ORIGINAL ARTICLE

EVALUATION OF TRIGLYCERIDES LEVELS IN SERUM AND TEARS OF OBESE AND NON-OBESE NORMAL ADULT HUMANS AND ITS EFFECTS ON PUPILLARY RESPONSE

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Background: Triglycerides are a type of storage fat present in serum of both normal and obese individuals. Triglycerides are normally present in human tears. The presence of qualitative fats in the tears affect the pupillary response to the external light stimulus which is measured through portable field dark adaptometer (PFDA) device. The current study was conducted to evaluate the levels of triglycerides in serum and tears of obese and non-obese humans and its relationship with pupillary response. **Methods:** This descriptive cross-sectional study was conducted at Institute of Basic Medical Sciences, Khyber Medical University, Peshawar. A total of 500 participants were recruited out of which only 50 qualified for the study as per inclusion criteria. Out of these 50, 25 (50%) were obese and 25 (50%) were non-obese. Serum triglycerides were measured by using Micro lab 300 biochemistry analyzer, while thin layer chromatography was used to detect triglycerides in tear samples. **Results:** Triglycerides were detected only in tears of obese individuals. No statistically significant difference was observed in the serum levels of triglycerides between the two groups (p=0.849). **Conclusion:** The presence of triglycerides in tears of obese adults caused a low pupillary response as compared to normal individuals.

Keywords: Triglycerides; Pupillary response; Tears; adaptometer

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INTRODUCTION

Lipids, cholesterol, phospholipids triglycerides (TG) and fatty acids, are important for the human body.[1] They form the basic structure of cell membranes (phospholipids) and are also the precursor to the steroid hormones, vitamin D and bile acids.[2] Lipoproteins, allow the solubilization and transportation of lipids, usually hydrophobic substances, in aqueous blood plasma[3]. In humans, triglycerides makeup of 98% of lipid content and contributes about 40–50% of the total energy content.[4] Triglycerides are lipid segments used for storage of energy that is both fundamentally synthesized in the liver and derived from external sources through uptake in the intestine.[5] Long chain fatty acids and monoglycerides convert into triglycerides in the liver. Triglyceride mainly in the form of very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) will be secreted into the blood. Very low-density lipoprotein (VLDL) is then converted to intermediate-density lipoprotein. Further intermediate density lipoprotein is broken down into LDL. Low density lipoprotein stores either in peripheral tissue or circulates back to the liver.[6]

The cholesterol content of triglyceride-rich lipoproteins (remnant cholesterol) is the major cause of atherosclerosis and cardiovascular disease.[7] Human tears are a complex mixture containing lipids, proteins, carbohydrates, salts and other different compounds of high and low molecular weight.[8] Tear fluid in the eye forms a thin layer on the cornea and conjunctiva.[9] Its major functions are to moisturize the ocular surface while blinking; provides immunity to corneal epithelial cells and adjusting the refractive index of the cornea to expand optical properties. Tear film of the eye is composed of three layers, mucous layer, a middle aqueous layer, and an outer lipid. The middle aqueous layer consists of many different types of proteins and electrolytes like sodium. Proteins are also helpful in wound healing, inflammation and give protection to the cornea against different diseases.[10] Lysozyme, secretory immunoglobin A, lactoferrin, serum albumin, lipophilic and lipocalin are major proteins present in the tear[11] and provide protection to the eye against infections[12]. Lipid layer of the tear film is made up of polar lipid (phosphatidylcholine) and non-polar lipid (glycerol) mainly triglycerides. Tear metabolome has mostly been studied in polar lipids such phosphatidylcholine.[13] The lipid layer reduced the overall surface free energy, i.e., surface tension to stabilize the tear film and also control the evaporation of water from the surface. The lipid layer of the tear is in contact with the skin of eyelid acting as a barrier to the aqueous layer. When the lids are closed lipids also form a watertight.[14]

In the current study, we explored the impact of high plasma and tear lipids on pupillary response

in people with BMI of more than 30 kg/m$^{2.}$. To our knowledge, no such studies have been conducted on humans, this is the first one. We hypothesized that hyperlipidaemia causes increase levels of qualitative fats in the tears of these individuals, which causes lower pupillary response to the external light stimulus measured through PFDA device

MATERIAL AND METHODS

This cross-sectional study was conducted at Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU), Peshawar from March 2017 to Mar2018. The study was approved by ethical review board of KMU-IBMS. Potential participants were recruited from the Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU), Peshawar Pakistan. Vision of the participants was tested prior to adaptometry. Participants having 6/6 vision with glasses, <25 kg/m^2 BMI for non-obese and >30 kg/m^2 BMI for obese, were included in the study, whereas participants with cardiovascular and visual problems and diabetes mellitus were excluded from the study. Written informed consent was taken from all the participants.

The participants were requested to keep the eyes gently closed without squeezing. Schirmer strips were kept on the eyes and allowed to remain in place until the strip gets moist. The strips were then carefully removed from the eyes and the moist area was marked. The strips were covered in a clean paper and allowed to dry for 10 minutes. Figure 1 shows the sampling procedure.

Portable Field Dark Adaptometry was done according to the standard protocols previously described by Alain B. Labrique, *et al.*[15] PFDA goggles have a camera element and a solid-state flash. First, the goggles were adjusted comfortably on the participant face and it was ensured that no light is entering from the outside. Participants were requested to keep their eyes open and look straight forward. After that, the participant's eyes were subjected to a flash of white light subsequent to allowing them to adjust to dark conditions. Stimuli of dim light, with an increase in 0.4 log cd/m^2 occur at the increment of 1 second. Participants were exposed to a total of nine sessions. In between each session, 10 seconds rest was given to the participants in order to re-position their pupillary constriction. At the end of the nine session's real time, the monitored video was recorded at the end of the nine sessions. In order to quantify the participant's pupillary light reflex, the Custom developed software was used to analyze pre-and post-flash images (Figure-2).

Thin layer chromatography (TLC) was used for the detection of triglycerides in tear samples. Tears samples were first mixed with 100 µl of mixture of chloroform: methanol (67 µl :33 µl) and vortexed it for 2 minutes at room temperature. The samples were kept for one hour at room temperature and then centrifuged at 10,000 rpm for 3 minutes. The supernatant was discarded and the lower organic layer was transferred to pre-labelled clean glass vials.

The sample were carefully applied to TLC Silica gel 60 glass plates. The separation was done for both polar and non-polar lipids. For polar lipid, we used eluent composition of chloroform, methanol acetic acid, formic acid, and water and for non-polar lipids hexane, diethyl ether and acetic acid was used. After separation, the plate was dipped in copper sulphate and phosphoric acid solution. The solution was then heated for 30–45 minutes at 180℃. The lipids were detected and identified by lipid standards.

Dark adaptation video analysis was performed by using "Tracker 5.0" software. For this analysis, the saved video was first imported to the software. Then pre-and post-stimulus of the pupillary response was measured with the help of measuring tool by calculating the pupillary response to the different intensities of dim light. Pupillary response, pupillary dynamic and pupillary threshold of each video was measured by using this software. All the pupillary response videos were analyzed by the same data analyst using the same software Tracker 5.0

Triglycerides were measured in the serum of the participants. For this 2–3 ml venous blood was collected in plain gel tubes. The tubes were centrifuged at 5000 rpm for 10 minutes. Triglycerides were measured from the serum by using Micro lab 300 biochemistry analyzer. Results in mg/dl were recorded and statistically analyzed.

The data collected were in Microsoft Excel® 2016 and then reviewed to check for any random errors. All the data organized were analyzed through Minitab Version 16 (Minitab® V. 16 USA) &Stata® version 15 (Stata® V. 15 USA). For testing normality of the data Anderson-Darling test was applied to check whether the data was attributed to the normal/parametric or non-normal/non- parametric distribution of trial variables. Data expressed from probability plots were presented either mean/standard deviation or

median/inter-quartile range (IQR) by descriptive statistics. Correlation of triglycerides with different variables was determined by Pearson correlations. Multi-effect linear regression models were used to associate pupillary threshold with study variables. Probability-values ≤0.05 was considered statistically significant.

RESULTS

A total of 500 participants were initially recruited in the study. Two hundred (n=200) candidates had responded positively. Out of these 200, 50 (25%) were healthy and the remaining 150 (75% were excluded from the study as they didn't qualify the inclusion criteria. Among these fifty participants (n=50), 25 (n=25) were having normal BMI and the other 25 (n=25) were obese.

The mean age of the participants was 31.86±9.01 years; their mean height was 164±6.92cm with the mean weight of 76.88±10.73 kg. The mean BMI of the participants was 28.6±2.95 kg/m^2. The median age of the participants having normal BMI was 25 (IQR = 2.5) years while those of obese participants was 36.04±10.49 years. The height of the normal BMI participants was (165.9±7.86) cm with a mean weight of (62.72±9.65) kg and median waist size of 97 (IQR = 8) cm while, height of obese BMI participants was (162.1±5.98) cm with mean weight of (91.04±11.82) kg and median waist size of 100 (IQR = 22.5) cm. The BMI of normal participants were (22.6±2.2) kg/m^{2}and the obese participants BMI were (34.6±3.71) kg/m^2.

Serum triglycerides levels of the participants were measured in both the groups. No statistically significant difference was found between the two groups (p=0.849), as shown in Table-1.

The mean pupillary diameter in normal participants at low intensity was (9.72±5.24) and in obese participants, it was 8.1 (IQR= 7.7). At high intensity mean pupillary diameter of the normal participant was (25.76±4.86) and in the obese it was (25.02±4.84). Similarly, the mean pupillary dynamics of normal participants at low intensity was 0.60 (IQR= 0.15) and in obese it was 0.50 (IQR= 0.2). Pupillary dynamics of normal participants at high intensity was 0.55 (IQR= 0.10) and obese participants were 0.55 (IQR = 0.10) as shown in Table-2.

The Mean pupillary threshold of participants was (-1.39cd/m^2). The first stimulus at which pupil diameter decreased by 20% or more is known as pupillary threshold. Abnormal pupillary threshold is > (0.5cd/m^2). Good, adequate and impaired pupillary threshold of the normal and obese participants is shown in Table-3.

Correlation analysis of triglycerides pupillary threshold, high and low intensity pupillary responsiveness and pupillary dynamics at low intensity (p=0.02) was observed in obese adults as compared to normal adults with pupillary dynamics at low intensity (p=0.581) as shown in Table-4.

Association of pupillary threshold was found significant with pupillary responsiveness at low & high intensities in both the BMI groups as shown in Table-5.

Triglycerides levels in tears of obese and non-obese participants were detected on TLC plates at 254nm under UV transilluminator. In normal participants, triglycerides levels were not detected in tears as shown in Table-6.

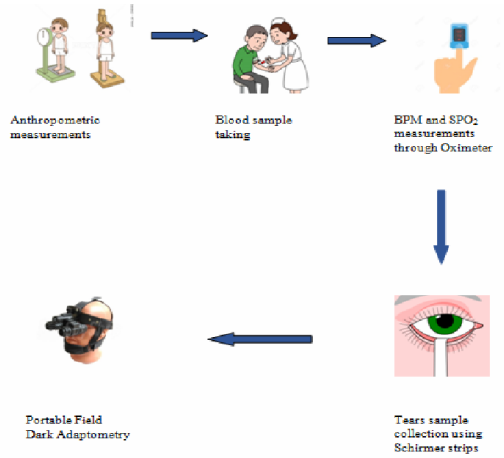

Figure-1: **Flow chart diagram showing tears sample collection using Schirmer strips**

Figure-2: Portable field dark adaptometry

Table-1: Serum triglycerides levels of normal and obese adults

Study participants	Normal BMI (*n=25*) Median, IQR*	Obese BMI (*n=25*) Median, IQR*	*p-value*
Triglycerides (mg/dl)	129, 37*	152, 62*	0.849

Table-2: **Pupillary diameter and pupillary dynamics**

Study participants	Normal BMI (*n=25*) Mean±SD Median, IQR*	Obese BMI (*n=25*) Mean±SD Median, IQR*	*p-value*
% CHANGE IN PUPIL DIAMETER			
All stimulus (-2.9 to 0.1 cd/m^2)	16.85± 9.70	15.86±9.86	0.722
Low intensity increments (-2.9 to -1.3 cd/m^2)	9.72±5.24	8.1, 7.7*	0.413
High Intensity increments (-0.9 to 0.1 cd/m^2)	25.76±4.86	25.02±4.84	0.592
PUPILLARY DYNAMICS			
All stimulus (-2.9 to 0.1 cd/m^2)	0.50, 0.15*	0.60, 0.10*	0.118
Low intensity increments (-2.9 to -1.3 cd/m^2)	0.60, 0.15*	0.50, 0.2*	0.069
High Intensity increments (-0.9 to 0.1 cd/m^2)	0.55, 0.10*	0.55, 0.10*	1.000

Study participants	Normal BMI (*n=25*) Mean±SD Median, IQR*	Obese BMI (*n=25*) Mean±SD Median, IQR*	*p-value*
% CHANGE IN PUPIL DIAMETER			
All stimulus (-2.9 to 0.1 cd/m^2)	16.85± 9.70	15.86±9.86	0.722
Low intensity increments (-2.9 to -1.3 cd/m^2)	9.72±5.24	8.1, 7.7*	0.413
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Study participants	Normal BMI (n=25) Mean±SD Median, IQR*	Obese BMI (n=25) Mean±SD Median, IQR*	p-value
% CHANGE IN PUPIL DIAMETER			
All stimulus (-2.9 to 0.1 cd/m^2)	16.85± 9.70	15.86±9.86	0.722
Low intensity increments (-2.9 to -1.3 cd/m^2)	9.72±5.24	8.1, 7.7*	0.413
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Low intensity increments (-2.9 to -1.3 cd/m^2)	0.60, 0.15*	0.50, 0.2*	0.069

Table 3: Pupillary Threshold (Adequate, good, impaired)

Study participants	Normal BMI (*n=25*) Mean ± S. D	Obese BMI (*n=25*) Mean ± S. D	Difference	*p-value*
Pupillary threshold ≥ 15 cd/m^2	-1.39 ± 0.58	-1.27 ± 0.67	-0.12	0.502
Pupillary threshold ≥ 15 distribution	N (%)	N (%)		
xGood (-2.9 to -2.1 cd/m^2)	1 (4)	2 (8)		
Adequate (-1.7 to -0.9 cd/m^2)	20 (80)	18 (72)		
Impaired (-0.5 to 0.1 cd/m^2)	4 (16)	5 (20)		

Table 4: Correlation analysis of triglycerides with study variables

Study participants Variables	Normal BMI (*n=25*)		Obese BMI (*n=25*)	
	Pearson-correlation coefficient	*P-value*	Pearson-correlation coefficient	*P-value*
Pupillary Threshold ≥ 15	-0.101	0.647	0.069	0.742
Pupillary Responsiveness- Low intensity (-2.9 to -1.3 cd/m^2)	-0.007	0.976	-0.163	0.437
Pupillary Responsiveness- High intensity (-0.9 to 0.1 cd/m^2)	0.091	0.680	0.033	0.877
Pupillary Dynamics- Low intensity (-2.9 to -1.3 cd/m^2)	0.121	0.581	0.469	0.02
Pupillary Dynamics- High intensity (-0.9 to 0.1 cd/m^2)	-0.002	0.993	0.302	0.142

Table-5: Pupillary threshold multi-effect linear regression analysis with study variables

Study Variables	Normal BMI (*n=25*)		Obese BMI (*n=25*)	
	Coef. [95% Conf Interval]	*p-value*	Coef. [95% Conf Interval]	*p-value*
Triglycerides (mg/dl)	0.000 [-0.000, 0.001]	0.918	0.007 [-0.000, 0.016]	0.069
Pupillary Responsiveness- Low intensity (-2.9 to -1.3 cd/m^2)	-0.068 [-0.102, -0.033]	0.00	-0.086 [-0.135, -0.038]	0.00
Pupillary Responsiveness- High intensity (-0.9 to 0.1 cd/m^2)	-0.037 [-0.496, -0.025]	0.00	-.026 [-0.046, -0.005]	0.01
Pupillary Dynamics- Low intensity (-2.9 to -1.3 cd/m^2)	-0.161 [-0.481, 0.157]	0.321	0.104 [-0.709, 0.917]	0.802
Pupillary Dynamics- High intensity (-0.9 to 0.1 cd/m^2)	0.261 [-0.266, 0.788]	0.332	-0.228 [-1.614, 1.15]	0.747

Table-6: Percentage of triglycerides in tears of normal and obese

No. of participants	Triglyceride TLC results (Yes) n (%)	Triglyceride TLC results (No) n (%)
Obese	25(50)	-
Normal	-	25(50)

DISCUSSION

In this pilot study triglyceride levels in blood and tears were evaluated followed by measurement of pupillary response in healthy and obese subjects by Portable field dark adaptometer (PFDA).

In this study, we measured pupillary threshold, pupillary responsiveness and pupillary dynamics by using PFDA. In our study we found that 16% of normal BMI adults have impaired pupillary threshold while 20 % impaired pupillary threshold was found in obese BMI adults based on standard ≥15% pupil change suggested by Labrique, *et al*.[15]

This study was conducted on two groups, one group of obese adults with BMI (≥30) and the other group of normal healthy individuals with BMI ≤25. In this study, we measured pupillary threshold, pupillary responsiveness and pupillary dynamics by using PFDA. In our study we found that 16% of normal BMI adults have impaired pupillary threshold while 20% impaired pupillary threshold was found in obese BMI adults based on standard ≥15% pupil change suggested by Labrique, *et al.*[15] A study conducted in 2012 in Zambia on pre-School children showed 24% impaired pupillary threshold which is greater than our study results.[16] Similarly, a study conducted on School-aged children in 2010 in Kenya showed 33.7% impaired pupillary threshold.[17] Both of the studies showed high impaired pupillary threshold than our study. The reason might be due to the difference in vitamin A status which was not measured in all these studies. However, controversial findings regarding the impaired pupillary threshold have been documented in previous studies. A study conducted on normal adults in Peru showed 11% impaired pupillary threshold. Similarly, another study conducted on pregnant women in Bangladesh in 2010 showed 8.7% impaired pupillary threshold.[15] Both the studies conducted in Peru and Bangladesh shows low pupillary threshold than our study. However, controversial findings regarding the impaired pupillary threshold have been documented in previous studies. Other studies conducted on obese and non-obese groups showed that obese patients have higher mean triglyceride levels than mean triglyceride level of non-obese patients.[18]

We also measure pupillary responsiveness in our study by PFDA in both the BMI groups. In the normal BMI group, the mean pupillary responsiveness at low intensity was 9.72±5.24 cd/m^2 and at high intensity, it was 25.76±4.86) cd/m^2. In the obese group, the mean pupillary responsiveness at low intensity was8.52±5.04 cd/m^2 and at high intensity increment it was25.02±4.84 cd/m^2. The previous study conducted in Peru in 2013 on normal adults showed mean pupillary responsiveness of (−16.3±7.6) cd/m^2 at low intensity increments and (−30.5±8.8) cd/m^2 at high intensity increment. Congdon *et al*.[19] suggested that the negative pupillary responsiveness show better dark adaptation. In our study, the pupillary response of normal adults and the obese adults was positive which showed that the participants of our study have poor dark adaptation.

Similarly, in this study, we also measured pupillary dynamics in normal adults as well as obese adults. The mean pupillary dynamics of normal adults at low intensity increments was 0.604±0.13) cd/m^2, while at high intensity increments it was 0.556±0.147) cd/m^2. On the other hands, the mean pupillary dynamics at low intensity increments, was 0.636±0.230 cd/m^2 and at high intensity increments, it was 0.62±0.141 cd/m^2. One previous study conducted on normal adults showed mean pupillary dynamics of 1.07±0.22 at low intensity increments and 1.20±0.22) at high intensity increments which were greater than the pupillary dynamics of our healthy adult participants.[15]

Our study showed that Pupillary threshold ≥15 was found to be strongly significant with pupillary responsiveness of low intensity (-2.9-1.3 cd/m^2) as well as high intensity (-0.9 to 0.1 cd/m^2), in both the BMI groups indicating ≥15% change, cut off value for our population.

We performed multi-effect linear regression to find the association of pupillary threshold with study variables. We found statistically significant association of pupillary threshold with high & low intensities pupillary responsiveness in both the BMI groups.

We have measured triglyceride levels in the serum as well as in tears in both the BMI groups. We did not find statistically significant difference in triglycerides levels of both normal and obese BMI adults. Other studies conducted on obese and non-obese

groups showed that obese patients have higher mean triglyceride levels as compared to participants.[20]

Our study has some limitations. We were unable to control some confounding factors as the participants of our study have different sleeping habits. Some of the participants were students and they used to study at night which might have an impact on our PFDA results. Moreover, we had explained the whole procedure to the participants, and the participants were instructed to keep their eyes opened during PFDA.
During the procedure of PFDA, few of the participants complained that they developed headache, dizziness, nausea and itching of the eyes which might have an impact on the results. Finally, we detected triglycerides in the tear samples of only obese participants and not in the tears of normal non-obese participants.

CONCLUSION

The presence of triglycerides in the human eyes can be attributed to cause delay in the pupillary response of PFDA. However, further comprehensive study with large sample size is needed to find out the relationship between pupillary threshold and triglycerides in tears.

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AUTHORS' CONTRIBUTION

SF, RN: Designed and conceptualized the research. SH: Collected the samples and performed experiments. NU, SF: Wrote the manuscript. U: Collected the data and did adaptometry. FZ: Helped in data interpretation.

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