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ORIGINAL ARTICLE

Association of serum homocysteine with type II diabetic retinopathy.

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ABSTRACT… Objective: To elucidate the association of serum homocysteine with diabetic retinopathy. **Study Design:** Case Control study. **Setting:** Department of Physiology and Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, in Collaboration with Armed Forces Institute of Ophthalmology, Rawalpindi. **Period:** December 2019 to December 2020. **Material & Methods:** A total of ninety subjects were enrolled in the study which were subdivided into three groups; healthy subjects, diabetic subjects and patients with diabetic retinopathy (DR). The permission for carrying out the study was obtained from ethical review committee. Confidentiality of the data was maintained. The data obtained was analyzed and processed using SPSS software. **Results:** The mean Fasting Blood Glucose (FBG) levels were found to be 5.51 ± 0.34 (mmol/l), 8.11 ± 0.67 (mmol/l) and 8.73 ± 0.90 (mmol/l) in healthy controls, diabetic subjects and patients with DR respectively (p=0.001). The mean serum homocysteine levels were found to be 10.12 + 1.95 (μmol/l), 24.99 ± 4.25 (μmol/l) and 45.78 + 9.66 (μmol/l) in healthy controls, diabetic subjects and patients with DR respectively (p=0.001). **Conclusion:** Our research can be concluded that serum homocysteine levels have a strong association with the development of diabetic retinopathy. Monitoring the serum levels of this inflammatory biomarker can therefore be helpful in obviating the development of diabetic microangiopathic complications, particularly diabetic retinopathy. Serum homocystein can be used a prognostic tool in the progression of microangiopathic complications of diabetes.

Key words: Diabetic Retinopathy, Fasting Blood Glucose, Serum Homocysteine.

INTRODUCTION

Diabetic retinopathy is one of the major ocular complications of Type 2 Diabetes Mellitus. It affects retinal micro vasculature and causes gradual vision loss and blindness.[1] Prevalence of diabetic retinopathy directly depends upon duration and control of T2DM. Globally, total number of diabetic patients having DR is approximately 95 million (35.4%). Per year incidence of diabetic retinopathy is 2.2%–12.7% and progression 3.4%–12.3-%.[2] In the world blindness has a prevalence of approximately 1.5 billion, in which diabetic retinopathy comprises 0.4 million. Although visual impairment and blindness has decreased globally but blindness due to diabetic retinopathy has increased from 0.2 million to 0.4 million.[3,4] Risk factors of diabetic retinopathy are prolonged uncontrolled diabetes mellitus, anemia, hypertension, smoking, abnormal lipid metabolism, and nephropathy.[5]

Homocysteine is an amino acid consisting of glycine and a side chain made up of 2-mercaptoethyl. A thiol-containing amino acid formed by a demethylation of Methionine (Met).[6] It is considered that homocysteine is also involved in vaso-occlusive disorders of eye and this can be a useful biomarker for increased risk of DR in patients of T2DM. Increased levels of homocysteine causes platelet activation, increase coagulability, vascular smooth muscle cell (VSMC) proliferation, production of reactive oxygen species and decreased antioxidant action.[7]

Glutamate is an excitatory amino acid found in the retina and brain. Diabetic retinopathy is also initiated by disruption of homeostasis

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of glutamate. Glutamate causes activation of N-methyl D-aspartate (NMDA) receptors that result in production of free radicals that induce apoptosis of neuronal cells.[8] Therefore, by decreasing the level of extracellular glutamate or blocking the activation of NMDA receptors may reduce cell death and neurotoxicity. Studies suggest that homocysteine acts as an agonist at the glutamate site of NMDA receptors.[8,9] Homocysteine has sulfhydryl (-SH) group that increases the oxidative stress because it act as a pro-oxidant molecule. Also, sulfhydryl (-SH) group of homocysteine makes disulfide bonds which blocks protein function and causes endoplasmic reticulum (ER) stress.[10] Increase ER stress in eye increases retinal expression of VEGF and TNF-α, and vascular leakage, while blocking of ER stress minimize these changes in diabetic retina indicating the role of ER stress in the breakdown of the blood retinal barrier.[11] Increased homocysteine in type 2 diabetic patients elevates oxidative stress and decreases nitric oxide formation and is therefore responsible of endothelial dysfunction.[12]

Homocysteine increases smooth muscle proliferation and affects the extracellular matrix. Thus elevated homocysteine level may act as a pathogeneic factor in the development of diabetic retinopathy.[13] Deficiency of folate and vitamin B12 has a strong association with elevated serum homocysteine levels. Dietary supplementation can be managed at a very affordable cost. Role of hyperhomocysteinemia in the pathogenesis of DR may help in identifying a novel target to fight against this potentially blinding disease.[14,15]

MATERIAL & METHODS

Our study was carried at Department of Physiology/ CREAM/ Army Medical College / National University of Medical Sciences in collaboration with Pak Emirates Military Hospital and Armed Forces Institute of Ophthalmology, Rawalpindi from December 2019 to December 2020. A formal approval from Ethical Review Committee (ERC) was taken by ethical review Committee of Army Medical College (ref ID ERC ID 92). It was a cross sectional and analytical study. Study duration was one year. Sample size was calculated as ninety subjects using open Epi website calculator using a reference study carried out in Peshawer Pakistan by taking 37% prevalence of increased Homocystein in type II diabetes.[15] We divided it into three groups, control, diabetics and diabetic retinopathy. We included normal healthy individuals in group I, diabetic patients without any complication in group II and diabetic patients with retinopathy in group III.

Inclusion criteria was subjects of both genders, age between 30 to 60 years, patients diagnosed with diabetes mellitus type 2 and willing to participate in the study.

Subjects having ocular or systemic disease affecting retinal vasculature, previous ophthalmic surgical intervention, patients with a history of non diabetic macro and micro vascular complications, patients receiving medication like vitamin B12 and folate supplementation, patients with any other chronic or neoplastic diseases and patients taking NSAIDS for last two weeks were excluded from our study.

Blood sampling was done under strict aseptic conditions. Samples for determination of serum homocysteine were collected in serum separating tubes they were allowed to clot then centrifuged and stored at -80 Celsius and then analyzed by using technique of Chemi Luminscence Immuno Assay (CLIA).

Data collected was analyzed using computer software SPSS version 25. Quantitative variables like age, BSF, BMI, HbA1c and serum homocysteine were expressed as mean and standard deviation (SD). Repeated measures Analysis of Variance (ANOVA) was applied among three groups to find the statistical significant value. Significant variables were compared between two groups by applying Post Hoc Tukey's test which confirmed the previous result. Pearson correlation coefficient was applied to assess the relationship of serum homocysteine with other parameters. P value of ≤ 0.05 was considered to be significant.

RESULTS

In this study 90 patients were included and divided them into 3 groups of 30 each. Age comparison, blood glucose fasting, HbA1c and serum homocysteine are shown in Table-I. Accordingly the groups were compared by applying one way ANOVA.

We compared our variables in two groups by applying post hoc tukey test. It further confirmed our previous results and it also confirmed our hypothesis (Table-II). We correlated serum homocysteine with other variables and it showed us that serum homocysteine has strong positive correlation with FBG, RBG and HbA1c with significant p value (Table-III).

We compared our variables in two groups by applying post hoc tukey test and it confirmed our hypothesis (Table-II). We found that serum homocysteine has strong positive correlation with FBG, RBG and Hb1Ac with significant p value (Table-III)

DISCUSSION

Diabetes mellitus is considered a burden on the whole global economy. It is directly a burden for whole world economy by causing excess health expenditure. One of the major microvascular complications of T2DM is diabetic retinopathy. Almost one third of diabetic patients develop diabetic retinopathy and one third of diabetic retinopathy patients experience permanent loss of vision. Unfortunately, diabetes mellitus is a silent killer and patients of diabetic retinopathy remain asymptomatic till the irreversible stages, so it is important to diagnose DR in its early stage. In this way we can reduce the chances of development and progression of DR.

WHO criteria were selected to diagnose diabetic patients and it is a normal routine practice.[18] According to these criteria, we did Fating Blood Glucose (FBG), Random Blood Glucose (RBG) and HbA1c of all participants. This criteria is a standard and has been used in many studies of similar nature.[16] The mean age was found to be 44.90 ± 5.83 and 45.07 ± 5.73 in diabetic and diabetic retinopathic group respectively.

Variables	Group I Normoglycemic (n=30)	Group II DM (n=30)	Group III DR (n=30)	P-Value
Age years	44.63 + 4.88	44.90 + 5.83	45.07 + 5.73	0.954
FBG mmol/l	5.51 + 0.34	8.11 + 0.67	8.73 + 0.90	0.0001
RBG mmol/l	6.55 + 0.43	12.27 + 0.76	12.84 + 0.85	0.0001
HbA1c mmol/l	5.08 + 0.27	7.70 + 0.89	9.02 + 1.76	0.0001
S.homocysteine μmol/l	10.12 + 1.95	24.99 + 4.25	45.78 + 9.66	0.0001

All values have been expressed as Mean + SD, P value ≤ 0.05 is significant, FBG: Fasting Blood Glucose, RBG: Random Blood Glucose, HbA1c: Glycosylated hemoglobin

Table-I. Comparison of age, FBG, RBG, HbA1c and serum homocysteine among normal healthy, diabetic and diabetic retinopathic groups by one way ANOVA

Variables	Group 1 Vs Group II	Group 1 Vs Group III	Group II Vs Group III
FBG mmol/l	0.0001	0.0001	0.002
RBG mmol/l	0.0001	0.0001	0.006
HbA1c mmol/l	0.0001	0.0001	0.0001
S.homocysteine μmol/l	0.0001	0.0001	0.0001

Table-II. Comparison of FBG, RBG, HbA1c and serum homocysteine between 2 groups by Post-Hoc Tukey test

Variable	Parameters correlated	r-value	P-Value
Serum homocysteine μmol/l	Age	0.083	0.43
	FBG mmol/l	0.771	0.0001
	RBG mmol/l	0.781	0.0001
	HbA1c mmol/l	0.721	0.0001

Table-III. Correlation of age, FBG, RBG and HbA1c with serum homocysteine

We selected this age criteria because many studies suggest that levels of homocysteine increase with age due to loss of intrinsic factor which decreases the absorption of vitamin B12 which is an important enzyme required for reconversion of homocysteine to methionine. As a result levels of homocysteine increase.

HbA1c shows average glycemic status, which was significantly poor in diabetic patients as compared to normoglycemic healthy controls (7.70 ± 0.89 versus 5.08 ± 0.27). In DR patients, the mean value of HbA1c was 9.02 + 1.76. The existence of a statistically significant difference in HbA1c of diabetic and DR patients (p= 0.0001 by post Hoc Tukey test) is showing the role of poorly controlled hyperglycemia in expediting the onset and development of diabetic retinopathy. Studies suggest that four weeks after chronic hyperglycemia, leukocytes begin to adhere to retinal microvasculature followed by migration into retina. These changes impair vascular wall integrity and it is responsible of vascular wall permeability which is a leading cause of DR.

This is consistent with many clinical studies, such as the Diabetes Control and Complications Trial (DCCT) study, the Kumamoto study and the U.K. Prospective Diabetes Study (UKPDS) which show that lifestyle modification and keeping HbA1c at a target level is helpful in preventing the complications of diabetes.[17]

Serum homocysteine was also found to have a significant correlation with fasting blood glucose (r=0.771 and p=0.001) and HbA1c (r=0.721 and p=0.001). A positive correlation between fasting blood glucose and homocysteine levels, consistent with the results of our study showed by Parsad. This shows that diabetes has strong association with mutation of MTHFR and as a result levels of homocysteine rise in blood of type 2 diabetic patients.[18]

A similar study was conducted by Umayahara et al., this study showed a strong positive correlation of duration of diabetes and homocysteine levels. These findings were similar to a study conducted by Sonkar in which plasma homocysteine levels were correlated with duration and complications of T2DM. A study conducted on patients with DR also showed a strong positive correlation of duration of diabetes with homocysteine. A strong positive correlation of homocysteine and HbA1c was not found (r=-0.052 and p=0.576).[19] This difference may have arisen due to selection criteria for patients.

CONCLUSION

Serum homocysteine has a strong association with development of diabetic retinopathy. Serum homocystein can be used as a diagnostic and prognostic tool in the treatment of diabetic retinopathy.

LIMITATIONS

1. The severity of diabetic retinopathy was not considered. A correlation between this inflammatory biomarker and severity of disease was not included in this study.
2. For identification of correlation, selected biomarker have been measured only once. However, follow up of same can potentially be helpful in improving prognosis.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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5	Lubna Gohar	Statistical analysis.	
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