 to 30 years old	AoA for UK female non-smoker 25 to 30 years old
Mean	9.7	11.5	12.0	9.0
N	11	14	5	5
Std. Deviation	2.3	2.6	3.8	1.3
Median	9.3	10.7	10.3	9.0
P value	0.1		0.08	

Table 7.8: showing descriptive data for the amplitude of accommodation for Pakistani and United Kingdom female non-smokers groups of 18 to 24 years and 25 to 30 years old

For 18 to 24 years old age group, a Paired Samples t-test (Shapiro-Wilk test, $p > 0.05$) showed a non-significant difference of mean AoA between two cohorts of the data, $t(10) = -1.8$, $p = 0.11$. For 25 to 30 years old age group, a Wilcoxon Signed Rank test showed a non-significant result, $Z = -1.7$, $p = 0.08$.

Rest of the groups (i.e. 31 to 35, 36 to 40 and 41 to 50 years of age groups) were not analysed due to insufficient number (i.e. less than five participants per group) of participants present in one of the comparing groups.

The mean, S.D and median of Pakistani male non-smokers and UK male non-smokers are laid out in table 7.9 as below:

	AoA for Pakistani male non-smoker 18 to 24 years old	AoA for UK male non-smoker 18 to 24 years old	AoA for Pakistani male non-smoker 25 to 30 years old	AoA for UK male non-smoker 25 to 30 years old
Mean	9.6	10.5	9.5	9.2
N	9	23	5	10
Std. Deviation	2.3	2.1	2.0	2.4
P value	0.3		0.3	

Table 7.9: showing descriptive data for the amplitude of accommodation for Pakistani and United Kingdom male non-smokers groups of 18 to 24 years and 25 to 30 years old

For 18-24 years age category, a Paired Samples t-test (Shapiro-Wilk test, $p > 0.05$) showed a non-significant difference of mean AoA between two cohorts of the data, $t(8) = -1.0$, $p = 0.34$, suggesting that there was no difference in mean AoA in both cohorts.

For the 25-30 years age category, a Wilcoxon Signed Rank test (Shapiro-Wilk test, $p < 0.05$) showed a non-significant result, $Z = -0.9$, $p = 0.34$.

The other age categories i.e. 31-35, 36-40, and 41-50 years were not analysed due to the insufficient number (i.e. less than five participants per group) of participants present in one of the comparing groups.

The mean, S.D and median of Pakistani male smokers and UK male smokers are shown in table 7.10 as below:

	AoA for PAK male smoker 18 to 24 years old	AoA for UK male smoker 18 to 24 years old	AoA for PAK male smoker 25 to 30 years old	AoA for UK male smoker 25 to 30 years old
Mean	7.6	10.6	7.7	9.3
N	23	30	21	9
Std. Deviation	1.3	1.34	1.5	1.5
Median	7.8	10.5	7.8	9.2
P value	0.001*		0.04*	

**P value < 0.05*

Table 7.10: showing descriptive data for the amplitude of accommodation for Pakistani and United Kingdom male smokers groups of 18 to 24 years and 25 to 30 years old

For the 18-24 years age category, a Paired Samples t-test (Shapiro-Wilk test, $p > 0.05$) showed a significant difference, $t(22) = -7.7$, $p = 0.001$, suggesting that smoker participants of UK cohort of 18-24 years old had higher AoA compared to the Pakistani smoker counterparts. For 25 to 30 years of age category, a Wilcoxon Signed Rank test showed a significant difference between mean AoA of both cohorts, $Z = -2.1$, $p = 0.04$. The test suggested that smokers of UK cohort had higher AoA compared to smokers of Pakistani cohort of the similar age group (i.e. 25 to 30 years).

The other age categories i.e. 31-35, 36-40 and 41-50 years were not analysed due to insufficient number (i.e. less than five participants per group) of participants present in one of the comparing groups.

7.6 Defocus curves comparison between UK and Pakistani cohorts

For the defocus curves comparison, only age groups, which had minimum of five participants in each cohort, were compared.

7.6.1 Comparison of defocus curves for female non-smokers for age group 18 to 24 years

The mean LogMAR VA attained by defocus lenses (ranged from +1.50 DS to - 5.0 DS) of UK cohort of non-smokers female was numerically higher than the mean LogMAR attained from Pakistani female non-smokers. Table 7.11 displays the mean LogMAR VA for non-smoker female participants of both cohorts as below:

Defocus lens power (DS)	Mean LogMAR VA for Pakistani non-smoker female	Mean LogMAR VA for UK non-smoker female	Significance (P-value)
+1.5	0.73 ± 0.24	0.52 ± 0.30	[a]0.2
+1.0	0.43 ± 0.20	0.27 ± 0.17	[a] 0.1
+0.5	0.13 ± 0.09	0.04 ± 0.08	[a] 0.06
± 0.0	-0.03 ± 0.04	-0.06 ± 0.10	[b]0.2
-0.5	-0.02 ± 0.04	-0.08 ± 0.03	[b] 0.01*
-1.0	-0.02 ± 0.04	-0.08 ± 0.03	[b] 0.01*
-1.5	0.01 ± 0.04	-0.08 ± 0.03	[b] 0.007*
-2.0	0.09 ± 0.15	-0.08 ± 0.03	[b] 0.004*
-2.5	0.13 ± 0.21	-0.04 ± 0.07	[b] 0.01*
-3.0	0.31 ± 0.42	-0.07 ± 0.04	[b] 0.005*
-3.5	0.37 ± 0.48	-0.07 ± 0.04	[b] 0.005*
-4.0	0.42 ± 0.47	-0.05 ± 0.05	[b] 0.003*
-4.5	0.49 ± 0.47	-0.02 ± 0.06	[b] 0.003*
-5.0	0.56 ± 0.48	-0.01 ± 0.06	[b] 0.003*

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test
**p value significant*
Table 7.11: mean LogMAR visual acuity of non-smoker female participants from Pakistani and UK cohort of data

A graphical representation of mean LogMAR VA for 18-24 years female non-smokers group participants from Pakistan and the UK is displayed below in figure 7.1:

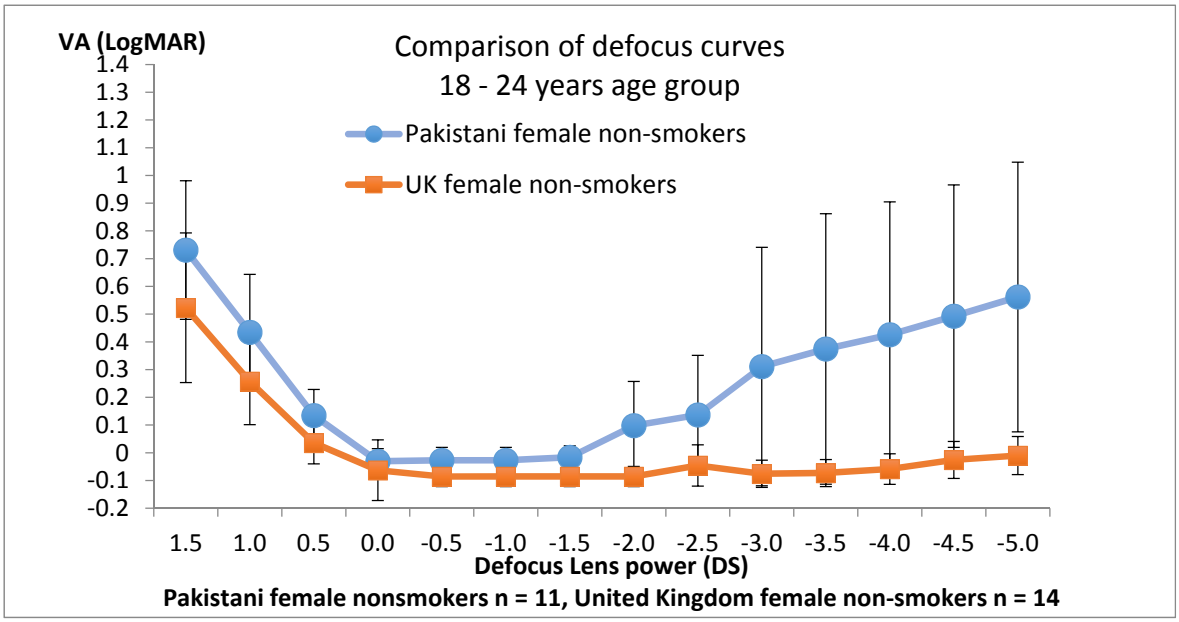


Figure 7.1: shows mean LogMAR visual acuity of 18-24 years old female non-smoker participants from Pakistan and the United Kingdom

7.6.2 Comparison of defocus curves for female non-smokers for age group 25 to 30 years

The mean LogMAR VA attained by defocus lenses (ranged from +1.50 DS to -5.0 DS) of UK cohort of non-smokers female was numerically lower than the mean LogMAR attained from Pakistani female non-smokers. Table 7.12 displays the mean LogMAR VA for non-smoker female participants of both cohorts age 25 to 30 years as below:

Defocus lens power (DS)	Mean LogMAR VA for UK non-smoker female	Mean LogMAR VA for Pakistani non-smoker female	Significance (P-value)
+1.5	0.59 ± 0.23	0.94 ± 0.09	[a]0.04*
+1.0	0.30 ± 0.19	0.55 ± 0.14	[a] 0.06
+0.5	0.04 ± 0.11	0.17 ± 0.08	[a] 0.2
± 0.0	-0.06 ± 0.05	-0.02 ± 0.04	[a] 0.1
-0.5	-0.05 ± 0.08	-0.02 ± 0.04	[a] 0.3
-1.0	-0.05 ± 0.08	0.008 ± 0.05	[a] 0.06
-1.5	-0.05 ± 0.08	-0.02 ± 0.04	[a] 0.04*
-2.0	0.05 ± 0.08	0.12 ± 0.11	[a] 0.07
-2.5	0.006 ± 0.10	0.20 ± 0.18	[a] 0.07
-3.0	0.02 ± 0.15	0.35 ± 0.31	[a] 0.01
-3.5	0.03 ± 0.16	0.51 ± 0.45	[a] 0.08
-4.0	0.05 ± 0.19	0.54 ± 0.45	[a] 0.1
-4.5	0.07 ± 0.18	0.61 ± 0.49	[a] 0.1
-5.0	0.12 ± 0.20	0.66 ± 0.49	[a] 0.1

[a] *Wilcoxon Signed Rank test,*
**p value significant*
Table 7.12: mean LogMAR visual acuity of non-smoker female participants from Pakistani and UK cohort of data

A graphical representation of mean LogMAR VA for 18-24 years female non-smokers group participants from Pakistan and the UK is displayed below in figure 7.2:

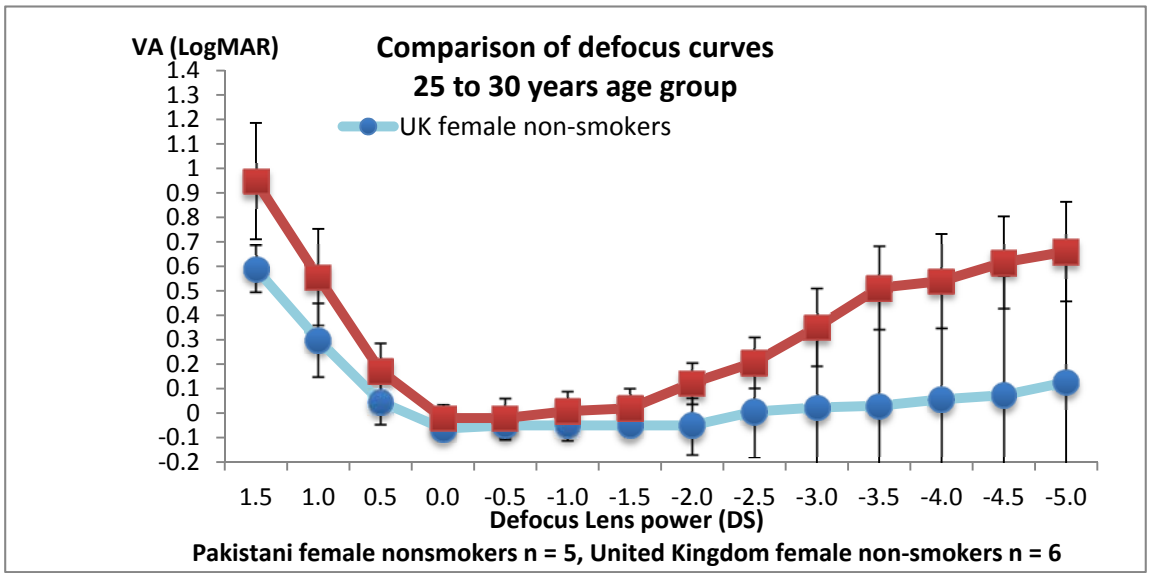


Figure 7.2: shows mean LogMAR visual acuity of 25-30 years old female non-smoker participants from Pakistan and the United Kingdom

7.6.3 Comparison of defocus curves for male non-smokers for age group 18 to 24 years

The mean LogMAR VA attained by defocus lenses (ranged from +1.50 DS to -5.0 DS) of UK cohort of non-smokers male was numerically lower than the mean LogMAR attained from Pakistani male non-smokers.

Table 7.13 displays the mean LogMAR VA for non-smoker male participants of both cohorts aged 18 to 24 years as below:

Defocus lens power (DS)	Mean LogMAR VA for UK non-smoker male	Mean LogMAR VA for Pakistani non-smoker male	Significance (P-value)
+1.5	0.47 ± 0.17	0.86 ± 0.12	[a]0.008*
+1.0	0.25 ± 0.15	0.53 ± 0.13	[a] 0.02*
+0.5	0.02 ± 0.08	0.13 ± 0.06	[a] 0.04*
± 0.0	-0.07 ± 0.05	-0.01 ± 0.03	[a] 0.7
-0.5	-0.07 ± 0.05	-0.01 ± 0.03	[a] 0.05
-1.0	-0.06 ± 0.05	0.004 ± 0.01	[a] 0.1
-1.5	-0.05 ± 0.06	0.004 ± 0.12	[a] 0.2
-2.0	-0.04 ± 0.09	0.06 ± 0.10	[a] 0.1
-2.5	-0.03 ± 0.10	0.16 ± 0.19	[a] 0.007*
-3.0	0.01 ± 0.11	0.25 ± 0.26	[a] 0.005*
-3.5	0.01 ± 0.21	0.37 ± 0.34	[a] 0.005*
-4.0	0.02 ± 0.21	0.56 ± 0.37	[a] 0.008*
-4.5	0.04 ± 0.21	0.75 ± 0.40	[a] 0.007*
-5.0	0.06 ± 0.23	0.79 ± 0.36	[a] 0.007*

[a] *Wilcoxon Signed Rank test, *p value significant*

Table 7.13: mean LogMAR visual acuity of non-smoker male participants from Pakistani and UK cohort of data

A graphical representation of mean LogMAR VA for 18-24 years male non-smokers group participants from Pakistan and the UK is displayed below in figure 7.3:

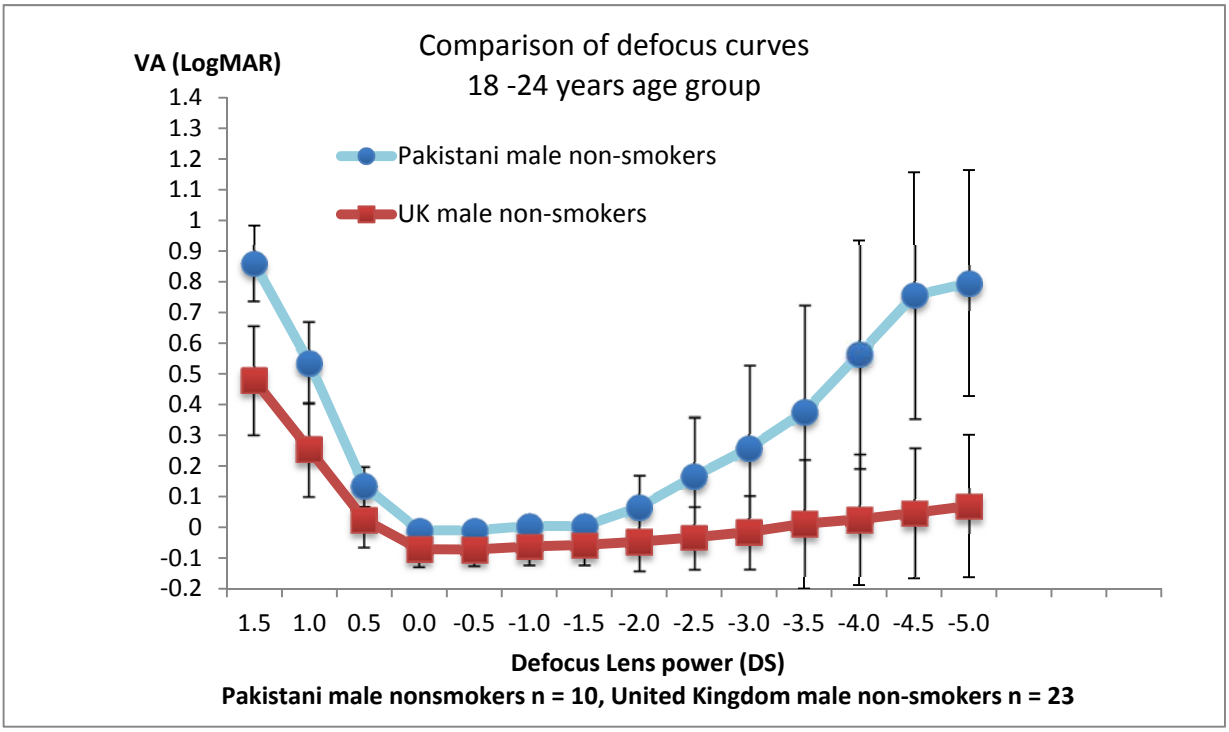


Figure number 7.3: showing defocus curves comparison between Pakistani male non-smokers and non-smoker males from the United Kingdom of 18 to 24years age group

7.6.4 Comparison of defocus curves for male smokers for the age group 18 to 24 years

The mean LogMAR VA attained by defocus lenses (ranged from +1.5 DS to -5.0 DS) of UK cohort of smokers male was numerically lower than the mean LogMAR attained from Pakistani male smokers. Table 7.14 displays the mean LogMAR VA for smoker male participants of both cohorts aged 18 to 24 years as below:

Defocus lens power (DS)	Mean LogMAR VA for Pakistani smoker male	Mean LogMAR VA for UK smoker male	Significance (P-value)
+1.5	0.63 ± 0.23	0.49 ± 0.14	[a]0.005*
+1.0	0.39 ± 0.17	0.25 ± 0.13	[a] 0.1
+0.5	0.11 ± 0.09	0.05 ± 0.07	[a] 0.1
± 0.0	0.00 ± 0.00	-0.06 ± 0.03	[a] 0.008*
-0.5	0.01 ± 0.03	-0.06 ± 0.04	[a] 0.004*
-1.0	0.04 ± 0.10	-0.05 ± 0.04	[a] 0.008*
-1.5	0.07 ± 0.14	-0.05 ± 0.05	[a] 0.01*
-2.0	0.19 ± 0.39	-0.03 ± 0.08	[a] 0.005*
-2.5	0.26 ± 0.35	-0.03 ± 0.08	[a] 0.005*
-3.0	0.41 ± 0.36	0.03 ± 0.12	[a] 0.005*
-3.5	0.62 ± 0.35	0.08 ± 0.18	[a] 0.005*
-4.0	0.80 ± 0.25	0.16 ± 0.25	[a] 0.004*
-4.5	0.90 ± 0.24	0.22 ± 0.28	[a] 0.005*
-5.0	1.00 ± 0.17	0.32 ± 0.33	[a] 0.005*

[a] *Wilcoxon Signed Rank test, *p value significant*

Table 7.14: mean LogMAR visual acuity of smoker male participants from Pakistani and UK cohort of data

A graphical representation of mean LogMAR VA for 18-24 years male smokers group participants from Pakistan and the UK is displayed below in figure 7.4:

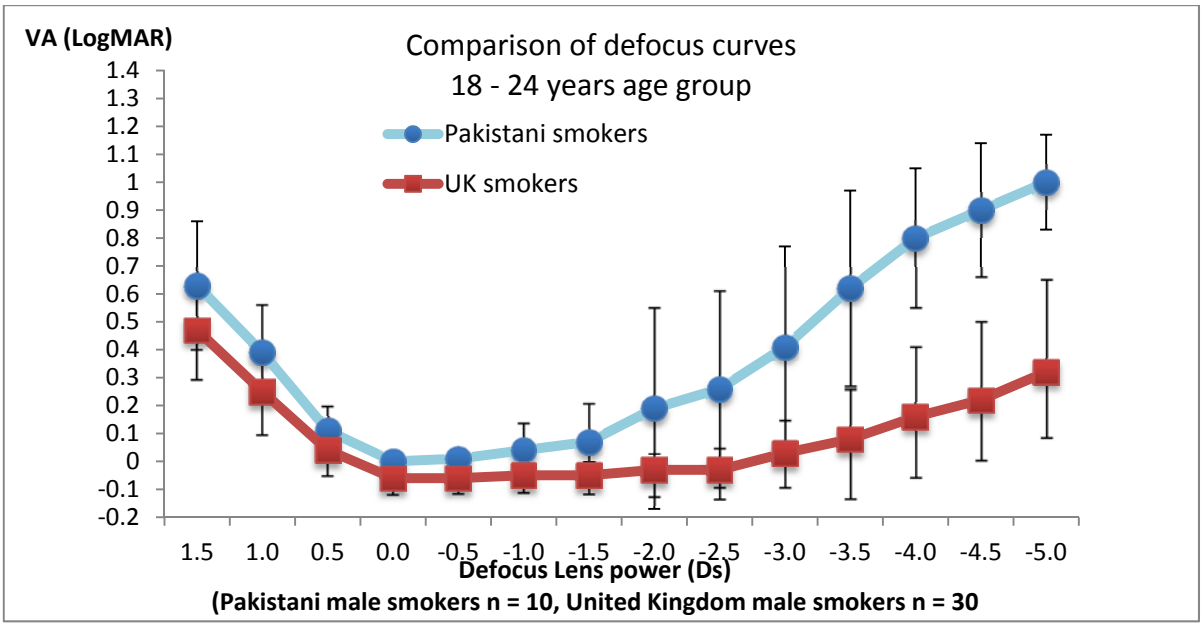


Figure number 7.4: showing defocus curves comparison between Pakistani smokers and smokers from the United Kingdom of 18 to 24 years age group.

7.6.5 Comparison of defocus curves for male smokers for the age group 25 to 30 years

The mean LogMAR VA attained by defocus lenses (ranged from +1.5 DS to -5.0 DS) of UK cohort of smokers male was numerically higher than the mean LogMAR attained from Pakistani male smokers. Table 7.15 displays the mean LogMAR VA for smoker male participants of both cohorts aged 25 to 30 years as below:

Defocus lens power (DS)	Mean LogMAR VA for Pakistani smoker male	Mean LogMAR VA for UK smoker male	Significance (P-value)
+1.5	0.85 ±0.18	0.44 ± 0.19	[b] 0.002*
+1.0	0.5 ± 0.14	0.25 ± 0.15	[b] 0.004*
+0.5	0.13 ± 0.06	0.06 ±0.07	[a] 0.01*
± 0.0	-0.009± 0.03	-0.04 ± 0.05	[a] 0.3
-0.5	-0.009 ± 0.03	-0.04 ±0.05	[a] 0.3
-1.0	-0.001 ± 0.02	-0.04 ± 0.05	[a] 0.2
-1.5	0.01 ± 0.05	-0.04 ± 0.05	[a] 0.1
-2.0	0.05 ± 0.09	-0.02 ± 0.06	[a] 0.2
-2.5	0.15 ± 0.18	-0.03 ± 0.06	[a] 0.007*
-3.0	0.29 ± 0.30	0.02 ± 0.13	[a] 0.1
-3.5	0.40 ± 0.34	0.10 ± 0.15	[a] 0.1
-4.0	0.55 ± 0.39	0.21 ± 0.25	[a] 0.2
-4.5	0.6 ± 0.40	0.27 ± 0.27	[a] 0.2
-5.0	0.70 ± 0.39	0.39 ± 0.30	[a] 0.4

*[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test *p value significant*

Table 7.15: mean LogMAR visual acuity of smoker male participants aged 25 to 30 years from Pakistani and UK cohort of data

A graphical representation of mean LogMAR VA for 25 to 30 years male smokers group participants from Pakistan and the UK is displayed below in figure 7.5:

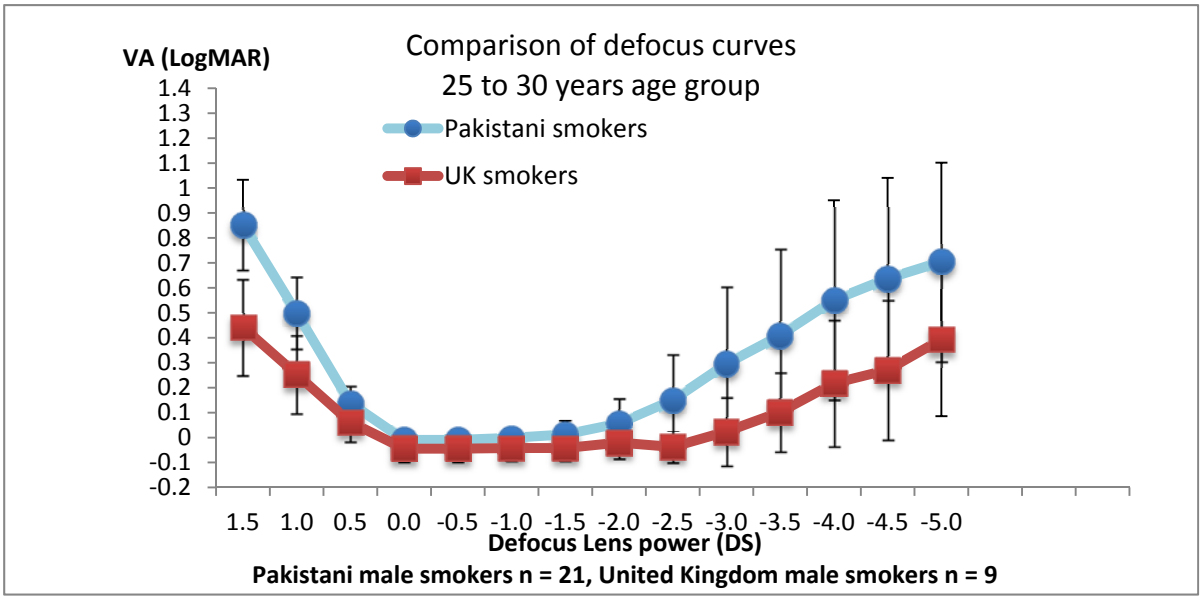


Figure number 7.5: showing defocus curves comparison between Pakistani non-smokers and non-smokers from the United Kingdom of 25 to 30 years age group.

7.7 Dietary analysis comparison between Pakistani and UK participants

The mean intake of vitamin A (both IU and RAE), vitamin D (measured in micrograms), lutein, and zeaxanthin (measured in micrograms) and polyunsaturated trans-fatty acids (measured in grams) are shown in table 7.16 as below:

Dietary elements	UK participants	Pakistani Participants	Significance (P value)
Vitamin A intake (IU)	1970.3 ± 2712.0	2032.1 ± 2458.5	[a] 0.7
Vitamin A (RAE) intake in micrograms	368.6 ± 376.3	556.7 ± 701.6	[a] 0.001*
Vitamin D intake in micrograms	2.5 ± 3.5	1.2 ± 1.0	[a] 0.004*
Lutein and Zeaxanthin intake in micrograms	487.8 ± 1135.7	291.4 ± 1000.2	[a] 0.01*
Polyunsaturated trans-fatty acids intake in grams	14.3 ± 38.1	10.4 ± 5.8	[a] 0.7

[a] *Wilcoxon Signed Rank test, *p value significant*

Table 7.16: showing means and standard deviations of dietary elements intake in the United Kingdom and Pakistani participants

7.8 Comparison of dietary elements intake and tear break-up time

Aging is a significant risk factor for dry eye disease, but only after the age of 50 years (Chia *et al.*, 2003, de Paiva, 2017). Since the current study, population is below 50 years for both cohorts the comparison of NAFLTBUT will be done based on intake quantity of dietary elements and not separated into smaller age group brackets.

7.8.1 Comparison of NAFLTBUT against intake of vitamin A (mg) gradations between two cohorts

Vitamin A intake measured in international units (IU) were converted into milligrams (mg) for calculation purposes. Vitamin A (mg) intake measurements were then graded into seven subgrades as mentioned earlier in section 4.4.6. The mean NAFLTBUT for each category of both cohorts is shown below in table 7.17:

	UK cohort TBUT grade 1	PAK cohort TBUT grade 1	UK cohort TBUT grade 2	PAK cohort TBUT grade 2	UK cohort TBUT grade 3	PAK cohort TBUT grade 3	UK cohort TBUT grade 4	PAK cohort TBUT grade 4	UK cohort TBUT grade 5	PAK cohort TBUT grade 5	UK cohort TBUT grade 6	PAK cohort TBUT grade 6	UK cohort TBUT grade 7	PAK cohort TBUT grade 7
N	45	44	38	45	15	15	11	10	9	5	8	9	45	7
Mean	9	10.5	8.3	8.8	14	8.1	7.7	9.6	8.6	10.5	8.6	9.2	8.8	8.6
St. D	4.8	3.5	3.5	3.2	10.3	2.8	3.7	1.5	6	5.3	4.1	3.2	3.2	2
P-value	[a]0.03*		[a]0.8		[a]0.3		[b]0.2		[b]0.5		[b]0.6		[b]0.3	

*[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test *p value significant*
**Gradations of vitamin A (mg): grade one = up to 0.50 mg, grade two = 0.51 to 1.0 mg, grade three = 1.10 to 1.50 mg, grade four = 1.51 to 2.0 mg, grade five = 2.01 to 2.50 mg, grade six = 2.51 to 3.50 mg and grade seven = 3.51 or above.*
Table 7.17: showing descriptive data of mean fluorescein tear break-up time for seven sub grades of vitamin A intake measured in milligrams for Pakistani and United Kingdom cohorts

7.8.2 Comparison NAFLTBUT against intake of vitamin A (RAE) gradations between two cohorts

Vitamin A (RAE) intakes measured in micrograms (µg) for both cohorts were graded into three sub grades (as explained earlier in section 4.4.7). The mean NAFLTBUT for each category of both cohorts are shown below in table 7.18:

Vitamin A (RAE) grades	UK cohort TBUT grade 1	Pak cohort TBUT grade 1	UK cohort TBUT grade 2	Pak cohort TBUT grade 2	UK cohort TBUT grade 3	Pak cohort TBUT grade 3
N	94	84	21	19	16	34
Mean	9	9.6	10.5	8.5	8.7	8.8
Std. Deviation	5.3	3.4	7	3.2	5	2.5
P-value	[a] 0.06		[b] 0.3		[a] 1.0	

[a] Wilcoxon Signed Rank test,
**Grades of vitamin A (RAE): grade one up to 400 µg, grade two = 400.0 to 700.0 µg & grade three = 700.1 µg and above*
Table 7.18: showing descriptive data of mean fluorescein tear break-up time for three gradations of vitamin A (RAE) intake measured in micrograms for Pakistani and United Kingdom cohorts

7.8.3 Comparison NAFLTBUT against intake of vitamin D (micrograms) gradations between two cohorts

Vitamin D intakes measured in micrograms (µg) for both cohorts were further graded into seven grades as mentioned in section 4.4.8. For the comparison, grade one to grade four was compared. This is because the Pakistani cohort participants had maximum up to 4.0 µg consumption of vitamin D. The mean NAFLTBUT for each category of both cohorts is shown below in table 7.19:

Vitamin D (µg) grades	UK cohort TBUT grade 1	Pak cohort TBUT grade 1	UK cohort TBUT grade 2	Pak cohort TBUT grade 2	UK cohort TBUT grade 3	Pak cohort TBUT grade 3	UK cohort TBUT grade 4	Pak cohort TBUT grade 4
N	57	73	29	37	12	20	10	7
Mean NAFLTBUT	9.5	9.2	9	9.4	7.9	9.3	8.7	9.8
Std. Deviation	5.5	3	6	3.6	3.6	3.2	4	3
P-value	[a] 0.5		[a] 0.3		[b] 0.1		[b] 0.8	

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test
**Grades of vitamin D: grade one = up to 1.0 µg, two = 1.01 to 2.0 µg, three =2.01 to 3.0 µg, four = 3.01 to 4.0 µg*
Table 7.19: showing descriptive data of mean fluorescein tear break-up time for four gradations of vitamin D intake measured in micrograms for Pakistani and United Kingdom cohorts

7.8.4 Comparison NAFLTBUT against intake of lutein and zeaxanthin (micrograms) gradations between two cohorts

Lutein and Zeaxanthin (L/Z) intake measured in micrograms was graded into seven grades as mentioned in section 4.4.9. The mean NAFLTBUT for each gradations for both cohorts is shown in table 7.20 below:

L/Z grades in µg	UK cohort grade 1	Pak cohort grade 1	UK cohort grade 2	Pak cohort grade 2	UK cohort grade 3	Pak cohort grade 3	UK cohort grade 4	Pak cohort grade 4	UK cohort grade 5	Pak cohort grade 5	UK cohort grade 6	Pak cohort grade 6	UK cohort grade 7	Pak cohort grade 7
N	35	58	11	9	18	7	26	38	16	10	13	10	12	5
Mean	9.4	9.4	9.4	8.7	11.5	10.3	8.3	8.7	8.7	9.3	8.8	10.3	8.4	9.8
Std. Deviation	5.5	3.4	6.5	5	6.3	2.7	5	2.8	5	2.3	7.1	3.4	3.6	2
P-value	[a]0.3		[a]0.8		[b]0.4		[a]0.3		[b]0.2		[a]0.5		[b]0.1	

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test
**Gradations of L/Z intake: grade one = up to 50 µg, grade two = 50.1 to 100 µg, grade three = 100.1 to 200 µg, grade four = 200.1 to 350.0 µg, grade five = 350.1 to 500.0 µg, grade six = 500.1 to 1000.0 µg and grade seven = 1000.1 µg or above*
Table 7.20: showing the descriptive data of mean fluorescein tear break-up time for seven grades of lutein and zeaxanthin intake measured in micrograms for the Pakistani and United Kingdom cohorts

7.8.5 Comparison NAFLTBUT against intake of polyunsaturated trans-fatty acids grades (grams) between two cohorts

Polyunsaturated trans-fatty acids (PUFA) dietary intakes were graded into four gradations as mentioned in section 4.4.5. For the comparison, only grades one to grade three were compared, as Pakistani data did not have participants who had more than 30.0 grams of PUFA dietary intake. The mean NAFLTBUT for each gradation for both cohorts is shown in table 7.21 below:

PUFA grades in grams	UK cohort grade 1	UK cohort grade 2	UK cohort grade 3	Pak cohort grade 1	Pak cohort grade 2	Pak cohort grade 3
N	75	43	8	70	59	8
Mean	9.2	9	12.3	9.6	9	8.8
Std. Deviation	5.7	4.8	8	3.6	2.7	2.1
P-value	0.2		0.8		0.6	

[a] *Wilcoxon Signed Rank test*
**Grades of PUFA: grade one = under 10.0 g, grade two = 10.1 to 20.0 g, grade three = 20.1 to 30.0 g*
Table 7.21: showing descriptive data of mean fluorescein tear break-up time for three gradations of polyunsaturated trans-fatty acids intake measured in grams for Pakistani and United Kingdom cohorts

7.9 Age group wise dietary comparison for AoA

With increased age, there is a decrease in the AoA and this would be in all age groups between 10 and 60 years of age. In this study, there was a significant age difference present between the two cohorts so it is important to sub-group the data into smaller age group brackets and compare age-matched samples. In the 18 to 24 years age group there were 83 participants from the UK cohort) and 29 participants from the Pakistani cohort. The mean AoA and dietary elements intake is displayed in table 7.22 for these subjects.

Age group 18-24 years	Mean value for UK cohort	Mean value for Pakistani cohort	Significance (p-value)
Amplitude of Accommodation (Dioptres)	10.7 ± 1.8	9.1 ± 2.1	[a] 0.001*
Vitamin A (as beta-carotene) intake (milligrams)	1.0 ± 1.0	1.2 ± 1.3	[a] 0.9
Vitamin A intake (retinol activity equivalent) (micrograms)	331.7 ± 318. 8	537.3 ± 657.3	[a] 0.1
Vitamin D intake (micrograms)	2.6 ± 4.0	1.5 ± 1.0	[a] 0.6
Lutein and zeaxanthin intake (micrograms)	565.5 ± 1392.2	220.0 ± 224.3	[a] 0.5
Polyunsaturated trans-fatty acids intake (grams)	11.0 ± 8.5	11.3 ± 5.5	[a] 0.5

[a] *Wilcoxon Signed Rank test, *p < 0.*05
Table 7.22: showing mean and standard deviation of amplitude of accommodation and dietary elements intake for the age group of 18 to 24 years old from the Pakistani and United Kingdom cohorts

In the 25 to 30 years age group there were 27 participants from the UK cohort) and 29 participants from the Pakistani cohort. The mean AoA and dietary elements intake is displayed in table 7.23 for these subjects.

Age group 25-30 years	Mean value for UK cohort	Mean value for Pakistani cohort	Significance (p-value)
Amplitude of Accommodation (Dioptres)	9.3 ± 1.8	8.7 ± 2.7	[a] 0.3
Vitamin A (as beta-carotene) intake (milligrams)	1.8 ± 3.0	1.1 ± 1.0	[a] 0.6
Vitamin A intake (retinol activity equivalent) (micrograms)	454.6 ± 467.0	532.1 ± 535.1	[a] 0.5
Vitamin D intake (micrograms)	2.6 ± 3.0	1.3 ± 1.2	[a] 0.02*
Lutein and zeaxanthin intake (micrograms)	402.5 ± 410.6	257.2 ± 344.5	[a] 0.1
Polyunsaturated trans-fatty acids intake (grams)	25.3 ± 82.2	12.1 ± 6.4	[a] 0.3

*[a] Wilcoxon Signed Rank test, *p < 0.05*

Table 7.23: showing mean and standard deviation of amplitude of accommodation and dietary elements intake for the age group of 25 to 30 years old from the Pakistani and United Kingdom cohorts

In the 31 to 35 years age group there were nine participants from the UK cohort) and 16 participants from the Pakistani cohort. The mean AoA and dietary elements intake is displayed in table 7.24 for these subjects.

Age group 31-35 years	Mean value for UK cohort	Mean value for Pakistani cohort	Significance (p-value)
Amplitude of Accommodation (Dioptres)	7.8± 0.6	7.6 ± 1.6	[b] 0.9
Vitamin A (as beta-carotene) intake (milligrams)	1.0 ± 1.1	1.1 ± 1.8	[a] 0.2
Vitamin A intake (retinol activity equivalent) (micrograms)	497.0 ± 598.4	546.7 ± 901.0	[a] 0.3
Vitamin D intake (micrograms)	2.6 ± 2.0	1.2 ± 1.0	[b] 0.1
Lutein and zeaxanthin intake (micrograms)	395.6 ± 460.4	123.0 ± 178.1	[a] 0.6
Polyunsaturated trans-fatty acids intake (grams)	16.0 ± 15.3	10.0 ± 4.4	[a] 0.5

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test

Table 7.24: showing mean and standard deviation of amplitude of accommodation and dietary elements intake for the age group of 31 to 35 years old from the Pakistani and United Kingdom cohorts

In the 36 to 40 years age group there were five participants from the UK cohort) and 16 participants from the Pakistani cohort. The mean AoA and dietary elements intake is displayed in table 7.25 for these subjects.

Age group 36-40 years	Mean value for UK cohort	Mean value for Pakistani cohort	Significance (p-value)
Amplitude of Accommodation (Dioptres)	6.5 ± 1.1	5.7 ± 1.4	[b] 0.6
Vitamin A (as beta-carotene) intake (milligrams)	0.8 ± 0.8	1.1 ± 0.8	[a] 0.5
Vitamin A intake (retinol activity equivalent) (micrograms)	380.6 ± 414.8	541.5 ± 423.6	[a] 0.5
Vitamin D intake (micrograms)	2.3 ± 1.7	1.2 ± 0.8	[b] 0.4
Lutein and zeaxanthin intake (micrograms)	125.7 ± 118.4	168.8 ± 164.8	[a] 0.5
Polyunsaturated trans-fatty acids intake (grams)	15.3 ± 5.1	11.5 ± 4.8	[a] 0.1

[a] *Wilcoxon Signed Rank test,* [b] *Paired Samples t-test*

Table 7.25: showing mean and standard deviation of amplitude of accommodation and dietary elements intake for the age group of 36 to 40 years old from the Pakistani and United Kingdom cohorts

In the 41 to 50 years age group there were seven participants from the UK cohort) and 47 participants from the Pakistani cohort. The mean AoA and dietary elements intake is displayed in table 7.26 for these subjects.

Age group 41-50 years	Mean value for UK cohort	Mean value for Pakistani cohort	Significance (p-value)
Amplitude of Accommodation (Dioptres)	4.8 ± 1.4	4.4 ± 1.2	[b] 0.8
Vitamin A (as beta-carotene) intake (milligrams)	0.7 ± 0.4	1.3 ± 1.8	[b] 0.6
Vitamin A intake (retinol activity equivalent) (micrograms)	302.0 ± 243.6	592.3 ± 832.5	[b] 0.9
Vitamin D intake (micrograms)	0.8 ± 0.7	1.0 ± 1.0	[b] 0.5
Lutein and zeaxanthin intake (micrograms)	272.2 ± 380.1	455.7 ± 1670.1	[a] 0.8
Polyunsaturated trans-fatty acids intake (grams)	7.7± 7.0	8.7 ± 6.1	[b] 0.2

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test

Table 7.26: showing mean and standard deviation of amplitude of accommodation and dietary elements intake for the age group of 40 to 50 years old from the Pakistani and United Kingdom cohorts

7.10 Discussion

7.10.1 Comparison of TBUT

According to United Nations (UN) country classification, Pakistan is a developing economic country (UN, 2019), that is facing many financial challenges that adversely impact on the wellbeing of society (Murtaza *et al.*, 2015). It is evident that social and financial constraints are one of the main barriers for better healthcare and a healthy lifestyle (Shiell, 1991, Hall *et al.*, 2019).

This study compared UK based data of TBUT and AoA with the Pakistani data. Participants from the UK were significantly younger than (almost ten years) the participants from Pakistan. Apart from the age difference, there were no female smokers enrolled in Pakistani data. This was possibly due to the cultural difference where there were no female smokers presented, or they did not disclose themselves as smokers.

There was no significant difference observed in terms of TBUT between UK female non-smoker participants and Pakistani female non-smoker participants. The study did not find any significant difference in TBUT between male non-smokers from UK and male non-smokers from Pakistan. Similarly, there was no significant difference found in mean TBUT of smoker participants of UK and smokers from Pakistani cohort.

To date, there are no other published studies investigating the effects of smoking on the tear film conducted on Pakistani participants. Smoker participants had a lower TBUT compared to non-smokers, and this result was consistent in both cohorts. This result were consistent with previous studies results (mentioned above and in previous chapters).

With very few studies in the literature, it was difficult to compare the current study results with other of a similar demography. However, compared to the literature, the current study TBUT results contradicts a recent study conducted by Craig *et al.* (2019), which has shown an opposite result in terms of ethnic comparison of the ocular surface between Caucasian and East Asian participants. Craig *et al.* (2019) showed that East Asian participants had lower TBUT compared to Caucasian participants. In contrast, Kim *et al.* (2019) found no difference in TBUT) while comparing East Asian with Caucasian paediatric participants predisposition to dry eye disease.

There was no significant difference observed in OSDI scores between Pakistani females and UK females. Kim *et al.* (2019) found a similar result in terms of OSDI scores while investigating Asians and Caucasians paediatric participants. However, there was a significant difference observed in OSDI scores of Pakistani males and UK males. Pakistani males had significantly lower OSDI scores compared to UK males. This difference was due to Pakistani males smokers OSDI scores that were significantly lower than all of its comparatives, i.e. when compared to Pakistani non-smokers males or females or when compared to the UK non-smoker males and females.

The OSDI scores for Pakistani smoker males were even lower than UK smoker males as well. This result conflicts with previous studies conducted by Craig *et al.* (2019), which showed that Asian had higher OSDI scores compared to Caucasians and Kim *et al.* (2019) which showed no significant difference in OSDI scores, an explanation for this finding was not provided. However, a possible explanation for this is potential bias when in filling the OSDI questionnaire. Another possibility could be due to the design of OSDI scores. As OSDI scores, McMonnies Questionnaire (MQ), and the Salisbury Eye Evaluation Questionnaire (SEEQ)

were designed for the Western population, and it may not be suitable for other populations due to environmental differences as argued by Lu *et al.* (2018).

7.10.2 Comparison of accommodative ability

This study did an age group wise AoA comparison between the UK and Pakistani cohorts. There were no significant differences observed between the two cohorts for 18 to 24 years, 25 to 30 years, and 31 to 35 years of age. Although there were a limited number of participants in the UK cohort, who were 36 years old or above the Pakistani cohort had a significantly lower AoA compared to the UK cohort for the 36 to 40 years of age group. There could be several explanations for this finding; one possible reason could be a difference in ethnicity. Chattopadhyay and Seal (1984) observed a similar AoA for Indian participants and White participants up to 18 years and then observed ta decrease in AoA in the Indian population compared to European participants. Another reason could be due to the difference in the climate. Pakistani participants were from a hot climate, and it is seen that in hot climates the onset of presbyopia (which is directly linked with AoA) is earlier than in colder climates (Miranda, 1979).

For gender and smoking wise comparison, the current study's result showed that UK smokers of age group 18 to 24 years had better AoA compared to their Pakistani counterparts. A similar trend was seen for 25 t0 30 years of age group smoker participants of both cohorts. The difference in smoking intensity could be a possible reason as generally for Pakistani smoker participants smoked more cigarettes per day compared to UK smokers. Other possible reasons could be due to the difference in the geographical location (Hashemi *et al.*, 2017a) of participants and the difference in ethnicity (Edwards *et al.*, 1993). There was no significant difference observed in mean AoA for female non-smokers and male non-smokers of both cohorts.

Gender and smoking status wise defocus curves comparison performed for both cohorts showed that UK participants had better mean LogMAR VA compared to Pakistani cohort for all three smoking status (i.e. female non-smokers, male non-smokers and male smokers).

7.10.3 Comparison based on dietary elements intake

The average dietary elements intake of vitamin A (RAE) was significantly higher in the Pakistani cohort compared to the UK participants. Despite the fact that average vitamin A (RAE) intake of Pakistani participants was higher than UK participants, both cohorts had a low mean vitamin A consumption against the Recommended Dietary Allowance (RDA) of vitamin A RAE (i.e. 700 micrograms for females and 900 micrograms for males) by Office of Dietary Supplements, National Institutes of Health (NIH, 2018b). One of the possible reasons for this result is due to the foods eaten Pakistani participants. Typically, the Pakistani dietary pattern

is rich in vitamin A, e.g. wheat (in the form of chapatti, rice, green leafy vegetables, pulses and yoghurts meat (mostly beef), chicken(Gallup, 2011). Most of these food items also have a good amount of vitamin A (NIH, 2018b).

Vitamin D intake of UK participants was significantly higher than their Pakistani counterparts. Apart from sunlight, vitamin D is present in food items such as beef liver, milk, cheese, egg yolk, and mushrooms (Ovesen *et al.*, 2003, NIH, 2018a). This study found a low intake of vitamin D dietary elements in both cohorts against the normal RDA values suggested by Office of Dietary Supplements that is 15 micrograms per day for adults' males and females (NIH, 2018a, Ross *et al.*, 2011). The difference in vitamin D intake between the two cohorts could be due to the affordability of items. Food items such as milk, cheese, meat are accessible and affordable for a UK resident, however for Pakistanis living in Pakistan the socioeconomic burden and inflated food prices impact upon dietary intake.

Lutein and Zeaxanthin (L/Z) intake in the UK participants were significantly higher than the Pakistani counterparts' intake of L/Z. Some of L/Z enriched food sources are maize, kale, spinach, and egg yolks (Howells *et al.*, 2011, Gao *et al.*, 2011, Gammone *et al.*, 2015). As mentioned above affordability of dietary elements in Pakistan could explain this. Another possible explanation could be due to an increased awareness of L/Z benefits on ocular (especially on macula) health or more awareness of food items enriched in L/Z in the UK. There is no RDA set for L/Z intake, however according to the American Optometric Association, 10 milligrams of lutein and 2 milligrams of zeaxanthin are recommended for optimal ocular health.

This study did not find any significant difference of TBUT of both cohorts based on dietary elements intake. The study compared age group wise AoA and dietary elements intake for both cohorts and found that mean AoA of the UK cohort was significantly higher than mean AoA of the Pakistani cohort in the18 to 24 years age group. There was no dietary elements intake difference, which suggests that the difference in AoA was not due to dietary difference but may be due to other import factors such as ethnicity, geographical location and environmental differences as explained above in this section.

This chapter compared the UK subjects with the Pakistani subjects to investigate if dietary and environmental factors play a role when assessing the tears and AoA of the two groups. The next chapter will take the sub-group of British-Asians to compare those with the Pakistani subjects as this will remove the ethnic differences.

Chapter 8

Comparison of Pakistani results with British Asian participants results

The previous chapter compared all the UK data to that collected in Pakistan. The UK cohort consisted of a large number of subjects from South Asian origins (Indo–Pak). It would be interesting to compare these people to the data collected from Pakistan since the genetic make-up of both groups would be similar and the difference between them may be related to climate and diet. In this chapter, the UK data for British-Asian subjects only is compared to the data from Pakistani subjects in order to remove ethnic differences.

The total number (n) of British Asian female participants was 12, with a mean age of 22.1 ± 3.7 years and the total number of Pakistani female participants was (n = 36), with an average age of 34.7 ± 11.4 years.
The total number of British Asian male participants was (n = 51) with a mean age of 26.0 ± 7.7 years, and the total Pakistani male participants were (n = 104), with an average age of 34.6 ± 9.6 years.

8.1 Comparison of TBUT

8.1.1 British Asian female non-smokers versus Pakistani female non-smokers

The mean NAFLTBUT for British Asian non-smoker female participants (n = 9) was 12.1 ± 6.2 seconds (s), which was very similar to the mean NAFLTBUT of Pakistani female participants (n = 36) 12.1 ± 3.2 s. A Paired samples t-test (Shapiro-Wilk test, $p > 0.05$) was used to compare the NAFLTBUT of the two groups and showed a non-statistical significant difference, $t(8) = -1.2$, $p = 0.2$.

8.1.2 British Asian male non-smokers versus Pakistani male non-smokers

The mean NAFLTBUT for British Asian non-smoker male participants (n = 29) was 11.6 ± 6.0 s, which was marginally higher than Pakistani male non-smokers NAFLTBUT were (n =33) 10.7 ± 2.3 s. A Wilcoxon Signed Rank test (Shapiro-Wilk test, $p < 0.05$) was used to find any statistically significant difference in NAFLTBUT in both cohorts. The test showed a non-significant result, $Z = -0.1$, $p = 0.9$.

8.1.3 British Asian male smokers' versus Pakistani male smokers

The mean NAFLTBUT for British Asian smoker male participants (n = 22) was 6.5 ± 2.4 s, which was numerically lower than the mean NAFLTBUT of Pakistani male smoker participants were (n = 71) 7.2 ± 2.0 s.

A Paired Samples t-test (Shapiro-Wilk test, $p > 0.05$) was used that showed a non-statistical significant difference, $t(21) = 1.5$, $p = 0.1$. Figure 8.1 shows mean TBUT of both cohorts according to the different smoking statuses shown below:

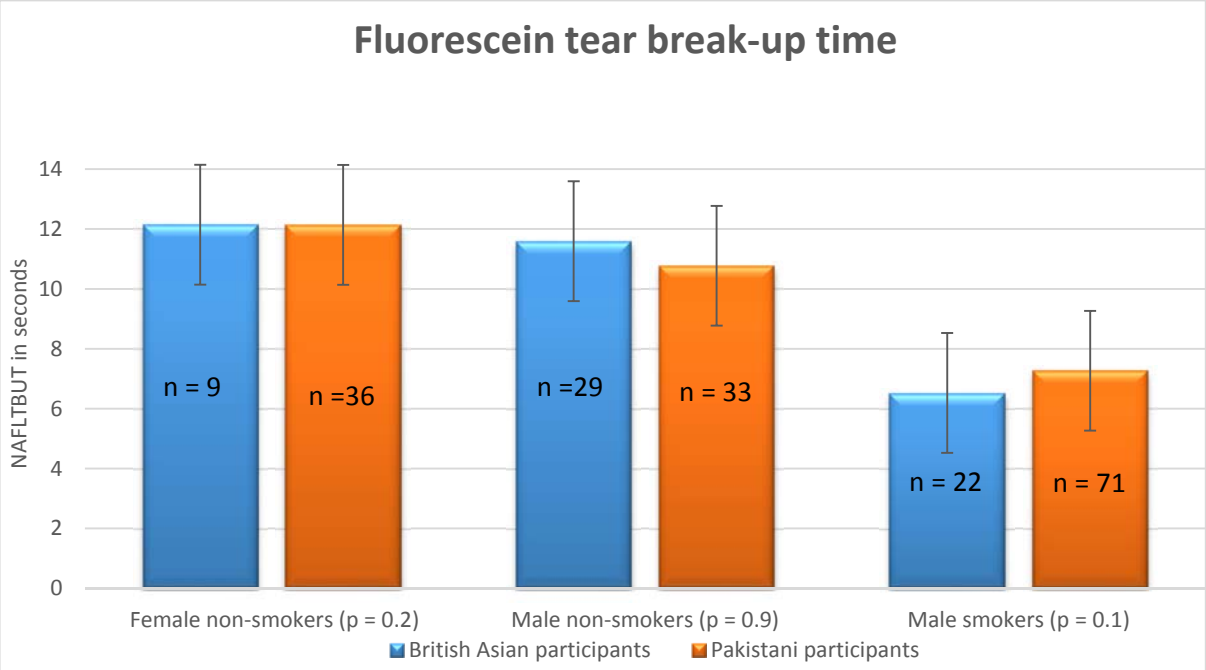


Figure 8.1: shows mean fluorescein tear break-up time (NAFLTBUT) measured in seconds for the different smoking status of British Asian and Pakistani data

8.2 Comparison of AoA

The mean ages of British Asian participants (for both female and male), was, younger than the mean age of Pakistani participants.

For comparing AoA between British Asian (Indo-Pak origin) and Pakistani participants, the data was further divided into different age groups and then a gender and smoking status wise comparison was conducted. Only age groups that had at least five participants in each cohort were compared.

8.2.1 Comparison of AoA between British Asian female non-smokers and Pakistani female non-smokers

A comparison was performed for the 18-24 years age non-smoker females' category only. The other age categories had an insufficient number of participants to be able carry out any statistical analysis.

The mean AoA for Pakistani non-smoker females was numerically less than the mean AoA of British Asian non-smoker females, as mentioned in table 8.1 a below:

Mean AoA (Dioptres)	Mean	N	Std. Deviation	Median	P-value
Pakistani female non-smokers18 to 24 years	9.8	11	2.3	9.3	0.3
British Asian non-smokers female 18-24	11.2	6	1.4	11.2	

Table 8.1 a: shows descriptive data of the amplitude of accommodation of Pakistani and British Asian non-smoker females of 18 to 24 years age group.

A Paired Samples t-test (Shapiro-Wilk test, $p > 0.05$) showed a non-statistical significant result, $t(5) = -1.0$, $p = 0.3$.

8.2.2 Comparison of AoA between British Asian male non-smokers and Pakistani male non-smokers

Only 18-24 years age group was compared, as other groups did not have sufficient numbers of participants for comparison.

The mean AoA for Pakistani male non-smokers was numerically lower than the mean AoA of British Asian counterparts. Table 8.1 b displays mean AoA for both cohorts as below:

Mean AoA (Dioptres)	Mean	N	Std. Deviation	Median	P-value
British Asian non-smokers male	11.1	17	2.1	10.7	0.1
Pakistani male non-smokers	9.6	9	2.3	8.5	

Table 8.1b: shows descriptive data of the amplitude of accommodation of Pakistani and British Asian non-smoker males of 18 to 24 years age group.

A Paired Samples t-test (Shapiro-Wilk test, $p > 0.05$) showed a non-statistical significant result, $t(8) = -1.7$, $p = 0.1$.

8.2.3 Comparison of AoA between British Asian male smokers and Pakistani male smokers

Two age groups (18 to 24 and 25 to 30 years) were compared. The other groups were not compared due to insufficient numbers of participants in them. For 18- 24 years old age group, the mean AoA for Pakistani participants was numerically lower than the mean AoA for British Asian male smokers, which is shown in table 8.1c as below:

Mean AoA (Dioptres)	Mean	N	Std. Deviation	Median	P-value
Pakistani male smokers	8	10	0.8	7.7	0.001*
British Asian non-smokers male	11.2	11	1.4	11	

Table 8.1 c: shows descriptive data of the amplitude of accommodation of Pakistani and British Asian male smokers of 18 to 24 years age group.

A Paired Samples t-test (Shapiro-Wilk test, $p > 0.05$) showed a statistically significant difference between mean AoA of both cohorts, $t(9) = -5.1$, $p = 0.001$.

Similarly, for 25 to 30 years old age category, the mean AoA of British Asian smokers was numerically higher than the mean AoA for Pakistani male smokers, which are mentioned in table, number 8.1d as below:

Mean AoA (Dioptres)	Mean	N	Std. Deviation	Median	P value
Pakistani male smokers AoA	7.7	21	1.5	7.8	0.001*
British Asian smokers male AoA	9.5	7	1.6	9.2	

Table 8.1 d: shows descriptive data for the amplitude of accommodation in Pakistani and British Asian male smokers of 25 to 30 years age group.

A Paired Samples t-test (Shapiro-Wilk test, $p > 0.05$) showed a statistically significant difference between mean AoA of both cohorts, $t(6) = -3.5$, $p = 0.01$.

8.3 Comparison of OSDI scores on the basis of gender and smoking status

The mean OSDI scores for Pakistani and British Asian participants are displayed in table 8.2 below:

OSDI scores	Pakistani female non-smokers	British Asian female non-smokers	Pakistani male non-smokers	British Asian male non-smokers	Pakistani male smokers	British Asian male smokers
Mean	18.1	9.5	11.1	8.4	9.2	17.4
Std. Deviation	3.6	10.1	12	8.6	7.7	15.4
P value	[a] 0.4		[a] 0.6		[a]0.007*	

[a] Wilcoxon Signed Rank test, * p value < 0.05

Table 8.2: shows comparison of Ocular Surface Disease Index scores between Pakistani and British Asian cohorts

8.4 Comparison of defocus curves of British Asians and Pakistani female non-smoker participants

Only the 18 to 24 years age group defocus curves were compared for the female non-smokers category. The other age groups, had fewer than five participants in each cohort and therefore statistical analysis was not possible. Table 8.3 displays the mean LogMAR VA of non-smokers female participants of both cohorts as below:

Defocus lens power (DS)	Mean LogMAR VA for British Asian female non-smokers	Mean LogMAR VA for Pakistani female non-smokers	Significance (P-value)
1.5	0.70 ±0.26	0.73 ± 0.24	[b] 1.0
1.0	0.37 ± 0.14	0.43 ± 0.20	[b] 0.4
0.5	-0.08 ± 0.08	0.13 ± 0.09	[b] 0.4
± 0.0	-0.03 ± 0.16	-0.03 ± 0.04	[a] 0.7
-0.5	-0.08 ± 0.04	-0.02 ± 0.04	[a] 0.06
-1.0	-0.08 ± 0.04	-0.02 ± 0.04	[a] 0.06
-1.5	-0.08 ± 0.04	-0.01 ± 0.04	[a] 0.04*
-2.0	-0.08 ± 0.04	0.09 ± 0.15	[a] 0.03*
-2.5	-0.05 ± 0.08	0.13 ± 0.21	[a] 0.1
-3.0	-0.08 ± 0.04	0.31 ± 0.42	[a] 0.04*
-3.5	-0.08 ± 0.04	0.37 ± 0.48	[a] 0.04*
-4.0	-0.07 ± 0.05	0.42 ± 0.47	[a] 0.03*
-4.5	-0.02 ± 0.04	0.49 ± 0.47	[a] 0.03*
-5.0	-0.02 ± 0.04	0.56 ± 0.48	[a] 0.04*

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test

*p value < 0.05

Table 8.3: mean LogMAR visual acuity of Pakistani and British Asian female non-smokers participants of 18 to 24 years age group.

A graphical presentation of both cohorts defocus curves is mentioned in figure 8.2 below:

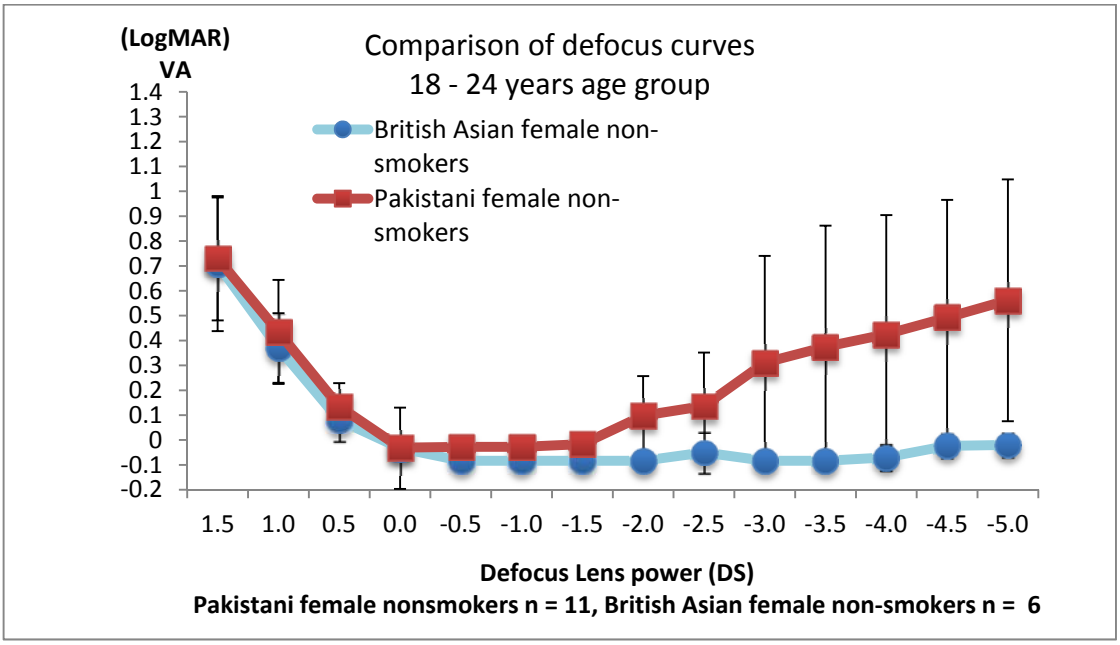


Figure 8.2: shows mean LogMAR VA attained from defocus lens power (ranged from +1.5 DS to -5.0 DS) for both Pakistani and British Asian female non-smokers of 18-24 years of age

8.5 Comparison of defocus curves of British Asians and Pakistani male non-smoker participants

The 18 to 24 years age group defocus curves were compared for the male non-smokers category. The other age groups had fewer than five participants in each cohort. Table 8.4 displays the mean LogMAR VA of non-smokers male participants of both cohorts as below:

Defocus lens power (DS)	Mean LogMAR VA for Pakistani male non-smokers	Mean LogMAR VA for British Asian male non-smokers	Significance (P-value)
1.5	0.87 ± 0.12	0.49 ± 0.18	[a] 0.04*
1.0	0.54 ± 0.14	0.20 ± 0.15	[b] 0.01*
0.5	0.13 ± 0.06	0.04 ± 0.09	[a] 0.1
± 0.0	-0.01 ± 0.03	-0.05 ± 0.05	[a] 0.3
-0.5	-0.01 ± 0.03	-0.06 ± 0.05	[a] 0.2
-1.0	0.004 ± 0.01	-0.05 ± 0.06	[a] 0.1
-1.5	0.004 ± 0.01	-0.04 ± 0.07	[a] 0.3
-2.0	0.07 ± o.10	-0.03 ± 0.10	[a] 0.1
-2.5	0.15 ± 0.19	-0.01 ± 0.11	[a] 0.01*
-3.0	0.24 ± 0.28	-0.01 ± 0.13	[a] 0.008*
-3.5	0.36 ± 0.36	0.02 ± 0.24	[a] 0.008*
-4.0	0.54 ± 0.38	0.03 ± 0.24	[a] 0.01*
-4.5	0.72 ± 0.41	0.06 ± 0.24	[a] 0.01*
-5.0	0.77 ± 0.38	0.07 ± 0.26	[a] 0.01*

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test
*p value < 0.05
Table 8.4: mean LogMAR visual acuity of Pakistani and British Asian male non-smokers participants of 18 to 24 years age group.

A graphical presentation of both cohorts defocus curves is mentioned in figure 8.3 below:

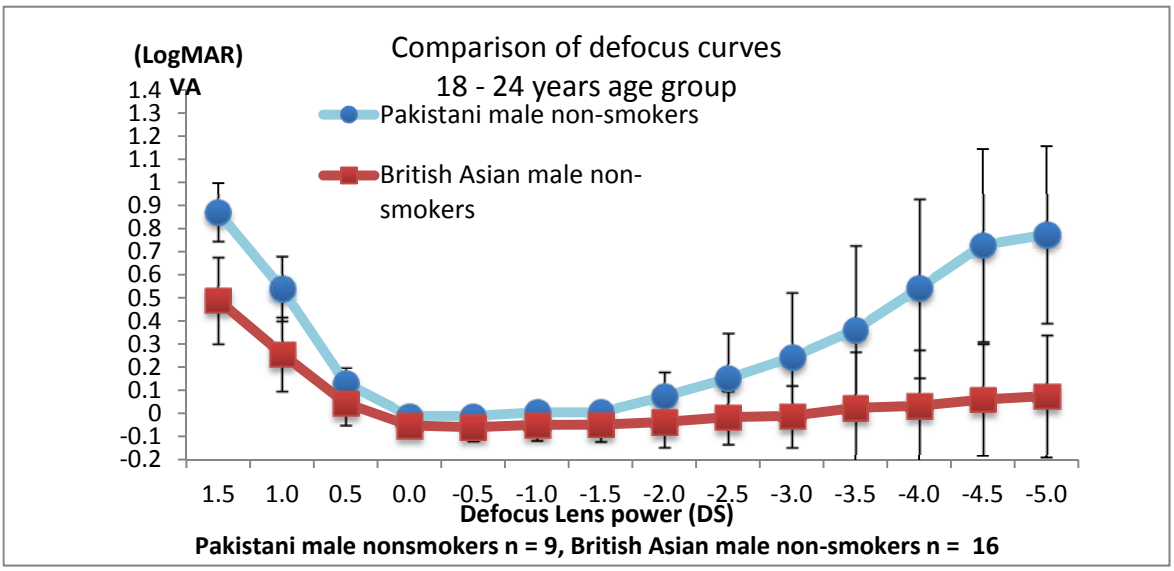


Figure 8.3: shows mean LogMAR VA attained from defocus lens power (ranged from +1.5 DS to -5.0 DS) for both Pakistani and British Asian male non-smokers of 18-24 years of age group.

8.6 Comparison of defocus curves of British Asians and Pakistani male smoker participants

The 18 to 24 years age group and the 25 to 30 years age group defocus curves were compared for the male smokers' category. The other age groups had fewer than five participants in each cohort. Table 8.5 displays the mean LogMAR VA of non-smokers male participants of both cohorts as below:

Defocus lens power (DS)	Mean LogMAR VA for Pakistani male smokers	Mean LogMAR VA for British Asian male smokers	Significance (P-value)
1.5	0.83 ± 0.17	0.48 ± 0.16	[b] 0.001*
1.0	0.44 ± 0.12	0.23 ± 0.13	[a] 0.02*
0.5	0.12 ± 0.03	0.04 ± 0.06	[a] 0.008*
± 0.0	0.00 ± 0.00	-0.07 ± 0.04	[a] 0.008*
-0.5	0.00 ± 0.00	-0.07 ± 0.04	[a] 0.008*
-1.0	0.00 ± 0.00	-0.07 ± 0.04	[a] 0.008*
-1.5	0.00 ± 0.00	-0.07 ± 0.04	[a] 0.008*
-2.0	0.02 ± 0.04	-0.04 ± 0.05	[a] 0.01*
-2.5	0.09 ± 0.14	-0.02 ± 0.09	[a] 0.04*
-3.0	0.18 ± 0.26	0.03 ± 0.13	[a] 0.04*
-3.5	0.30 ± 0.35	0.08 ± 0.19	[a] 0.04*
-4.0	0.39 ± 0.37	0.14 ± 0.26	[a] 0.02*
-4.5	0.49 ± 0.38	0.19 ± 0.32	[a] 0.01*
-5.0	0.59 ± 0.38	0.29 ± 0.39	[b] 0.01*

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test
*p value < 0.05
Table 8.5: mean LogMAR visual acuity of Pakistani and British Asian male smokers participants of 18 to 24 years age group.

A graphical presentation of both cohorts defocus curves is mentioned in figure 8.4 below:

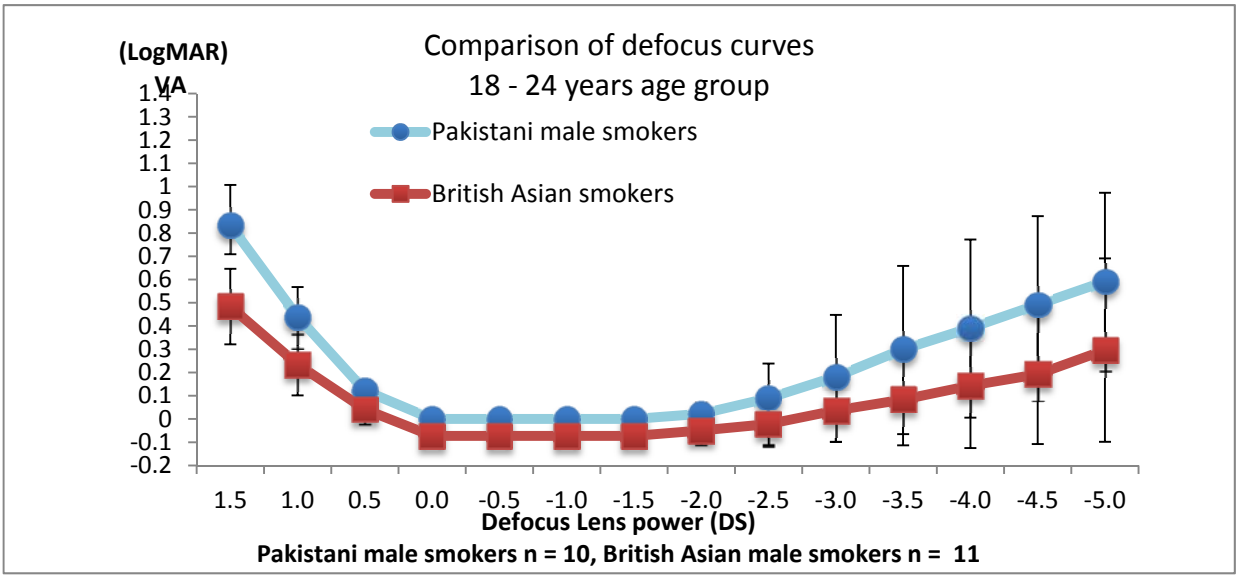


Figure 8.4: shows mean LogMAR VA attained from defocus lens power (ranged from +1.5 DS to -5.0 DS) for both Pakistani and British Asian male smoker participants of 18-24 years of age group.

The mean LogMAR VA attained by defocus lenses (ranged from +1.5 DS to +0.0 DS) of British Asian cohort of smokers male was numerically lower than the mean LogMAR attained from Pakistani male smokers in 25 to 30 years old age group. Table 8.6 displays the mean LogMAR VA of non-smokers male participants of both cohorts as below:

Defocus lens power (DS)	Mean LogMAR VA for Pakistani male smokers	Mean LogMAR VA for British Asian male smokers	Significance (P-value)
1.5	0.85 ± 0.18	0.45 ± 0.22	[b] 0.01*
1.0	0.50 ± 0.14	0.26 ± 0.17	[b] 0.02*
0.5	0.13 ± 0.06	0.05 ± 0.08	[b] 0.001*
± 0.0	-0.009 ± 0.03	-0.05 ± 0.05	[a] 0.3
-0.5	-0.009 ± 0.03	-0.05 ± 0.05	[a] 0.3
-1.0	-0.001 ± 0.02	-0.05 ± 0.05	[a] 0.2
-1.5	0.01 ± 0.05	-0.05 ± 0.05	[a] 0.2
-2.0	0.06 ± 0.09	-0.02 ± 0.07	[b] 0.3
-2.5	0.15 ± 0.18	-0.04 ± 0.06	[a] 0.01*
-3.0	0.30 ± 0.30	0.03 ± 0.15	[a] 0.3
-3.5	0.41 ± 0.33	0.13 ± 0.17	[a] 0.04*
-4.0	0.56 ± 0.38	0.26 ± 0.26	[b] 0.6
-4.5	0.64 ± 0.39	0.33 ± 0.28	[b] 0.6
-5.0	0.71 ± 0.38	0.43 ± 0.33	[b] 0.8

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test
*p value < 0.05

Table 8.6: mean LogMAR visual acuity of Pakistani and British Asian male smokers participants of 25 to 30 years age group.

A graphical presentation of both cohorts defocus curves is mentioned in figure 8.5 below:

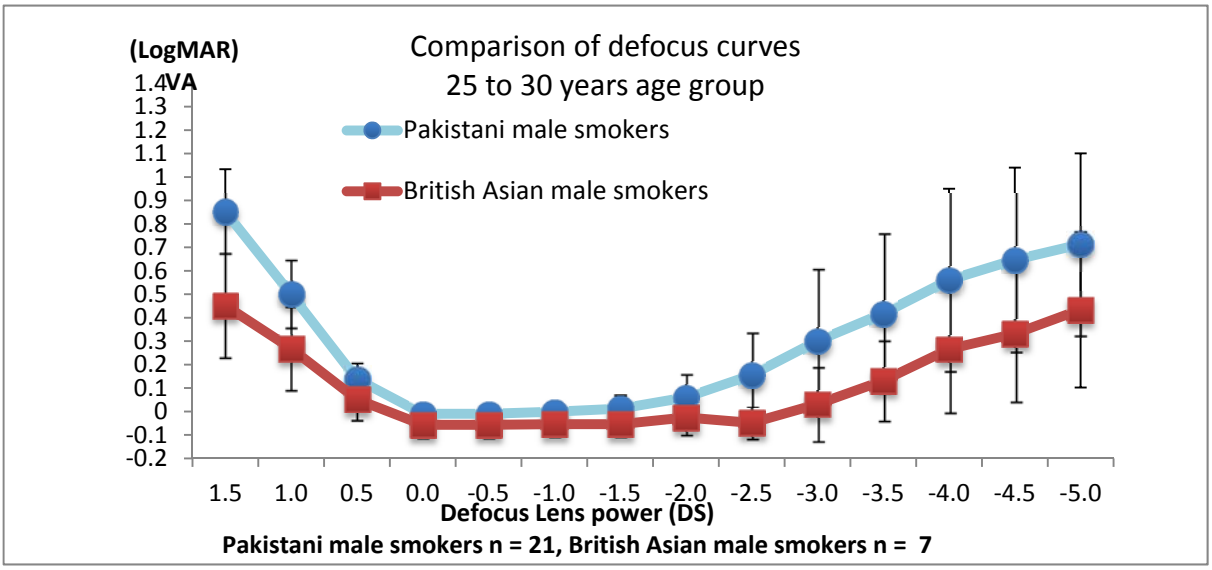


Figure 8.5: shows mean LogMAR VA attained from defocus lens power (ranged from +1.5 DS to -5.0 DS) for both Pakistani and British Asian male smoker participants of 25-30 years of age group.

8.7 Dietary analysis of British Asian versus Pakistani participants

Table 8.7 displays the mean intake of dietary elements for Pakistani cohort and British Asian cohort as below:

Dietary elements	British Asian (Indo-Pak) participants	Pakistani Participants	P value
Vitamin A (IU) intake (vit A IU)	1721.0 ± 1490.4	2032.1 ± 2458.5	[a] 0.02*
Vitamin A (RAE) intake in micrograms (vit A RAE)	333.4 ± 295.5	556.7 ± 701.6	[a] 0.001*
Vitamin D intake in micrograms (vit D)	2.6 ± 4.0	1.2 ± 1.0	[a] 0.001*
Lutein and Zeaxanthin (L/Z) intake in micrograms	412.6 ± 764.2	291.4 ± 1000.2	[a] 0.003*
Polyunsaturated trans-fatty acids (PUFA) intake in grams	12.6 ± 10.1	10.4 ± 5.8	[a] 0.001*

[a] Wilcoxon Signed Rank test,
*p value < 0.05

Table 8.7: shows mean intake of vitamin A (measured in IU and RAE), vitamin D, lutein and zeaxanthin, and poly-unsaturated trans-fatty acids for Pakistani and British Asian cohorts

These results suggest that Pakistani participants had significantly higher vitamin A (IU and RAE). In contrast, all other dietary elements intake was significantly higher in British Asian participants.

8.8 Comparison of NAFLTBUT versus dietary elements for both cohorts

As mentioned in the previous chapter (section 7.8) although age is a risk factor for dry eye disease above the age of 50 years, this study only had patients below that age so the data is not sub divided to be age-matched.

8.8.1 Comparison of NAFLTBUT against intake of vitamin A (mg) gradations for Pakistani and British Asian (Indo-Pak origin)

Intakes of vitamin A dietary element was graded into seven categories. Only grade one to grade five categories was analysed. There were fewer than five participants in British Asian cohort who had vitamin A intake value of more than 3.10 mg per day thus analysis on the other grades as not possible. Table 8.8 shows the mean NAFLTBUT against each grade of vitamin A (mg) as below:

Vitamin A grades in mg	PAK cohort grade 1	British Asians grade 1	PAK cohort grade 2	British Asians grade 2	PAK cohort grade 3	British Asians grade 3	PAK cohort grade 4	British Asians grade 4	PAK cohort grade 5	British Asians grade 5
N	44	21	45	21	15	5	10	6	7	5
Mean NAFLTBUT	10.5	9.5	8.8	8.8	8.1	17.1	9.6	7.2	7.7	9.9
Std. Deviation	3.5	5.5	3.2	3.3	2.8	9	1.5	3.1	2.7	7
P-value	[a] 0.2		[a] 0.6		[a] 0.1		[b] 0.048*		[a] 0.9	

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test,
*P value < 0.05
Gradations of vitamin A (mg): grade one = up to 0.50 mg, grade two = 0.51 to 1.0 mg, grade three = 1.10 to 1.50 mg, grade four = 1.51 to 2.0 mg, grade five = 2.01 to 2.50 mg.
Table 8.8: shows descriptive data of fluorescein tear break-up time against five gradations of vitamin A intake measured in milligrams for Pakistani and British Asian (Indo-Pak origin)

This result suggested that Pakistani participants, who had 2.01 to 2.50 mg intake of vit A, had better NAFLTBUT compared to ethnically similar British Asian participants with similar vit A intake.

8.8.2 Comparison of NAFLTBUT against intake of vit A RAE gradations for Pakistani and British Asian (Indo-Pak origin)

Table 8.9 displays the mean NAFLTBUT against each grade of vit A (RAE) as below:

Vitamin A (RAE) grades	British Asians grade 1	Pak cohort grade 1	British Asians grade 2	Pak cohort grade 2	British Asians grade 3	Pak cohort grade 3
N	47	84	10	19	6	34
Mean NAFLTBUT	9.1	9.6	12.5	8.5	8.8	9
Std. Deviation	5	3.4	7.3	3.2	5.2	2.5
P-value	[a] 0.2		[a] 0.9		[b] 0.049*	

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test,
*p < 0.05
Gradations of vitamin A (RAE): grade one up to 400 µg, grade two = 400.0 to 700.0 µg & grade three = 700.1 µg and above
Table 8.9: shows descriptive data of fluorescein tear break-up time against three gradation of vitamin A (RAE) intake measured in micrograms for the Pakistani and British Asians (Indo-Pak origin)

This result suggested that British Asians participants, who had 400 to 700 micrograms intake of vit A (RAE), had better NAFLTBUT compared to their genetically similar Pakistani participants with similar vit A intake.

8.8.3 Comparison of NAFLTBUT against intake of vit D gradations for Pakistani and British Asian (Indo-Pak origin)

Vit D measurements were graded into seven grades, but only grade one to grade four were compared due to the limitation in vit D intake (up to 4.0 µg) in Pakistani participants. Table 8.10 shows the mean NAFLTBUT against each grade of vit D as below:

Vitamin D grades	British Asians grade 1	Pak cohort grade 1	British Asians grade 2	Pak cohort grade 2	British Asians grade 3	Pak cohort grade 3	British Asians grade 4	Pak cohort grade 4
N	28	73	15	37	6	20	6	7
Mean NAFLTBUT	9.5	9.2	10.6	9.4	8	9.3	8.6	9.8
Std. Deviation	4.8	3	7.2	3.6	4	3.2	4.3	3
P-value	[a] 0.5		[a] 0.5		[a] 0.6		[b] 0.7	

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test
**Gradations of vitamin D: grade one = up to 1.0 µg, two = 1.01 to 2.0 µg, three =2.01 to 3.0 µg, four = 3.01 to 4.0 µg*
Table 8.10: shows descriptive data of fluorescein tear break-up time against four gradations of vitamin D intake measured in micrograms for Pakistani and British Asians (Indo-Pak origin)

There was no significant difference observed between NAFLTBUT values of Pakistani and British Asian participants against vitamin D intake gradations.

8.8.4 Comparison of NAFLTBUT against intake of L/Z gradations for Pakistani and British Asian (Indo-Pak origin)

L/Z intake measured in micrograms was categorised into seven gradations. For comparison between Pakistani cohort and British Asian cohort, grade two and, grade six were excluded, as British Asian cohort did not have sufficient participants for these categories (i.e. five or more).

Table 8.11 shows the mean NAFLTBUT against each grade of L/Z intake as below:

L/Z intake grades	British Asians grade 1	Pak cohort grade 1	British Asians grade 3	Pak cohort grade 3	British Asians grade 4	Pak cohort grade 4	British Asians grade 5	Pak cohort grade 5	British Asians grade 7	Pak cohort grade 7
N	15	58	10	7	16	38	8	10	6	5
Mean NAFLTBUT	9.6	9.4	11.52	10.3	8.6	8.7	9.7	9.3	7.7	9.8
Std. Deviation	5.3	3.4	6.3	2.7	4.7	2.8	6.5	2.3	3.2	2
P-value	[b] 0.5		[b] 0.9		[a] 0.4		[a] 1.0		[a] 0.4	

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test

**Gradations of L/Z intake: grade one = up to 50 µg, grade two = 50.1 to 100 µg, grade three = 100.1 to 200 µg, grade four = 200.1 to 350.0 µg, grade five = 350.1 to 500.0 µg, grade six = 500.1 to 1000.0 µg and grade seven = 1000.1 µg or above*

Table 8.11: shows descriptive data of fluorescein tear break-up time against seven gradations of lutein and zeaxanthin intake measured in micrograms for Pakistani and British Asians (Indo-Pak origin)

8.8.5 Comparison of NAFLTBUT against intake of PUFA gradations for Pakistani and British Asian (Indo-Pak origin)

Polyunsaturated trans-fatty acids (PUFA) dietary intakes were graded into four grades, as mentioned in chapter two. For comparison, only grade one and grade two were compared as British Asian participants did not have more than five participants in grade three and grade four. The Pakistani data did not have sufficient participants (i.e. five or more) who had more than 30.0 grams of PUFA dietary intake. Table 8.12 displays the mean NAFLTBUT for each grade for both cohorts as shown below:

PUFA grades	British Asians grade 1	Pak cohort grade 1	British Asians grade 2	Pak cohort grade 2
N	32	70	23	59
Mean NAFLTBUT	9.5	9.6	9.5	9
Std. Deviation	5.2	3.6	4.7	2.7
P-value	[a] 0.3		[a] 0.9	

[a] Wilcoxon Signed Rank test

**Gradations of PUFA: grade one = under 10.0 g, grade two = 10.1 to 20.0 g, grade three = 20.1 to 30.0 and grade four = above 30.1 g*

Table 8.12: shows descriptive data of fluorescein tear break-up time against two gradations of polyunsaturated trans-fatty acids intake measured in grams for Pakistani and British Asian cohorts (Indo-Pak origin)

8.9 Dietary comparison of AoA in different age groups

As mentioned in section 7.9, since AoA decreases with age the data will be age matched for comparison.

In the 18 to 24 years age group there were 37 participants from the British-Asian cohort and 29 participants from the Pakistani cohort. The mean AoA and dietary elements intake is displayed in table 8.13 for these subjects.

Age group 18-24 years	Mean value for British-Asian	Mean value for Pakistani cohort	Significance (p-value)
Amplitude of Accommodation (Dioptres)	11.0 ± 1.7	9.1 ± 2.1	[a] 0.001*
Vitamin A (as beta-carotene) intake (milligrams)	1.0 ± 1.0	1.2 ± 1.3	[a] 0.4
Vitamin A intake (retinol activity equivalent) (micrograms)	298.8 ± 276.6	537.3 ± 657.3	[a] 0.1
Vitamin D intake (micrograms)	2.7 ± 4.7	1.5 ± 1.0	[a] 0.9
Lutein and zeaxanthin intake (micrograms)	500.4 ± 952.3	220.0 ± 224.3	[a] 0.2
Polyunsaturated trans-fatty acids (grams)	12.8 ± 10.2	11.3 ± 5.5	[a] 0.7

[a] Wilcoxon Signed Rank test, *p < 0.05

Table 8.13: showing mean and standard deviation of amplitude of accommodation and dietary elements intake for the age group of 18 to 24 years old from the Pakistani and British-Asian cohorts

In the 25 to 30 years age group there were 14 participants from the British-Asian cohort and 29 participants from the Pakistani cohort. The mean AoA and dietary elements intake is displayed in table 8.14 for these subjects.

Age group 25-30 years	Mean value for British-Asian	Mean value for Pakistani cohort	Significance (p-value)
Amplitude of Accommodation (Dioptres)	9.4 ± 1.6	8.7 ± 2.7	[a] 0.3
Vitamin A (as beta-carotene) intake (milligrams)	1.2 ± 1.0	1.1 ± 1.0	[a] 0.6
Vitamin A intake (retinol activity equivalent) (micrograms)	383.6 ± 304.3	532.1 ± 535.1	[a] 0.5
Vitamin D intake (micrograms)	2.6 ± 3.1	1.3 ± 1.2	[a] 0.02*
Lutein and zeaxanthin intake (micrograms)	348.0 ± 335.6	257.2 ± 344.5	[a] 0.1
Polyunsaturated trans-fatty acids (grams)	9.5 ± 3.7	12.1 ± 6.4	[a] 0.3

[a] *Wilcoxon Signed Rank test, *p < 0.05*

Table 8.14: showing mean and standard deviation of amplitude of accommodation and dietary elements intake for the age group of 25 to 30 years old from the Pakistani and British-Asian cohorts

In the 31 to 35 years age group there were six participants from the British-Asian cohort and 16 participants from the Pakistani cohort. The mean AoA and dietary elements intake is displayed in table 8.15 for these subjects.

Age group 31-35 years	Mean value for British-Asian	Mean value for Pakistani cohort	Significance (p-value)
Amplitude of Accommodation (Dioptres)	7.6± 0.4	7.6 ± 1.6	[b] 0.2
Vitamin A (as beta-carotene) intake (milligrams)	0.8 ± 0.7	1.1 ± 1.8	[a] 0.4
Vitamin A intake (retinol activity equivalent) (micrograms)	365.3 ± 356.8	546.7 ± 901.0	[a] 0.3
Vitamin D intake (micrograms)	2.8 ± 2.3	1.2 ± 1.0	[b] 0.3
Lutein and zeaxanthin intake (micrograms)	300.4 ± 472.4	123.0 ± 178.1	[a] 0.4
Polyunsaturated trans-fatty acids (grams)	19.2 ± 18.2	10.0 ± 4.4	[a] 0.6

[a] Wilcoxon Signed Rank test, [b] Paired Samples t-test

Table 8.15: showing mean and standard deviation of amplitude of accommodation and dietary elements intake for the age group of 31 to 35 years old from the Pakistani and British-Asian cohorts

8.10 Discussion

8.10.1 TBUT comparison

The study did a sub-group analysis between Pakistani participants and British Asian participants from an Indo-Pak background. These participants were genetically the same, but they were exposed to different environments (UK and Pakistan). There was a significant difference present between the mean ages of British Asian participants and Pakistani participants. Pakistani participants were approximately nine years older than their British Asian counterparts were.

The current study did not find any significant difference of TBUT between British Asian participants and Pakistani participants based on gender and smoking status. This result was similar to the result of previous chapter comparison between the UK and Pakistani participants, where the study did not find any significant difference between Pakistani participants and UK participants.

Recently, two studies compared East Asian participants' ocular surface parameters with Caucasian participants who were genetically different but had a similar environment. Kim *et al.* (2019) found no significant difference in TBUT between East Asians and young Caucasian population. In contrast, Craig *et al.* (2019) found a substantial decrease in TBUT in East Asian participants when compared to Caucasians. To date, this is the first study, which has compared TBUT of participants with same ethnic background but living in different geographical locations.

There was a significant difference observed in mean OSDI scores between Pakistani smokers and British Asian smokers. Pakistani smokers had lower OSDI scores when compared to British smokers and non-smokers. One of the possible reason for this result could be because the OSDI questionnaire may not be compatible for Southeast Asians participants, as argued by Lu *et al.* (2018).

8.10.2 Accommodative ability comparison

The current study compared AoA between two cohorts based on smoking status, age group, and gender. The study did not find any significant difference in mean AoA between Pakistani and British Asian male and female non-smoker participants. However, the study found British Asian male smokers had better AoA than Pakistani male smokers' participants. The difference in UV exposure, high temperature, the geographical difference could be possible reasons this finding (Miranda, 1979, Hashemi *et al.*, 2017b) The difference in smoking habits could also be another factor influencing this outcome. On average, Pakistani smokers were smoking more cigarettes per day than British Asian smokers were. It is evident from the literature that

smoking has a dose-response relationship with its associated diseases (Hammond *et al.*, 1999b, Thomas *et al.*, 2012).

The current study found a significant difference in the subjective clear vision range attained from defocus lenses in both cohorts. British Asian non-smokers and smokers had better subjective clear vision range compared to Pakistani non-smokers and smokers of similar age groups.

Vit A intake (IU and RAE) intakes were significantly higher in Pakistani cohort compared to British Asian cohort. This was probably due to the nature of Pakistani diet (Gallup, 2011), which is usually rich in vitamin A (NIH, 2018b). In contrast, vit D and lutein and zeaxanthin intakes were found to be significantly higher in British Asian compared to their Pakistani counterparts. Socio-economic difference and lack of awareness can lead to the problem of affordability of food items in Pakistan can be a possible explanation for this difference.

This study did not find any significant difference in TBUT against dietary elements intakes of vit D, PUFA, and L/Z between British Asian cohort and Pakistani cohort. However, the study found a higher TBUT in the Pakistani cohort for grade four comparison of vit A compared to British Asian participants. In contrast, a higher TBUT was seen in British Asian participants of grade two intake of vit A (RAE) compared to Pakistani cohort.

A comparison of the dietary elements intake and AoA, showed a significant difference observed in mean AoA of both cohorts. British Asians had better AoA compared to their Pakistani counterparts against similar intake of dietary elements. A possible explanation for this is because of the age difference of both cohorts. British Asians participants were significantly younger than Pakistani participants, and it is well documented that with the increase in age, AoA will decrease (Benzoni and Rosenfield, 2012, Hashemi *et al.*, 2017b).

The study compared age group wise comparison of dietary elements intake and AoA and found a significant difference in mean AoA in the age group of 18 to 24 years old participants. British-Asian participants of 18-24 years old age group had significantly better AoA compared to the Pakistani participants of similar age group without any significant difference of dietary elements intake and hence ruling out the ethnic and dietary differences that can influence the results.

Chapter 9

Transient effects of smoking on ocular health

Whilst conducting the main data collection from UK subjects the investigatory team noted that some subjects had just had a cigarette before they presented for their study visit. The idea was considered to investigate the immediate effects of smoking on the tears and accommodation (AoA and defocus curves). The MPOD values were not measured as it was felt that the measurement time would be too long for any immediate effects to be still present.

9.1 Introduction

The cumulative effect of long-term smoking may be responsible for many ocular and systematic conditions by producing an allostatic load on the body. Smoking is also responsible for the short-term effects on the body such as; increased heart rate and blood pressure, reduction of blood flow to hands and feet, short breath etc. (CDC, 2014, Piano *et al.*, 2010). Nicotine is one of the primary active chemical agents in cigarette smoke, is responsible for short term/acute pharmacological effects of smoking (Tutka *et al.*, 2005).

It is believed that nicotine has a hemodynamic impact and it acts as a sympathomimetic drug to increase heart rate, blood pressure, and cardiac contractility and to constrict some blood vessels (Tachmes *et al.*, 1978, Piano *et al.*, 2010). The other compounds present in cigarette smoke, e.g. carbon monoxide, hydrogen cyanide, nitrogen oxides, benzene, cadmium (Haussmann, 2012) are responsible for long-term adverse effects (Piano *et al.*, 2010).

The effects of smoking on the pre-corneal tear film are well reported in the literature, but in terms of the immediate effect of smoking on the tear film, there is little literature. Rummenie *et al.* (2008) investigated the brief effects of smoking on the ocular surface and the tear film in healthy non-smokers eyes and found worsening signs for TBUT, tears evaporation rate, tear lipid spread time and vital staining scores after five minutes of exposure to smoking and after 24 hours to smoke when compared to the baseline values. Although after 24 hours to smoke ocular exposure signs were getting better and approaching near to normal (baseline values) but those were still statistically different from the baseline values. Changes in tear cytokines provide evidence for adverse effects of smoking, as an increase in tear inflammatory cytokines, tears lipid peroxidation, and decrease of mucosal defence was found in the tear samples and impression cytology specimens (Rummenie *et al.*, 2008).

Few studies have shown that smoking affects pupil size (Erdem *et al.*, 2015, Lie and Domino, 1999). Lie and Domino (1999) investigated pupil diameter, heart rate, blood pressure, pre, and

post smoking in smokers and non-smokers. After conducting pupil measurement with an instant camera, they concluded that the pupil constricts slightly but statistically significant after sham (fake) or tobacco smoking for both smoking categories. Erdem *et al.* (2015) conducted a study to evaluate the acute effects of smoking on pupil sizes and wavefront aberrations by using a NIDEK OPD-Scan II system. The study concluded that photopic and mesopic pupil sizes decreased after smoking.

The human iris receives parasympathetic and sympathetic nervous innervations, the sphincter iris muscle under the control of sympathetic nerves and dilator iris muscles under the control of parasympathetic nerves (Winn *et al.*, 1994). Unlike heart rate and blood pressure (BP) where nicotine acts as a sympathomimetic drug to increase the heart rate and BP as a result of releasing epinephrine(Piano *et al.*, 2010). In the case of the iris, these results are surprisingly contraindicated and it appears that the sphincter iris muscle is relatively more activated by the parasympathetic system than sympathetic system (Erdem *et al.*, 2015).

In this body of work, it was noted that some participants in chapter number two had just smoked a cigarette and this raised the question if there were immediate effects on the eye that would be transient in their nature.

9.2 Study aim

The purpose of this study was to assess the immediate effects of smoking on the pre-corneal tear film, pupil size, and accommodative status of the eyes in healthy smokers.

9.3 Methods

The study design was a prospective cross-sectional study. The smokers were recruited from Aston University and were students and staff. The Aston University research ethics committee approved the study and the research followed the tenets of the Declaration of Helsinki. Participants received a participant information sheet, which detailed what the research entailed. If they were happy to proceed, written informed consent was obtained.

9.3.1 Inclusion and exclusion criteria

Inclusion criteria:

- Aged between 18 to 50 years old
- No current contact lens use
- Subjects able to give written informed consent
- A regular cigarette smoker of one or more cigarettes per day
- LogMAR visual acuity of 0.0 or better

Exclusion criteria:

- Any active ocular disease/condition
- Systematic disease condition (e.g. hypertension or diabetes)
- Known diagnosis of dry eyes
- E-cigarette smokers

9.3.2 Study instruments

Following instruments were used in this research study.

1. Tearscope (EASYTEAR®view+, Trento, Italy) for analysing the TBUT and lipid layer non–invasively.
2. RAF near point rule (Clement Clarke Ltd, Essex, UK) for measuring an amplitude of accommodation.
3. CSO Slit lamp (SL990, Costruzione Strumenti Oftalmici, Firenze, Italy) for measuring TBUT invasively.
4. Bio fluoro fluorescein strips (Biotech Vision Care, Luzern, Switzerland)
5. CSO computerised visual acuity (Costruzione Strumenti Oftalmici, Firenze, Italy) chart for measuring defocus curves
6. Topcon vision meter VT-SE((Topcon, Tokyo, Japan) measuring an amplitude of accommodation by defocus curves
7. An Auto–refractor (NIDEK OPD-Scan III, Gamagori, Japan) for measuring participant's refractive error objectively (if participant visual acuity is not 20/20).
8. Keratograph 5M (Oculus Optigerate GmbH, Wetzlar, Germany) with tear film scan software for measuring TBUT non–invasively and lipid layer non–invasively.

Instruments are provided in the previous chapter (chapter 2).

9.3.3 Sample size

The maximum Sample size was calculated using G*Power 3.1(Faul *et al.*, 2007) using a paired t-test to show a medium effect size with 90% power and an alpha level of 0.05. The maximum number of subjects required was 44 and therefore, 45 subjects were recruited to ensure adequate statistical power and allow for attrition.

9.3.4 Experimental procedure

A study advertisement and research participant information sheet were distributed to the participants via email and in person. An appointment was then scheduled via email. The full study procedure was explained to the participants at their appointment. Any procedural queries

were answered before taking written consent. Participants were asked to abstain from smoking at least one hour before baseline measurements.

The only right eye was examined in this experiment. Subjective refraction was done if the participant's visual acuity was less than 0.0 LogMAR with the help of Topcon vision meter/phoropter after taking an estimation of refractive error with the help of Nidek OPD Scan III. The endpoint criterion was a maximum plus sphere and minimum minus cylinder power maintaining the best visual acuity. In most cases, the participant's glasses or prescription was used as a starting point of refraction. Before smoking, tear break up (TBUT) time was measured non-invasively with Keratograph K5Mand Tearscope and invasively on Slit lamp with the help of fluorescein strips. Three readings were recorded from each instrument in dim light conditions. Additionally, a single measurement reading was obtained for tear meniscus height (TMH), average pupil diameter (PD) and tear film lipid layer from the Keratograph 5m machine.

Three readings for the amplitude of accommodation (AoA) were recorded with RAF near point rule in bright light conditions. Subjective clear vision range was calculated by performing a defocus curves technique. LogMAR visual acuity was recorded with Landlot’s C chart by different lenses (range from +1.50 Ds to –1.50 Ds) in a randomised manner. A single letter was shown to the participant with four different directions, and the participant was asked to tell the direction of the letter. An approximately 30-second gap was given for each measurement where three repeated measurements were taken, and a gap of 1 minute was taken between different instruments. After this baseline data was collected participants were allowed to smoke one cigarette. Post–smoking examination was then performed on the same eye and all the instruments mentioned above were used again. NL took all measurements for the study.

9.3.5 Statistical Analysis

All measurements from a case report form (CRF) was noted down in the Microsoft Excel spreadsheet which was later exported to an SPSS sheet. Statistical analysis was performed by using SPSS 23.0 statistical package program for Windows (SPSS Inc., Chicago, IL, USA). Normality was confirmed for the data sets using Shapiro–Wilk test $p > 0.05$. A parametric data underwent a parametric statistical analysis (Paired Samples t-test). Non-parametric data underwent a non-parametric statistical analysis (Wilcoxon Signed Rank Test). A value of less than 0.05 was considered as statistically significant.

9.4 Results

Forty-five participants (30 male and 15 female) with the mean age of 22.0 ± 4.4 years old (range 18 to 42, median 21.0 years) were included. The average age for male participants was 21.5 ± 3.4 years old, and for female participants, 23.1 ± 5.9 years old.

Table 9.1 shows summary of findings (excluding defocus curves) as below:

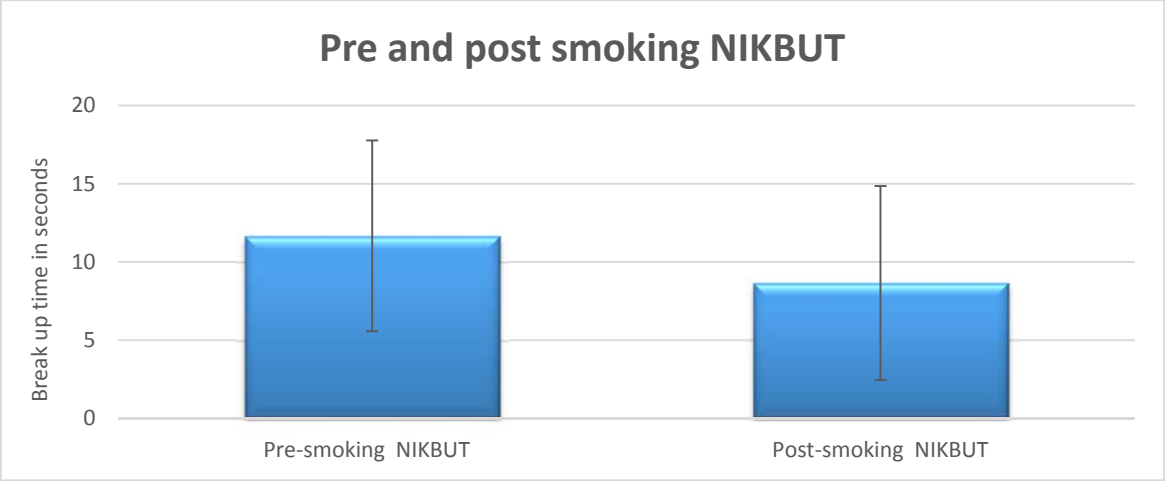
Ocular tests	Pre-smoking measurements	Post-study measurements	P-value
NIKBUT (seconds)	11.6 ± 6.1	8.6 ± 6.2	0.001*
NITBUT (seconds)	9.8 ± 4.0	7.0 ± 2.3	0.001*
NAFLTBUT (seconds)	6.7 ± 3.2	4.6 ± 2.6	0.001*
K5M lipid layer thickness (nanometres)	79.1 ± 30.1	61.8 ± 18.7	0.001*
Tearscope lipid layer thickness (nanometres)	65.7 ± 20.7	54.2 ± 19.3	0.001*
TMH (millimetres)	0.4 ± 0.1	0.3 ± 0.1	0.1
Pupil size (millimetres)	5.6 ± 1.1	5.3 ± 1.0	0.008*
AoA (Dioptres)	10.5 ± 1.8	9.9 ± 1.7	0.001*

* Significant results $p < 0.05$

Table 9.1: difference in measurements before and after smoking

9.4.1 TBUT (non–invasive and invasive)

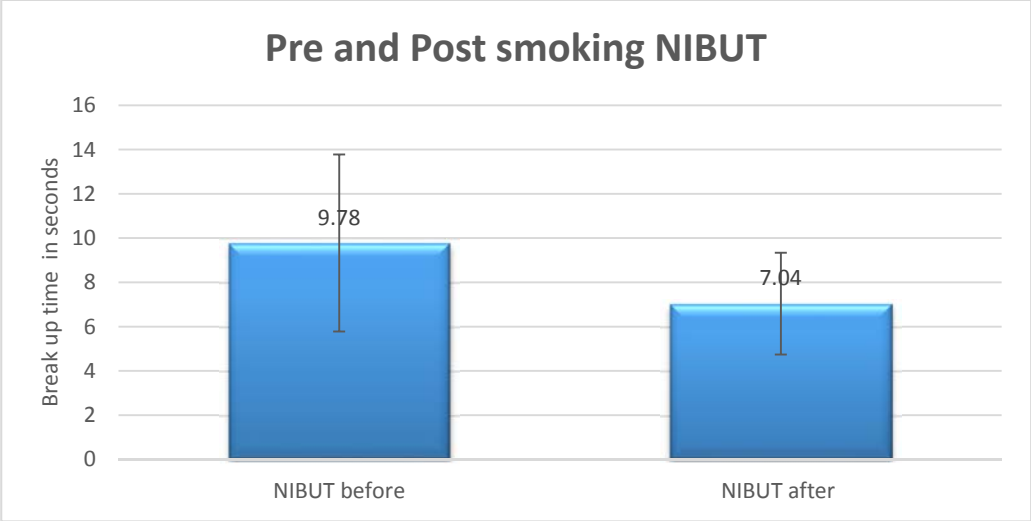
The post–smoking mean non–invasive Keratograph tear break up time (NIKBUT) was 8.7 ± 6.2 seconds (s), which was numerically lower than the pre-smoking NIKBUT 11.7 ± 6.1(s). A Wilcoxon Signed Rank test (Shapiro-Wilk test, $p < 0.05$) indicated a significant difference between pre and post smoking NIKBUT, $Z = -3.88$, $p < 0.001$, $r = -0.40$. A graphical representation of means and 95 % confidence intervals are displayed in figure 9.1.



**P value = 0.001*

Figure 9.1: showing mean non-invasive Keratograph tear break-up time before and after five minutes of posting smoking

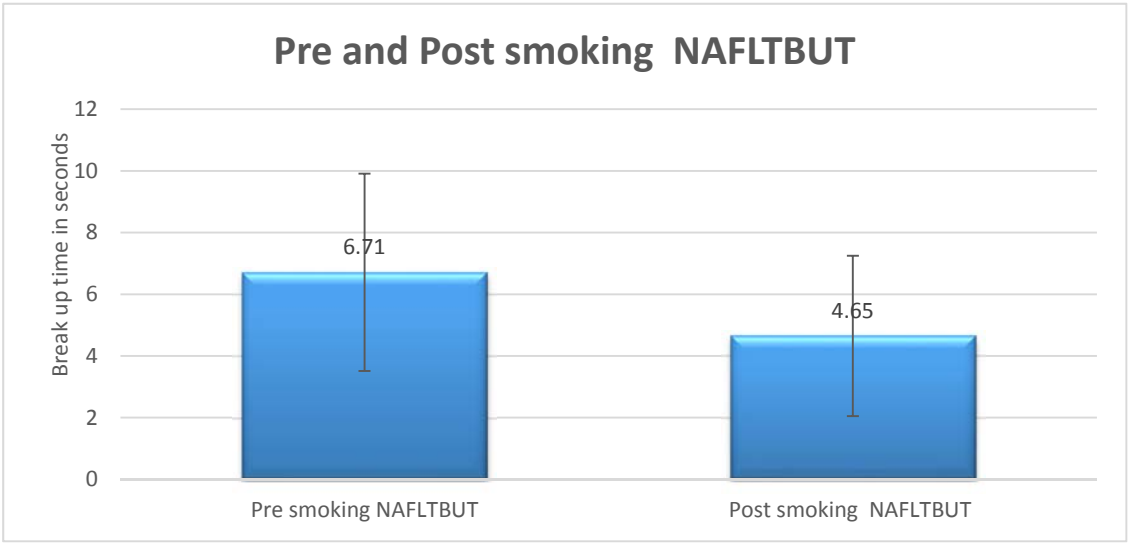
The post–smoking mean non–invasive Tearscope tear break up time (NIBUT) was 7.0 ± 2.3 (s), which was numerically lower than the pre-smoking NIBUT 9.8 ± 4.0 (s). A Wilcoxon Signed Rank test (Shapiro-Wilk test, $p < 0.05$) indicated a significant difference between pre and post smoking, $Z = -5.60$, $p < 0.001$, $r = -0.59$. A graphical representation of means and 95 % confidence intervals are displayed in figure 9.2.



**P value = 0.001*

Figure 9.2: showing mean non-invasive Tearscope tear break-up time before and after five minutes of posting smoking

The post–smoking mean invasive fluorescein tear break up time (NAFLTBUT) was 4.7 ± 2.6 (s), which was numerically lower than the pre-smoking NAFLTBUT 6.7 ± 3.2 (s). A Wilcoxon Signed Rank test (Shapiro-Wilk test, $p < 0.05$) indicated a significant difference between pre and post smoking $Z = -5.52$, $p < 0.001$, $r = -0.58$. A graphical representation of means and 95 % confidence intervals are displayed in figure 9.3.

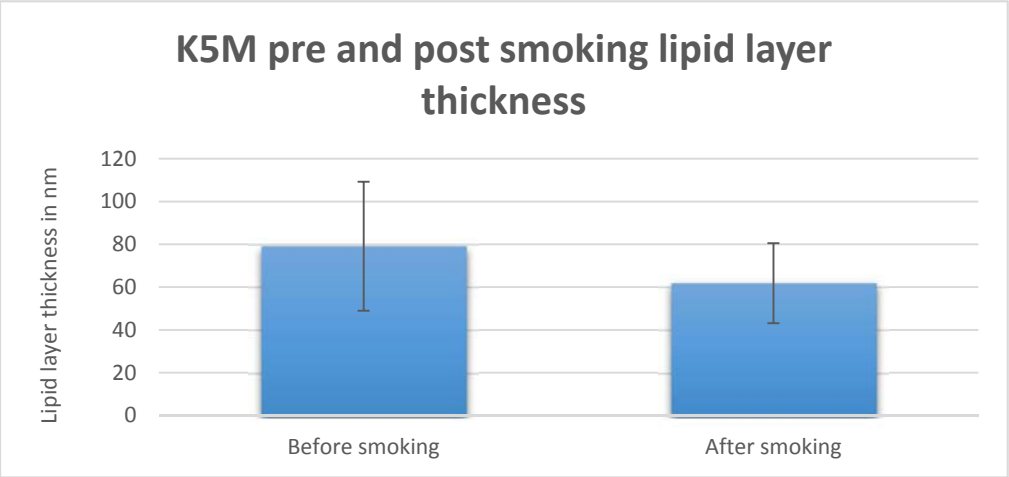


**P value = 0.001*

Figure 9.3: showing mean invasive fluorescein tear break-up time before and after five minutes of posting smoking

9.4.2 Tear film lipid-layer thickness

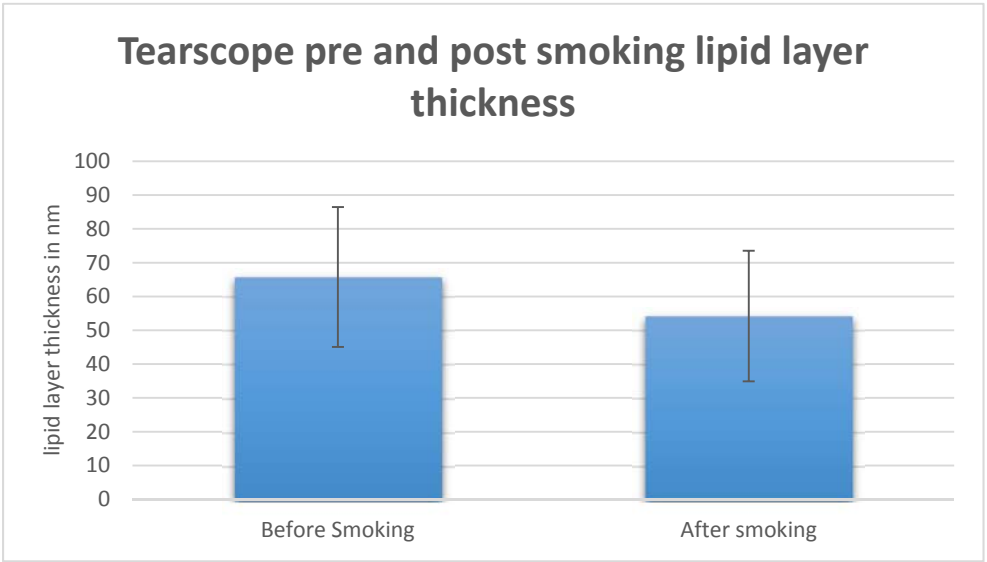
The post–smoking test mean K5 lipid layer thickness was 61.8 ± 18.7 nanometres (nm), which was numerically lower than pre-smoking test mean K5 lipid layer thickness 79.1 ± 30.1 nm. A Wilcoxon Signed Rank test (Shapiro-Wilk test, $p < 0.05$) indicated a significant difference between pre and post smoking K5M lipid layer thickness, $Z= -4.91$, $p = 0.001$, $r = -0.52$. A graphical representation of means and 95 % confidence intervals are displayed in figure 9.4



**P value = 0.001*

Figure 9.4: Keratograph (K5M) lipid layer thickness measured in nanometres before and after five minutes of cigarette smoking.

Similarly, the post–smoking test mean tearscope lipid layer thickness was 54.2 ± 19.3 nm, which was numerically lower than pre-smoking test mean lipid layer thickness 65.8 ± 20.7 nm. A Wilcoxon Signed Rank test (Shapiro-Wilk test, $p < 0.05$) indicated a significant difference between pre and post smoking tearscope lipid layer thickness, $Z= -5.09$, $p = 0.001$, $r = -0.53$. A graphical representation of means and 95 % confidence intervals are displayed in figure 9.5.



**P value = 0.001*

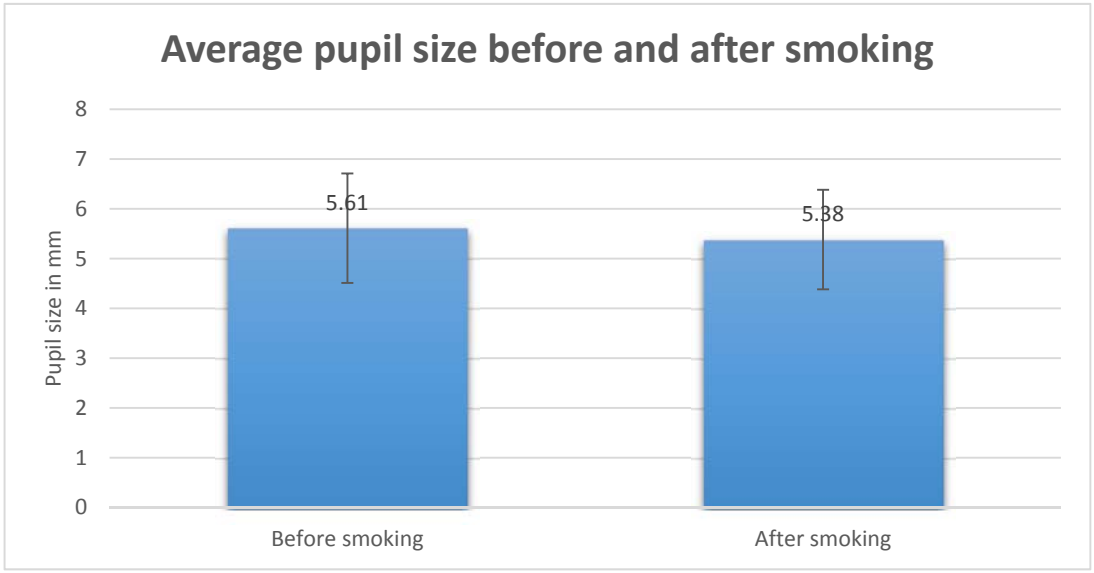
Figure 9.5: Tearscope lipid layer thickness before and after five minutes post cigarette smoking measured in nanometres.

9.4.3 Tear meniscus height (TMH)

The mean post–smoking TMH was 0.4 ± 0.1 millimetres (mm), which was marginally higher than mean TMH of pre-smoking 0.3 ± 0.1 mm. A Wilcoxon Signed Ranked test (Shapiro–Wilk test, $p < 0.05$) indicated no statistically significant difference between pre and post smoking TMH, $Z = -1.40$, $p = 0.16$.

9.4.4 Pupil size

A Paired t-test (Shapiro-Wilk test, $p > 0.05$) was conducted to compare post smoking average pupil size with pre-smoking average pupil size. There was a significant difference observed in mean (M) pre-smoking pupil size (M = 5.6 ± 1.1 mm) and post-smoking pupil size (M = 5.38 ± 1.0); t (43) 2.76, $p = 0.008$, $r = 0.41$. A graphical representation of means and 95 % confidence intervals are displayed in figure 9.6.

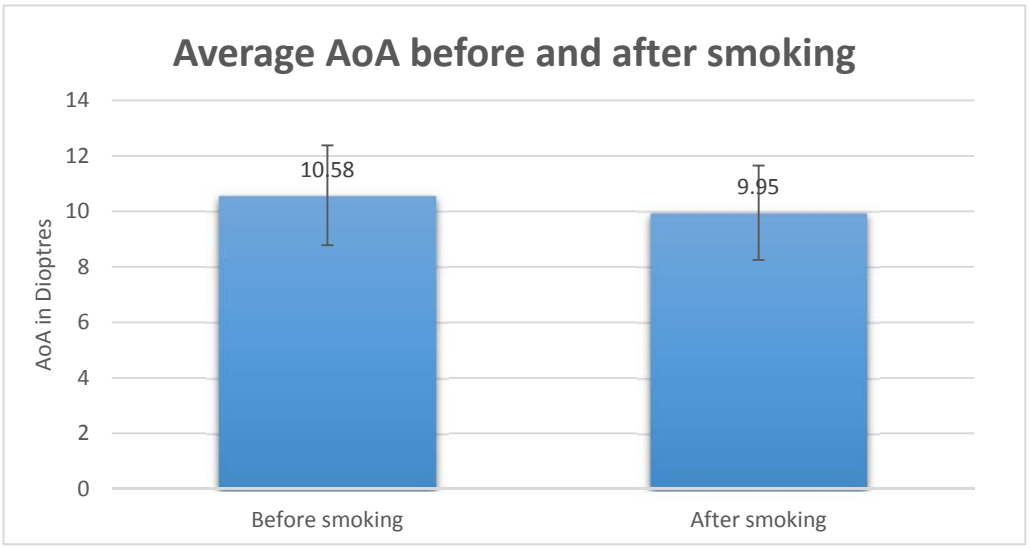


**P value = 0.008*

Figure 9.6: Average pupil size measured in millimetres before and after five minutes of smoking

9.4.5 Amplitude of Accommodation (AoA)

The post–smoking test mean AoA lipid was 9.9 ± 1.7 Dioptres (D) which were numerically lower than pre-smoking test mean AoA 10.6 ± 1.8 D. A Paired Samples t-test (Shapiro-Wilk test, $p > 0.05$) indicated that the difference was statistically significant t (44) 4.67, $p = 0.001$, $r = 0.69$. A graphical representation of means and 95 % confidence intervals are displayed in figure number 9.7.



**P value = 0.001*

Figure 9.7: Average amplitude of accommodation measured in Dioptres before and after five minutes of smoking

9.4.6 Defocus curves

The mean post–smoking LogMAR defocus curves from +0.5Ds to -5.00Ds were either marginally lower or equal to pre–smoking defocus curves and hence no significant statistical or numerical difference found. However, post–smoking LogMAR defocus curve difference for +1.50 Ds and + 1.00 Ds was numerically different (mean 0.51 ± .1 LogMAR & 0.28 ± 0.10 LogMAR for +1.50 and + 1.00 Ds lenses respectively) compared to pre–smoking LogMAR defocus curves values (0.47 ± 0.10 LogMAR & 0.25 ± 0.10 LogMAR).

A Wilcoxon Singed Rank Test (Shapiro-Wilk test, $p < 0.05$) indicated that median post–smoking test ranks were lower (Mdn for +1.50 Ds = 0.52 & Mdn for +1.00 Ds = 0.24 to median pre–smoking test ranks (i.e. Mdn for +1.50 Ds = 0.50 & Mdn for +1.00 Ds = 0.22). The mean difference was still statistically significant, $Z= -2.45$ $p = 0.014$ and $Z = -2.06$, $p = 0.04$ for +1.50 Ds & +1.00 Ds respectively. A graphical representation of means and 95 % confidence intervals are displayed for defocus curves ranging from +1.50 Ds to – 5.00 Ds in figure 9.8.

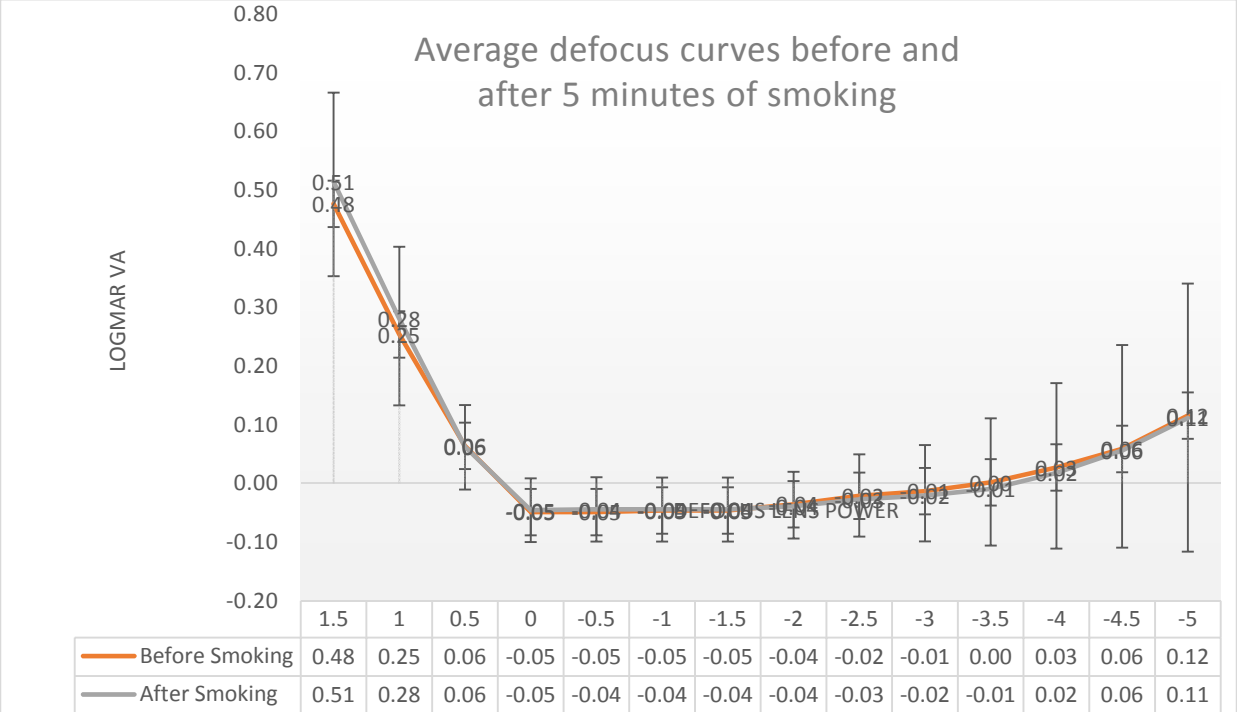


Figure 9.8: Subjective clear vision range measure in LogMAR, attained by defocus lenses (ranged +1.5 to -5.0 Dioptres sphere) before and after five minutes of smoking

9.5 Discussion

9.5.1 Transient effects of smoking on tear film

The ocular surface is the most exposed mucosal surface of the human body and it encounters challenges from the environment such as wind, extreme temperatures, UV radiation, pollen particles, and tobacco smoke. The effects of cigarette smoke on ocular tear film have been well documented from as early as the late 1970s (Basu *et al.*, 1978). Since then, there are several articles published on the effects of smoking on tear film and ocular surfaces (Satici *et al.*, 2003, Yoon *et al.*, 2005b, Altinors *et al.*, 2006, Matsumoto *et al.*, 2008, Thomas *et al.*, 2012, Masmali *et al.*, 2016). All of the studies presented in the literature have reported that chronic active smoking is associated with tear instability and adverse effects on the ocular surface. Low corneal and conjunctival sensitivities, increased conjunctival squamous metaplasia, alternation of tears proteins and reduced goblets cell among smokers were also reported in many articles (Yoon *et al.*, 2005b, Rummenie *et al.*, 2008, Thomas *et al.*, 2012).

A single study was identified from the literature that researched the transient effects of smoking on tear film (Rummenie *et al.*, 2008) on healthy non-smokers who were exposed to secondary smoking by a smoking chamber. Additionally, transient effects of smoking on pupil size and accommodative ability of eye are new concepts and only a few studies have been conducted on the acute effects of smoking on them. Smoking is related to an immediate constriction of

the pupil (Erdem *et al.*, 2015) and on increasing objective accommodation (Bardak *et al.*, 2017).

With respect to the fluorescein TBUT results, this study's results are consistent with Rummenie *et al.* (2008), as a significant decrease in TBUT was seen followed by brief cigarette smoke. The study investigated two different methods of measuring non–invasive TBUT with the help of Keratograph K5 and EASYTEAR®view+ Tearscope and found a significant decrease in TBUT; these results are also consistent with fluorescein TBUT results. The study found a substantial reduction in the thickness of the tear film lipid layer after exposure of cigarette smoke. This study did not perform lipid layer interferometry as previous studies (Rummenie *et al.*, 2008, Altinors *et al.*, 2006) did but still tear lipid layer instability has been observed by comparing pre and post smoking lipid layer thickness.

There are many possible reasons why tear film instability is affected by smoking and it is a quite complex mechanism to understand. However, Lipid peroxidation of the outer layer of the tear film is one of the reported possible mechanisms that might be responsible for pre-corneal tear film break down as argued by Thomas *et al*. (2012) and Altinors' *et al.* (2006). Cigarette mainstream smoke contains over 4000 active compounds in its tar and gas phases, which includes nicotine and tar. The substances in the gas phase can enter through the airway epithelial barrier, enter the systemic circulation via the pulmonary circulation, and increase systemic oxidative damage, leading to the development of cigarette smoking-related diseases (Horinouchi *et al.*, 2016).

Nicotine activates macrophage reactions at the cellular level. Human macrophages interacting with carbonyl or cigarette smoke have been observed to modify extracellular matrix proteins that reduce their ability to phagocytose apoptotic neutrophils (Kirkham *et al.*, 2004). It is hypothesised that this mechanism may also apply to the ocular surface as increased apoptosis of conjunctival epithelial cells is observed in patients with Kerato-conjunctivitis sicca. Alternatively, smoking can cause ocular epithelial damage by its direct contact with the ocular surface, as suggested by Satici *et al.* (2003). Satici *et al.* (2003) observed a higher number of squamous cell metaplasia in the conjunctival epithelium of smoker, which could be caused by toxic and irritant materials present in cigarette smoke.

9.5.2 Transient effects of smoking on pupil size

Effects of smoking on pupillometry are still not studied extensively even though the first study that observed this relationship was conducted in 1969. So far, few studies are done on this topic, results from these studies are inconsistent, and the mechanism of action is still unclear. Roberts and Adams (1969) reported that after rapidly inhaling cigarette smoke, there is an average 0.75 mm increase in pupil size. Pupil size then returned to the original size in less than three minutes of post-smoking exposure. Sobaci *et al.* (2013) observed that photopic

pupil sizes of chronic smokers (5.36 ± 0.73 mm) was different from non-smokers (4.73 ± 0.58 mm; p = 0.001) and suggests that chronic smoking may dilate the pupil size. A recent pre and post–smoking study by Bardak *et al.* (2017) found no significant mean pupil size difference before (mean 5.72 ± 1.21 mm) and after smoking (mean 5.68 ± 1.14 mm) of a single cigarette (p = 0.62).

In contrast, Lie and Domino (1999) reported a decreased pupil size among smokers after smoking one cigarette. Erdem *et al.* (2015) reported results consistent with Lie and Domino (1999) findings in terms of pupil size, in their pre and post–smoking results and found a significant decrease in mean photopic pupil size from 3.52 ± 0.7mm to 3.29 ± 0.5mm (p = 0.001) after smoking. Mean mesopic pupil size was also decreased from 6.42 ± 0.7mm to 6.14 ± 0.7mm after smoking (p = 0.001). The results from this study are also consistent with Lie and Domino (1999) & Erdem *et al.* (2015) results. The current study found a significant constriction in mesopic pupil size and photopic pupil size.

In the current study, all participants were asked to abstain smoking at least one hour before their baseline measurements while a previous but recent study by Bardak *et al.* (2017) followed at least 12 hours abstinence period of smoking. The variation in abstinence time of smoking could be associated with pupillometry results. It has been noticed that previous studies (Erdem et al .2015 & Bardak et al. 2017) included only those smoker participants who smoked at least 10 or more cigarettes for at least five years whereas, in this study, participants were considered as a smoker if they smoked at least one cigarette per day. This variation in the smoking pattern can also be associated with different results as the smoking habit is a high dose–responsive.

9.5.3 Transient effects of smoking on accommodation

To date, there are only three studies found in the previous literature that has shown any relationship of smoking with an accommodation facility of the eye. Roberts and Adams (1969) observed that immediately after puffing in cigarette smoke, there was 1.25 D decrease in AoA, and participants retain their normal AoA back in less than five minutes of smoking cessation. Ide *et al.* (2012), found that there was a significant difference in the mean amplitude of accommodation in smokers (4.9 ± 2.7 D) compared to mean amplitude of accommodation of non–smokers (6.9 ± 3.1 D, p = 0.001) measured objectively by using a newly developed compact accommodator. The point to be noted here is the study design, although Ide *et al*. (2012) compared AoA between smokers and non-smokers, the study was not designed to observed pre and post- smoking accommodation change. It can be argued that their study can be referenced in a general context for the effects of smoking on AoA.

In contrast, Bardak *et al.* (2017) reported that after acute smoking, objective amplitude of accommodation increased with every dioptre of stimulus and the increase of accommodation

was significant at 2 D (pre-smoking -0.12 ± 1.5 to 0.10 ± 1.6 after smoking p =0.02) and 3 D (pre-smoking 0.05 ± 1.6 to 0.30 ± 1.8 after smoking, p= 0.03). The current study reported a decrease in AoA measured by subjective method (RAF rule) after smoking exposure. The study found an approximate of 0.50 D (average) decrease in AoA after a cigarette smoke (pre-smoking AoA 10.58 ± 1.7 D & post–smoking 9.95 ± 1.8 D, p = 0.001). There was however no significant decrease in subjective clear vision range by performing defocus curves before and after smoking exposure apart from 1.50 Ds lens and 1.0 Ds lens where the decrease was statistically significant (from 0.48 ± 0.1 to 0.51 ± 0.1 LogMAR for +1.50 Ds, p =0.01, from 0.25 ± 0.1 to 0.28 ± 0.1 LogMAR for +1.00 Ds, p =0.04).

The contradiction in Bardak *et al.* 2017 results and this study results are may be due to factors discussed above (i.e. the difference in abstinence smoking time and smokers' participants meeting criteria). Another, possible reason of variations in results could be due to the nature of the method used to measure accommodation, and this study uses a subjective method which may overestimate the objective accommodation due to a depth of focus phenomenon (López-Gil *et al.*, 2009). Lastly, it can be hypothesised that smoking might alter the elasticity of lens zonules or ciliary muscles which then affect the accommodative ability of the eye similar to corneal prospective where smoking is associated with increased corneal rigidity (Hafezi, 2009).

Chapter 10
Conclusion

10.1 Summary of thesis findings

This body of work initially attempted to determine the effects of lifestyle on the tear film, accommodation, and macular pigment optical density and originally this was planned only with a UK cohort of subjects. It was noted that a high number of participation were British-Asian and this led an investigation of participants from Pakistan as a comparator.

It was found by this work that smoking had a negative impact on the quality of the tears. Smokers had significantly lower TBUT compared to non-smokers and this result was consistent with the findings of the previous studies such as (Sayin *et al.*, 2014, Agrawal *et al.*, 2018). A dose-response relationship of smoking with TBUT was established as there was a positive correlation found between smoking pack years and low TBUT and between the number of cigarettes smoked per day and low TBUT. Indirect exposure to tobacco smoke has been linked with dry eye symptoms (El-Shazly *et al.*, 2012). A decreased TBUT was noted in the participants who were exposed to passive cigarette smoke compared to individuals who had no exposure to tobacco smoke.

Subjective symptoms of dry eyes (OSDI scores) are a rapid form of dry eye assessment. This piece of work found that smokers had high OSDI scores compared to non-smokers. This thesis found that a strong but negative correlation of age with AoA, but did not find a significant association of AoA with smoking status.

In the UK cohort, there was no significant difference observed in the dietary elements intake within different ethnicities. There was no significant difference observed between males and females dietary elements intake except for PUFA intake that was high in female participants. PUFA intake in females was probably higher due to its cosmetic effects on hair and skincare (Rees *et al.*, 2001, Di Nardo, 2019). The dietary analysis picture of Pakistani cohort was different from the UK cohort. In Pakistani cohort, there was a gender-based difference observed in the intake of dietary elements. This study found a trend of low dietary elements intake in Pakistani females, which may be linked with cultural taboos, and socio-economic status (Iqbal *et al.*, 2017). For example in many areas of Pakistan, it is often the case that the men of a household will eat first and the women afterwards and hence may consume lower quantities compared to the men. Similarly, it has been noted that anaemia is more common (Soofi *et al.*, 2017, McCormack *et al.*, 2018). There was no dietary intake difference observed in Pakistani male smokers and non-smokers.

In the UK cohort, there was no association or correlation found between dietary elements intake of vitamin A, vitamin D, lutein, zeaxanthin and TBUT. However, there was a small positive correlation observed between polyunsaturated trans-fatty acids intake and NIKBUT. There was no association or correlation found between dietary elements intake of vitamin D, lutein, zeaxanthin and TBUT in Pakistani participant. In the Pakistani cohort, however, there was a weak negative correlation found between vitamin A (IU and RAE) intake and NAFLTBUT. A negative correlation between OSDI scores with some dietary elements was seen in both cohorts. In the UK cohort, vitamin A (IU and RAE) and vitamin D showed a weak negative correlation with OSDI scores, whereas in the Pakistani cohort vitamin D showed a weak negative correlation with OSDI scores, and polyunsaturated trans-fatty acids showed a medium negative correlation with OSDI scores.

No correlation or relationship was observed between dietary elements intake and AoA, or between dietary elements intake and MPOD scores in the UK cohort. In the Pakistani cohort, a weak but positive correlation of vitamin D and polyunsaturated trans-fatty acids intakes with AoA and a significant association between polyunsaturated trans-fatty acid and AoA was noted.

When comparing data collated in the UK versus that in Pakistan there was no significant difference in TBUT observed between cohorts based upon smoking and gender statuses, neither there was any difference in the passive exposure of both cohorts. There was no significant difference observed in OSDI scores between non-smoker males and females in both cohorts, however, Pakistani smokers had lower OSDI scores compared to UK smokers.

There was no significant difference in mean AoA observed between female non-smokers and male non-smokers of both cohorts in a similar age group. UK smokers, however, had significantly better AoA compared to Pakistani smokers of similar age groups. Participants of the UK cohort had better subjective clear vision range attained by defocus lenses of similar power than the subjective clear vision range of Pakistani participants.

The Pakistani diet is generally composed of food contents rich in vitamin A (Gallup, 2011). This dietary pattern reflects in the dietary comparison of both cohorts. Pakistani participants had high vitamin A (RAE) dietary intake compared to the UK cohort. In contrast, the UK participants had a significantly higher amount of vitamin D, lutein, and zeaxanthin intake compared to Pakistani counterparts. Based on dietary elements intake, mean AoA of UK participants was higher than Pakistani participants for the age group of 18 to 24 years old participants.

A sub-group analysis of British-Asians versus Pakistani participants revealed almost similar results, as seen in the comparison of Pakistani participants versus the UK participants. There was no significant difference in TBUT based on gender and smoking statuses. The mean OSDI scores of Pakistani smoker males were significantly lower than British-Asian smoker males.

The mean AoA of non-smokers male and female was not statistically different but the British-Asian smokers had significantly higher AoA compared to Pakistani cohort. The British-Asian cohort had significantly better subjective clear vision range than Pakistani participants.

The mean intake of vitamin A (IU) was significantly higher in Pakistani participants than the mean intake of British-Asian participants. However, vitamin D, lutein, zeaxanthin and polyunsaturated trans-fatty acids intake was significantly higher in British-Asian participants. Based on the dietary elements intake, the mean AoA of British-Asian participants were significantly higher than the Pakistani participants were.

This body of work tried to evaluate the short-term effects of smoking on the tear film and accommodation of the eye. The results revealed that short-term exposure to tobacco smoke could decrease TBUT, alter the tear lipid layer, cause miosis of the pupil, and decrease the amplitude of accommodation. A possible reason for the short-term effects of smoking may be related to the short life span of nicotine. Nicotine rapidly changes into a less toxic and relatively inactive substance, which may cause a reversal in any changes caused by it.

10.2 Strengths

This body of work has added many new additions to the current science. Evaluating the effect of smoking on AoA as a primary outcome measure and on defocus curves was not studied before. This body of work also added value in the existing literature by conducting a study to investigate the effects of smoking on TBUT and accommodative ability in the Pakistani subjects. This piece of work added value in the literature by conducting a study that investigated the effects of vitamin A, vitamin D, lutein and Zeaxanthin and polyunsaturated fatty acids on the amplitude of accommodation. In addition, this work is unique as by reporting the dietary effects on the tear film and amplitude of accommodation of a Pakistani population. This piece of work added value in the existing literature by conducting the first direct study on effects of sunlight/UVR on the tear film and AoA.

This piece of work is first of its kind, which has compared smoking and dietary effect in Pakistani participants with the UK participants and with British-Asian participants with relation to its effects on ocular health. Transient effect of smoking on ocular health is a new research area, and this work adds to the existing literature by showing transient effects of smoking with one-hour short abstinence time.

10.3 Limitations

There are some limitations attached to this piece of work that needs to be considered while interpreting its results. One of the main limitations was a significant enrolment of young population (80 %) for the UK cohort participants who were aged 30 years or below. A large number of young study population could be the reason of getting some unexpected results such as not observing any significant difference in OSDI scores between males and females. Similarly, the younger ages of the subjects in this study may have not revealed changes in MPOD values. It may also be the case that MPOD measurements are not sensitive enough to show differences in the groups studies in this thesis over the periods tested. Inclusion of younger participants in the UK cohort of data also affected some age-related comparisons of AoA between Pakistan participants and the UK and British Asian participants.

Cumulative effects of smoking or any other lifestyle factor would probably be prominent after some decades. At younger ages, this effect could not be seen as prominent. Smoking can form a dose-response relationship with its related diseases. In the current piece of work, most of the smoker participants from both cohorts were light smokers, and only a fraction of them were heavy smokers. This fact applied to the smoking pack-years as well as only a few participants from both cohorts had more than ten-pack years of smoking exposure. Pakistani cohort of smokers did not have any female smoker participants. The lack of Pakistani female smoker participants has resulted in a gender bias for this study as UK based female smokers participants were not compared based on gender and smoking status.

There are chances of respondent based bias while reporting numbers of cigarette smoked per day or while filling the OSDI or baseline questionnaire as it was self-reported. This could also affect the results of this study. Lastly, around ten per cent of Pakistani smokers reported the use of drugs (marijuana) along with cigarette smoking and these added effects were not accounted for in this study.

There are some limitations associated with the dietary analysis and that need to be considered while interpreting the dietary outcome results. Some unmeasured variables may have confounded the results. The dietary analysis could be affected by self–reporting bias from participants while they detailed the quantity of food eaten they ate for the 24 hours of food recalls. Participants may have under-reported unhealthy diets or over-reported healthier diets.

There are some limitations associated with UVR related results, which need to be considered. Firstly, participants self-reported responses on sunlight exposure hours could affect the outcome of the results, and there could be a possibility that these results could be due to a bias factor or a false positive result. It is difficult to measure exact UVR exposure, as UVR exposure varies from place to place and by time to time regardless of any measurement method used. The use of protective eye wears e.g. sunglasses was not checked in the current

research. This can influence the results. Subjects will be mobile during the day and spend part of the day in direct sunlight and part of the day away from sunlight and will be exposed to reflected sunlight too. Finally, the environmental study only looked at sunlight (UVR) exposure, but there may be other environmental factors that could be considered. Humidity, air pollution, and temperature, for example, may have a role.

The ‘transient effects of smoking on the ocular health' study has limitations of its kind. First, the study did not use an objective method of measuring accommodation and defocus curves for near vision. Defocus curves take quite a long time to carry out and so the transient effects would be lost during this timeframe. Finally, this study did not restrict participants to use a single brand cigarette with a set level of nicotine and other ingredients, as they were free to use their preferred brand of cigarette this could affect the results achieved from the study.

10.4 Future work

Any future work in the thesis relevant area should consider the limitations of this work. Firstly, the inclusion of participants with presbyopia would be better to see any cumulative effects over more years of lifestyles on ocular health. Based on smoking effect on the pre-corneal tear film, the future work should use other diagnostic tests such as conjunctival impression cytology, fluorescein staining, and corneal and conjunctival sensitivities test as used in some recent studies (Aktaş *et al.*, 2017, Agrawal *et al.*, 2018). The current study was unable to add ex-smokers as study participants. It would be a point of interest to evaluate all three smoking statuses (smokers, ex-smokers, and non-smokers).

This piece of work only investigated cigarette and roll-ups smokers. Other types of smoking, such as cigar, water-pipe smoking, or electronic cigarettes may be different. One of the possible mechanism of the effect of smoking on the tear film is through its direct contact with the ocular tear film, as suggested by Satici *et al.* (2003). In this case, different style of smoking could have different results, and there may be a chance that spectacle users’ who smoke may have different result compared to smokers without spectacles as the lens may create a partial barrier. Similarly, contact lens wearers who smoke may have different effects.

Future dietary analysis studies should consider taking plasma levels of antioxidants/vitamins along with the collection of diet diaries in order to reduce the risk of respondent based bias factor. UV wristwatches or use of physical dosimeter might be helpful for future studies that can measure UVR from both artificial sources and the sunlight. Further studies are required to evaluate the role of environmental factor such as air pollution, level of humidity, the temperature with ocular diseases along with UVR calculation.

Finally, for future studies to investigate transient effects of smoking on the ocular health, measuring ocular inflammation before and post smoking would be a point of interest to know whether smoking is associated with any conjunctival hyperaemia or other inflammatory ocular effects. The short-term increase in blood pressure (BP) after smoking is reported in the literature (CDC, 2014). Measuring Intra-ocular pressure (IOP) before and after smoking would be a point of interest as a reduction in BP is associated with a reduction in IOP (Klein *et al.*, 2005). The current body of work suggests a decrease in subjective AoA after smoking , A study conducted by Bardak *et al.* (2017) suggested an increase in objective accommodation immediately after smoking. In this work it was found that there was a drop in AoA after smoking but this may be related to how soon after smoking the measurements are taken. In this study, the last cigarette was consumed within five minutes but each subject underwent the tear testing prior to AoA measurement, so by the time AoA was measured at least 15 minutes had elapsed since the consumption of the last cigarette. It would be interesting to explore this further by taking measurements of AoA every few minutes immediately after a cigarette was consumed.

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Appendices

Appendix 1 – Baseline data collection questionnaire

Lifestyle effects on ocular health

Date: _____

Personal identification no: _____

Section 1:

Dietary information:

What did you eat yesterday including all meals and snacks?

Breakfast_____

_____

Lunch

_____

_____

Tea

_____

_____

Dinner

_____

_____

Snacks_____

_____

Section 2:

Smoking & Drinking information:

Drinking Status:

How many units you drink per week

- Non- drinker
- 1-2 units
- 3-4 units
- 5-6 units
- 7-8 units
- 9-10 units
- More than 10 units

1 unit of alcohol is: ½ pint of ordinary beer, lager or cider (3.5% ABV) 1 small glass of wine (125ml of 8% ABV wine) 1 single pub measure of spirits (1/6th gill i.e. 25ml)

Has your drinking status changed in last few years? _____

Smoking status:

- Regular smoker
- Ex – smoker
- Never smoked regularly

If regular smoker, then number of cigarettes smoked per day: _____

Smoking years: _____

Regular smoker – someone who currently smokes one or more manufactured or hand rolled tobacco cigarettes per day.

Ex – smoker – someone who does not now, but used to smoke one or more manufactured or hand rolled tobacco cigarettes per day.

Never smoked regularly – someone who has never smoked manufactured or hand rolled tobacco cigarettes at all or smoked less than one per day.

Other forms of smoking use:

- Pipe smoking
- Cigar smoking
- Water pipe (Sheesha)

Have you ever been exposed to unintentional tobacco smoke (passive smoking)? ____

Has your smoking status changed in last few years? _____

Section 3: Ocular surface disease index (OSDI):

Have you experienced any of the following *during the last*	**All of the time**	**Most of the time**	**Half of the time**	**Some of the time**	**None of the time**
1. Eyes that are sensitive to light?	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Have problems with your eyes limited you in performing any of the	**All of the time**	**Most of the time**	**Half of the time**	**Some of the time**	**None of the time**	**N/A**
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Have your eyes felt uncomfortable in any of the following situations *during*	**All of the time**	**Most of the time**	**Half of the time**	**Some of the time**	**None of the time**	
10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned?	4	3	2	1	0	N/A

Section 4: Back ground & personal information:

Ethnicity:

Choose the option which best describe you:

- **White**
- **Asian/Asian British**
- **Black/ African/Caribbean/Black British**
- **Mixed race**
- **Other ethnic groups (e.g. Arabs, Chinese)**

How many hours per day do you perform near vision tasks within arms' length?

_____

(For example use of a tablet device, laptop, desktop, mobile phone, newspaper, books etc.)

How many hours a day do you drive? _____

How many hours you spend outside during daylight hours? _____

Age: _____

Gender: _____

END OF QUESTIONNAIRE

Appendix 2 - PARTICIPANT INFORMATION SHEET

Title of the Study: Lifestyle effects on ocular health

You are being invited to participate in a research study. Before you decide to participate it is important for you to understand why the study is being done and what it will involve. Please take the time to read the following information carefully, and discuss with friends and family, if you wish. Please feel free to ask us about anything that is not clear.

- Part 1 describes the purpose of this study, and what will happen to you if you decide to participate.
- Part 2 gives you more detailed information about the conduct of the study.

What is the purpose of the study?

The purpose of this study is to investigate if certain lifestyle habits like smoking, alcohol consumption, dietary intake and exposure to sunlight can contribute to eye disorders.

Why have I been invited to participate?

You have been invited because you are aged between 18 to 50 years old and have healthy eyes and do not suffer with dry eyes or wear contact lenses

Must I agree to take part in this study?

No, your participation in this study is voluntary and you are entitled to refuse. Your decision of refusal will not affect your employment or in the case of a student it will not affect your relationship with the University.

If you decide to take part in this study you will be asked to sign a Consent Form. You will be given a copy of this and an information sheet to keep. If you change your mind you are free to withdraw at any time and without giving any explanation.

What will happen to me if I take part?

After the study has been explained to you and you have agreed to take part you will be asked questions about your lifestyle (smoking, alcohol consumption, dietary intake etc.) and about your ocular history in a short questionnaire. Your eye health will be assessed by doing following procedures:

1. Tear film analysis: Tears are measured with an automated non-contact instrument and the examiner will use an orange drop (fluorescein) to enhance the tear contrast.

2. Amplitude of accommodation: This is measured with a 'ruler' where print is brought closer to you until it is blurred and the distance at which is blurs is recorded.. Also, you will be asked to read letters from a letter chart whilst different lenses are held in front of your eyes to blur the letters.

3. Macular pigment: a non-contact device is used to measure the level of pigment at the central region of the back of your eye.

Will I receive imbursement for my participation?

Yes, you will receive £10 for your participation in this study which will be given at the end of the investigations. You will receive the payment as 'Love2Shop' vouchers or Amazon vouchers.

What are the possible risks?

The risks associated with taking part in this study are very small. All of the study procedures are routinely undertaken by optometrists and have been shown to have very no side effects or adverse events. As part of the study we will use Fluorescein 1.0% eye drops. This is a staining agents used to aid external examination of your eye. When applied to the eye, they may sting for a few moments. Due to their colouring (orange/ yellow) they may cause the vision to take on a coloured appearance for a few minutes. If the eyelids and the skin around the eyes become coloured by the stain then this can be removed with cold water. If you require more information about possible risks and disadvantages please ask.

Contact details

For further information about the study please contact:

Investigator:
Dr Shehzad Naroo School of Life and Health Sciences Aston University, Birmingham, B4 7ET
Tel: 0121 2044132
Email: s.a.naroo@aston.ac.uk

What if there is a problem?

If you have a concern about any aspect of this study, you should in the first instance speak with the principal investigator or another member of the research team and they should be able to answer your questions.

Dr Shehzad Naroo School of Life and Health Sciences Aston University Birmingham, B4 7ET
Tel: 0121 2044132
Email: s.a.naroo@aston.ac.uk

Dr Frank Eperjesi School of Life and Health Sciences Aston University Birmingham, B4 7ET
Tel: 0121 2044114
Email: f.eperjesi@aston.ac.uk

Mr Nisar Latif School of Life and Health Sciences Aston University Birmingham, B4 7ET
Tel: 0121 20445303
Email: latifn@aston.ac.uk

Who do I contact if I wish to make a complaint about the way in which the research is conducted?

If you have any concerns about the way in which the study has been conducted, then you should contact the Director of Governance of the University:

Mr John Walter Director of Governance Aston University Birmingham, B4 7ET
Tel: 0121 20444869
Email: j.g.walter@aston.ac.uk

Will my taking part in this study be kept confidential?

Yes. All information which is collected about you during the course of the research will be kept strictly confidential and you name will not be used. Our procedures for handling, processing, storage and destruction of your data are compliant with the *Data Protection Act 1998*. You have the right to view the data we have on record about you and to correct any errors.

What will happen to the results of the research study?

It is intended that the results of the research will be presented at scientific meetings, and published in relevant clinical and academic journals. The study will also be written into the PhD thesis of Nisar Latif. You will not be identified in any report or publication.

Who is organising and funding the research?

The Ophthalmic Research Group, Aston University is organising this study.

Who has reviewed the study?

This study was reviewed and given a favourable opinion by the Aston University Research Ethics Committee.

Appendix 3 – Ethical approval

Aston University
Aston Triangle
Birmingham
B4 7ET
0121 204 3000

Date: 18/04/2016

Life and Health Sciences

Dear Dr Shehzad Naroo

Study title:	Lifestyle effects on ocular health
REC REF:	Ethics application #905

Confirmation of Ethical Opinion

On behalf of the Committee, I am pleased to confirm a favourable opinion for the above research based on the basis described in the application form, protocol and supporting documentation listed below.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Study Advertisment 1.3	3	15th April 2016
Research Participant Information sheet version 1.4	4	15th April 2016
research protocol version 1.2 30th March	2	30th March 2016
questionnaire v1.2 30th March	2	30th March 2016
Consent form v1.2	2	18th January 2016

With the Committee's best wishes for the success of this project.
Yours sincerely

Dr Nichola Seare
Chair of the University Research Ethics Committee

Appendix 4 – Consent form

Aston University

Personal Identification Number for this study:
____

CONSENT FORM

Title of Project:	**Lifestyle effects on ocular health**
Research Venue:	Aston University Optometry Clinic
Aston University Investigators:	Shehzad Naroo PhD
	Frank Eperjesi PhD
	Nisar Latif MSc BSc

Please initial box

1. I confirm that I have read and understand the information sheet dated .... for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary; the study tests are not part of any medical treatment or negate the need for regular eye examination. ☐

3. I understand that I am free to withdraw at any time, without giving any reason, without my legal rights being affected. ☐

4. I agree to take part in the above study. ☐

____________________	____________	____________________
Name of Research Participant	Date	Signature
____________________	____________	____________________
Name of Person taking Consent	Date	Signature

1 copy for research participant; 1 copy for Aston University