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Association of serum advanced glycation (AGEs) end products, apolipoprotein-B and zinc in severity of T2DM retinopathy

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Abstract: Advanced glycation end products (AGEs), lipids and lipoproteins and antioxidant enzymes are involved in the development of diabetic retinopathy (DR). AGEs and modified Apolipoprotein-B (Apo-B) lead to the formation of reactive oxygen species causing damage to the retina leading to DR. Zinc has antioxidant properties and protects the retina against reactive oxygen species. The current study aimed to compare the levels of serum AGEs, Apo-B and zinc in non-diabetics and type II diabetics without and with DR. Serum AGEs and Apo-B were measured by ELISA while zinc was measured by atomic absorption spectrophotometry. The impact of all three markers on the severity of DR was calculated, individually as well as together as a model, to determine the relationship of these markers with severity of diabetic retinopathy. Regression analysis showed that AGEs, Apo-B and zinc were all contributing significantly to the severity of DR, together having an 82.8% impact on it (R^2=0.828). The model of the three parameters was best fit to indicate the severity of DR (p-value = 0.553). This study provides a basis for further validation of the suggested model with prospective studies which can then be used in clinical setups to predict the individuals at risk.

Keywords: Advanced glycation end products, apolipoprotein-B, zinc, diabetic retinopathy, non-proliferative diabetic retinopathy, proliferative diabetic retinopathy.

INTRODUCTION

Diabetic retinopathy (DR) is amongst the most common complications of diabetes mellitus leading to blindness. The earlier stage of DR is called non-proliferative diabetic retinopathy (NPDR) and the advanced stage is called proliferative diabetic retinopathy (PDR) (Duh *et al*., 2017). Hyperglycemia, Advanced glycation end products (AGEs), ROS (Movahedi Rad *et al*., 2017), lipids and lipoproteins (Ahsan, 2015) etc. play a role in its development.

AGEs are formed by the nonenzymatic reaction of sugar with proteins and lipids. Its formation is accelerated in diabetes. AGEs have been shown to be involved in pathophysiology of DR through the increase in the production of reactive oxygen species (ROS) (Sharma *et al*., 2012). Studies suggest that elevated levels of lipids cause an increase in retinal hard exudates and subsequent severity of DR (Hewapathirana and Page, 2012). Apolipoproteins may be a better biomarker of DR than traditional lipids (Prakash *et al*., 2016). There is a strong association of Apo-B with the severity of DR (Rathnakumar Krishnamoorthy 2017). In diabetes, due to the abnormal capillary leakage, there is extravascular sequestration of lipoproteins resulting from their glycation and subsequent cross linkage (Wu *et al*., 2008). Modified lipoproteins cause neuronal and vascular damage to the retina resulting in the development of diabetic retinopathy (Yu and Lyons, 2013). Any decrease in the antioxidants within the body can cause an increase in ROS. Zinc has antioxidant properties. It is needed for the catalytic function of superoxide dismutase (Jarosz *et al*., 2017), protects the sulfhydryl groups of the proteins from the attack of the free radicals (Chasapis *et al*., 2020), inhibits NADPH oxidase thus reducing the formation of free radicals and helps in the inhibition of lipid peroxidation (Prasad, 2014). Its deficiency may be linked with the development of DR (Luo *et al*., 2015).

Diabetic retinopathy leads to blindness that can be prevented if timely measures are taken. The current study aimed to determine the relationship of AGEs, Apo-B and zinc with the severity of DR in type II diabetics to develop a model to indicate the patients at risk of development and progression of DR. This model can then be validated by prospective studies to see if it can be used to predict development and progression of DR in type II diabetics in clinical setups.

MATERIALS AND METHODS

The study was approved by Advanced Study and Research Board, University of Health Sciences, Lahore. Ethical approval was sought from Committee of Ethical Review, UHS, Lahore. Both male and female subjects with ages 40 years or above were taken. Duration of diabetes for patients was five years or more. Patients with glaucoma or history of lipid lowering drug intake were excluded. The samples were collected from outpatient department of Ophthalmology units in Sheikh Zayed

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Hospital, Lahore and Lahore General Hospital after a written consent was taken. Data was collected on a predesigned proforma. Diagnosis was done on fundoscopy by the ophthalmologists according to the clinical criteria (Wilkinson *et al*., 2003). Our study included 81 subjects divided into four groups. Group 1 included twenty healthy, non-diabetic controls with normal cholesterol levels, group 2 included twenty type 2 diabetic patients without DR, group 3 included twenty type 2 diabetic patients with NPDR and group 4 included twenty-one type 2 diabetic patients with PDR.

Levels of serum AGEs and Apo-B were measured by enzyme linked immunosorbent assay (ELISA) kit manufactured by Bioassay Technology Laboratory, China, using an automated analyzer. Serum zinc levels were measured by atomic absorption spectrophotometry. Random blood sugar levels and serum cholesterol levels were also measured in the controls.

STATISTICAL ANALYSIS

The data was analyzed using IBM SPSS software (version 25.0, SPSS Inc.). Mean ± SD was given for normally distributed while median and inter-quartile range (IQR) was given for skewed quantitative data. 1-way ANOVA test and post-hoc Tukey's test was used to compare the means of normally distributed homogenous data. Kruskal Wallis test and post-hoc Mann-Whitney U test (pairwise Kruskal-Wallis test) was used to compare the medians and IQR of skewed, non-homogenous data. Pearson correlation test was used to observe the correlation between AGEs, Apo-B and zinc while Spearman correlation test was used to determine the correlation between quantitative and qualitative parameters within each group. Logistic regression test was applied to establish the contribution of the AGEs, Apo-B and zinc to the severity of DR. R^2 gave the percentage impact of the markers on the severity of the disease and p-value ≥0.05

Table 1: Data of quantitative parameters in study groups

Parameters		Controls (Group 1) n=20	Diabetics without DR (Group 2) n=20	Diabetics with NPDR (Group 3) n=20	Diabetics with PDR (Group 4) n=21
Age (yrs)	Mean (SD)	47.9 ± 5.457	58.6 ± 8.9	52.35 ± 6.42	52.1 ± 6.04
	Median (IQR)	47.5 (8)	56 (14)	50 (12)	50 (8)
	p-value	0.62	0.072	0.235	0.35
Duration of DM (yrs)	Mean (SD)	-	9.15 ± 7.73	8.35 ± 3.2	13.48 ± 7.32
	Median (IQR)	-	6 (5)	8 (7)	12 (9)
	p-value	-	<0.001	0.004	0.019
AGE (ng/L)	Mean (SD)	394.447± 114.790	795.601 ± 168.125	1219.287±283.023	1116.552±311.614
	Median (IQR)	382.783 (166.971)	811.886 (265.127)	1123.547(572.941)	1026.129(545.248)
	p-value	0.156	0.684	0.015	0.013
Apo-B (µg/ml)	Mean (SD)	798.998± 103.393	874.203 ± 108.248	946.493± 138.541	989.610±107.968
	Median (IQR)	789.989 (171.485)	852.261 (193.572)	936.036 (243.367)	953.532 (178.159)
	p-value	0.413	0.054	0.206	0.153
Zinc (ppm)	Mean (SD)	0.467 ± 0.01	0.466 ± 0.017	0.448 ± 0.016	0.432 ± 0.017
	Median (IQR)	0.467 (0.018)	0.466 (0.025)	0.454 (0.025)	0.425 (0.028)
	p-value	0.717	0.766	0.272	0.175

p-value generated according to Shapiro Wilk test. Data normal if p-value≥0.05*

Table 2: Data of qualitative parameters in study groups

Parameters		Controls (Group 1) n=20	Diabetics without DR (Group 2) n=20	Diabetics with NPDR (Group 3) n=20	Diabetics with PDR (Group 4) n=21
Gender	Male	11 (55%)	10 (50%)	8 (40%)	11 (52.4%)
	Female	9 (45%)	10 (50%)	12 (60%)	10 (47.6%)
Use of insulin	Present	0 (0%)	4 (20%)	8 (40%)	16 (76.2%)
	Absent	20 (100%)	16 (80%)	12 (60%)	5 (23.8%)
Smoking	Yes	5 (25%)	4 (20%)	6 (30%)	4 (19%)

Table 3a: Individual parameter model

Parameter	Nagelkerke R^2	Hosmer Lemeshow Test p-value*
AGE	0.501	0.707
Apo-B	0.178	0.098
Zinc	0.457	0.812

p-value ≥0.05 is significant*

Table 3b: Variables in the equation

Parameter	B	S.E	Wald	df	p-value	Exp (B)
AGE	0.009	0.003	10.614	1	0.001	1.009
Apo-B	0.007	0.003	6.751	1	0.019	1.007
Zinc	-87.548	25.122	12.145	1	<0.001	0

p-value ≤0.05 is significant

Table 4a: Multiple parameter model

Parameter	Nagelkerke R square	Hosmer Lemeshow test (p-value)*
AGEs, Apo-B, Zinc	0.828	0.553

p-value ≥0.05 is significant*

Table 4b: Variables in the equation

Parameter	B	S.E	Wald	df	p-value	Exp (B)
AGE	0.014	0.005	7.071	1	0.008	1.014
Apo-B	0.008	0.006	1.957	1	0.162	1.008
Zinc	-99.985	34.664	8.320	1	0.004	0

p-value ≤0.05 is significant

generated by Hosmer Lemeshow test was significant and the model was best fit to indicate the disease severity.

RESULTS

Mean ± SD and median (IQR) of biochemical parameters is given in table 1. Our study showed that there was a significant difference in median levels of serum AGEs between the groups (p-value<0.001). Pairwise comparison showed that there was a clinically significant difference between the median of AGE amongst group 1 and 2 (p-value = 0.006), group 1 and 3 (p-value <0.001), group 1 and 4 (p-value <0.001), group 2 and 3 (p-value = 0.002) and group 2 and 4 (p-value = 0.048). However, there was no significant difference between the median of AGE amongst group 3 and 4 (p-value = 1). The mean levels of serum Apo-B showed a significant difference between the groups (p-value<0.001). Post-hoc test showed that there was a clinically significant difference between means of group 1 and 3 (p-value=0.001), group 1 and 4 (p-value<0.001) and group 2 and 4 (p-value=0.01) while difference between group 1 and 2 (p-value =0.175), group 2 and 3 (p-value =0.203) and group 3 and 4 (p-value= 0.631) was non-significant. In our study, there was a statistically significant difference between the mean of serum zinc amongst the four groups (p-value<0.001). Pairwise comparison showed that there was a clinically significant difference between the zinc values of group 1 and 3 (p-value = 0.001), group 1 and 4 (p-value <0.001), group 2 and 3 (p-value =0.002), group 2 and 4 (p-value <0.001), and group 3 and 4 (p-value =0.009). However, no significant difference was found between group 1 and 2 (p-value = 0.997).

No correlation was found between age of the subjects and AGEs, AGEs and duration of the DM or AGEs and zinc across the four groups. However, there was an association of use of insulin within the groups (p<0.001).

For regression analysis, the subjects were divided into two groups; one without DR and the other with DR. Controls were excluded. Logistic regression analysis showed that the impact of AGEs on the severity of DR was 50.1% (R^2=0.501, p=0.707), the impact of Apo-B was 17.8% (R^2=0.178, p=0.098) and that of zinc was 45.7% (R^2=0.457, p=0.812) as shown in table 3a. Together, the three markers had an impact of 82.8% on the severity of DR and the model was best fit to indicate the severity of the disease (R^2=0.828, p=0.553) as shown in table 4a.

DISCUSSION

The current study demonstrated a significant difference in median levels of serum AGEs between the groups (p-value<0.001). Post-hoc test showed that there was a clinically significant difference between median of all groups except group 3 and group 4 (p-value=1). The levels of AGEs were significantly higher in diabetic patients than controls. This is in congruence to several previous studies which documented similar results (Koska *et al*., 2018, Kilhovd *et al*., 1999, Ono *et al*., 1998). Movahedi and his team, however, did not find a significant difference between means of AGE in patients with NPDR and PDR (Movahedi *et al*., 2017) which was similar to our findings.

In our study, there was a statistically significant difference in the mean of the Apo-B levels amongst all four groups (p<0.001) with their levels being higher in diabetics without and with DR as compared to controls. Our results were similar to the results of Rathnakumar *et al* and Sasongko *et al* who demonstrated that there was an increase in Apo-B with the progression of DR (Rathnakumar 2017, Sasongko *et al*., 2011). Deguchi and his team, had also found out that the levels of Apo-B were lower in NPDR than PDR (Deguchi *et al*., 2011).

Our results were similar to observations made by Namitha and colleagues, who found that there was an increase in Apo-B in diabetic patients with DR as compared to controls but the difference in the Apo-B levels between diabetics without DR and with DR was not statistically significant probably because of higher number of NPDR patients in their study subjects (Namitha *et al*., 2017). We also did not find a significant difference in the mean of Apo-B between patients without DR and with NPDR. In another study, Hu and his colleagues showed that there was non-significant difference in the levels of Apo-B between patients with very mild NPDR and patients with PDR (Hu *et al*., 2012) agreeing with our results. Although there was a significant difference of the Apo-B levels between controls and patients with NPDR and PDR in our study, the mean values of Apo-B were in normal range (<98 mg/dl) (Kanani and Alam, 2010) in groups 1, 2 and 3 while that in group 4 was 98.9 mg/dl. Smoking and BMI may both be determinants of Apo-B (Frondelius *et al*., 2017). We did not find any correlation between Apo-B and smoking with increasing severity of DR. The relationship of Apo-B levels with BMI could not be determined as we did not record the height of the patients.

We found that there was significant difference in the means of serum zinc levels between our study groups (p-value<0.001). On applying post-hoc test, a clinically significant difference was found between all groups except group 1 and group 2 (p-value=0.997). This was similar to results achieved by Praveena *et al* and Kumari *et al* endorsing our idea that lack of antioxidants may promote the development and progression of DR in diabetic patients (Kumari *et al*., 2014, Praveena *et al*., 2013). Our results, however, were in contrast to a study done by Lou *et al* who did not find a significant correlation between zinc and DR (Luo *et al*., 2015). In our study, we found that the levels of zinc were considerably lower than the reference range (0.8-1.2µg/ml) even in control groups. An earlier study done in Pakistan had shown a deficiency of zinc in women and children (Khalid *et al*., 2014). In the current study, the serum zinc levels continued to show a decreasing trend in diabetic patients without DR and in patients with NPDR and PDR similar to the studies carried out by Praveena *et al*. and Kumari *et al*. but were lower than the reference range (Kumari *et al*., 2014, Praveena *et al*., 2013). This deficiency in our study group may be associated with nutritional effects which could not be ruled out because no data had been collected regarding their food intake. This could also be explained by certain factors that may be leading to decreased absorption or increased excretion of zinc from body or diets low in zinc due to deficient minerals in the regional soil where they are grown which needs to be probed into.

Our study indicated that even in the presence of normal or close to normal renal functions, the levels of AGE were higher in patients with DR as compared to controls. This was in contrast to study documented by Wagner and his co-workers who stated that there was no increase in AGEs in type II diabetics with normal creatinine levels (Wagner *et al*., 2001). Only 19 of our study subjects were smokers and we did not find any association between smoking and AGE levels on application of Spearman correlation. Inferring from our results, the levels of AGEs may be high in diabetic patients without and with DR and oxidative stress produced by hyperglycemia alone may contribute to the development of DR. Decreased clearance of AGEs from the kidneys or smoking may not have an impact on progression and severity of DR. This confirms the role of oxidative stress produced by AGEs due to hyperglycemia and the subsequent reactions taking place in the body leading to development of DR. The normal or near to normal renal functions may also be a reason for normal Apo-B levels as chronic kidney disease has been seen to affect Apo-B catabolism (Chan *et al*., 2009).

No study has been reported so far that attempted to establish a model to determine if the three parameters namely serum AGEs, Apo-B and zinc together had an impact on the severity of the DR. For the sake of statistical analysis, the study subjects were divided into two groups. The controls were excluded. The remaining 61 subjects were divided into two groups. Group 0 included diabetics without DR and group 1 included diabetics with DR. Logistic regression was initially applied on each parameter individually to see whether it fit best to indicate the severity of the disease. It showed that AGEs had an impact of 50.1%, Apo-B had an impact of 17.8% and zinc had an impact of 45.7% on the severity of the DR. Increase in AGEs may be contributing to the disease through their oxidative pathway and fall in zinc causing progression of the disease due to lack of defense against the oxidative stress. When the three parameters were assessed together, the test statistics showed that the three parameters together had an 82.8% impact on the severity of the disease (R^2=0.828) and the model was best fit to indicate the severity of the disease (p-value = 0.553) because both AGE and Apo-B (levels within normal range) showed a rise while zinc showed a decrease in levels from control group towards the groups with DR. Hence, the model consisting of AGEs, Apo-B and zinc together could be used to predict the development and progression of DR in type 2 diabetics. However, our results need to be validated with prospective studies for use in clinical setups.

CONCLUSION

DR is one of the most common causes of blindness which can be prevented if candidates at high risk are timely identified and warned. Since our model illustrates that serum AGEs, Apo-B and zinc together have a major (82.8%) impact on severity of DR, monitoring their levels

in such individuals can be helpful in preventing or delaying the development of DR. Our study provides a basis for larger scale researches on these markers for further validation and determination of timing of following up of their levels in type 2 diabetic patients. These markers can then be used for follow up in clinical setups.

REFERENCES

Ahsan H (2015). Diabetic retinopathy -Biomolecules and multiple pathophysiology. *Diabetes Metab. Syndr,* **9**(1): 51-54.

Chan DT, Dogra GK, Irish AB, Ooi EM, Barrett PH, Chan DC and Watts GF (2009). Chronic kidney disease delays VLDL-apoB-100 particle catabolism: Potential role of apolipoprotein C-III. *J. Lipid Res.,* **50**(12): 2524-2531.

Chasapis CT, Ntoupa PSA, Spiliopoulou CA and Stefanidou ME (2020). Recent aspects of the effects of zinc on human health. *Arch. Toxicl.,* **94**(5): 1443-1460.

Deguchi Y, Maeno T, Saishin Y, Hori Y, Shiba T and Takahashi M (2011). Relevance of the serum apolipoprotein ratio to diabetic retinopathy. *Jpn. J. Ophthalmol.,* **55**(2): 128-131.

Duh EJ, Sun JK and Stitt AW (2017). Diabetic retinopathy: current understanding, mechanisms and treatment strategies. *JCI Insight,* **2**(14): e93751.

Frondelius K, Borg M, Ericson U, Borne Y, Melander O and Sonestedt E (2017). Lifestyle and dietary determinants of serum apolipoprotein a1 and apolipoprotein b concentrations: Cross-sectional analyses within a Swedish cohort of 24,984 individuals. *Nutrients,* **9**(3): 211.

Hewapathirana N and Page S (2012). Diabetic microvascular complications - screening, diagnosis and prevention. *Clin. Focus Pri. Care,* **6**(3): 177-191.

Hu A, Luo Y, Li T, Guo X, Ding X, Zhu X, Wang X and Tang S (2012). Low serum apolipoprotein A1/B ratio is associated with proliferative diabetic retinopathy in type 2 diabetes. *Graefes Arch. Clin. Exp. Ophthalmol.,* **250**(7): 957-962.

Jarosz M, Olbert M, Wuszogrodzka G, Mlyniec K and Librowski T (2017). Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling. *Inflammopharmacology,* **25**(1): 11-24.

Kanani FH and Alam JM (2010). Apolipoprotein B in Type 2 diabetics a cross sectional study in a tertiary care set-up. *JPMA,* **60**(8): 653.

Khalid N, Ahmed DA, Bhatti M, Randhawa M, Ahmad A and Rafaqat R (2014). A question mark on zinc deficiency in 185 million people in Pakistan-possible way out. *Crit. Rev. Food Sci. Nutr,* **54**(9): 1222-40.

Kilhovd BK, Berg TJ, Birkeland KI, Thorsby P and Hanssen KF (1999). Serum levels of advanced glycation end products are increased in patients with type 2 diabetes and coronary heart disease. *Diabetes Care,* **22**(9): 1543-1548.

Koska J, Saremi A, Howell S, Bahn G, De Courten B, Ginsberg H, Beisswenger PJ and Reaven PD (2018). Advanced glycation end products, Oxidation products, and incident cardiovascular events in patients with type 2 diabetes. *Diabetes Care,* **41**(3): 570-576.

Kumari S, Pradhan T and Panda T (2014). Trace Minerals and Oxidative Stress in Diabetic Retinopathy. *Bangladesh J. M. Sci.,* **13**(2): 175-179.

Luo YY, Zhao J, Han XY, Zhou XH, Wu J and Ji LN (2015). Relationship between serum zinc level and microvascular complications in patients with type 2 diabetes. *Chin. Med. J.,* **128**(24): 3276.

Movahedi Rad M, Rabizadeh S, Salehi S, Mirmiranpour H, Esteghamati A, Jafari R and Nakhjavani M (2017). Advanced end glycation products, Advanced oxidation protein products and ferritin reducing ability of plasma as markers of diabetic retinopathy. *Austin J. Endo. Diabetes,* **4**(1): 1057-1062.

Namitha D, Nusrath A, Rajeswari A, Rani NA and Shilpashree YD (2017). Apolipoprotein A-1 and apolipoprotein B: Better indicators of dyslipidemia in diabetic retinopathy patients? *Indian J. Biochem.,* **21**(2): 142-146.

Ono Y, Aoki S, Ohnishi K, Yasuda T, Kawano K and Tsukada Y (1998). Increased serum levels of advanced glycation end-products and diabetic complications. *Diabetes Res. Clin. Pract.,* **41**(2): 131-137.

Prakash G, Agrawal R, Satsangi SK and Prakash S (2016). Comparison of Serum Apolipoproteins and Traditional Lipids in Eyes with Diabetic Retinopathy in Indian Population: A Case Series. *Middle East Afr. J. Ophthalmol.,* **23**(2): 212-214.

Prasad AS (2014). Zinc is an antioxidant and anti-inflammatory agent: Its role in human health. *Frontiers in Nutrition,* **1**(14): 1-10.

Praveena S, Sujatha P and Ganesh B (2013). Study of micro elements in Diabetic Retinopathy. *J. Med. Dent. Sci.,* **2**(6): 531-537.

Rathnakumar Krishnamoorthy RK, Ramesh Venkatachalam R Poovitha and Geetha Rajagopalan (2017). Apolipoproteins an early and better diagnostic marker for diabetic retinopathy. *J. Clin. Diagnostic Res.,* **11**(10): NC01-NC05.

Sasongko MB, Wong TY, Nguyen TT, Kawasaki R, Jenkins A, Shaw J and Wang JJ (2011). Serum apolipoprotein AI and B are stronger biomarkers of diabetic retinopathy than traditional lipids. *Diabetes Care,* **34**(2): 474-479.

Sharma Y, Saxena S, Mishra A, Saxena A and Natu SM (2012). Advanced glycation end products and diabetic retinopathy. *J. Ocul. Bio, Dis, Infor.,* **5**(3-4): 63-69.

Wagner Z, Wittmann I, Mazak I, Schinzel R, Heidland A, Kienysch-Engel R and Nagy J (2001). Nϵ-(carboxymethyl) lysine levels in patients with type 2 diabetes: Role of renal function. *AJKDD,* **38**(4): 785-791.

Wilkinson CP, Ferris FL, Klein RE, Lee PP, Agardh CD, Davis M, Dills D, Kampik A, Pararajasegaram R and Verdaguer JT (2003). Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology,* **110**(9): 1677-1682.

Wu M, Chen Y, Wilson K, Chirindel A, Ihnat MA, Yu Y, Boulton ME, Szweda LI, Ma JX and Lyons TJ (2008). Intraretinal leakage and oxidation of LDL in diabetic retinopathy. *IOVS,* **49**(6): 2679-85.

Yu JY and Lyons TJ (2013). Modified lipoproteins in diabetic retinopathy: A local action in the retina. *J. Clin. Exp. Ophthalmol.,* **4**(6): 314.