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Serum and vitreous levels of Interleukin-6, Leptin and Vascular Endothelial Growth Factor in patients with proliferative and non-proliferative diabetic retinopathy

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ABSTRACT

Introduction: Retinopathy is one of the most dreaded consequences of chronic diabetes mellitus, progressively leading to blindness, and is believed to result from inflammatory and cytokine mediators.

Objective: To document the levels of Interleukin-6, Leptin and Vascular Endothelial Growth Factor in serum and vitreous fluid of patients with proliferative and non-proliferative diabetic retinopathy.

Materials & Methods: It was a comparative study conducted in main three hospitals of Peshawar and Al-Shifa Hospital Rawalpindi. Laboratory analyses were done in IRNUM, Peshawar. A total of 274 diabetics with and without retinopathy participated in the study. Serum and vitreous levels of the aforementioned parameters were measured by ELISA. Data analysis was done by using SPSS version 17; $p \leq 0.05$ was taken as significant.

Results: Serum and vitreous IL-6 levels were significantly greater in PDR and NPDR patients ($p<0.001$). Serum leptin concentrations were also significantly greater in PDR and NPDR patients as compared to DNR patients and normal subjects ($p<0.001$) but vitreous leptin concentrations did not differ significantly between NPDR and PDR patients ($p=0.915$). Serum VEGF values were elevated in DNR, NPDR and PDR patients as compared to the normal subjects ($p<0.001$). Vitreous level of VEGF was significantly raised in PDR than NPDR ($p<0.001$).

Conclusion: Patients with PDR had significantly high serum and vitreous levels of IL-6 and VEGF. Serum leptin levels were significantly high in PDR and NPDR but vitreous levels of leptin did not show significant difference in both PDR and NPDR.

Keywords: Interleukin-6, Leptin, Vascular Endothelial Growth Factor, Diabetic Retinopathy.

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INTRODUCTION

Diabetes mellitus is a chronic disease associated with abnormally high levels of glucose in blood due to inadequate sensitivity of the cells to action of insulin.[1] The prevalence of diabetes is on a rise worldwide and has reached to 16.98% in Pakistan during 2019[2] and it was 4·0% in Nepal and 8·8% in India in 2017.[3]

Diabetic Retinopathy (DR) being the most prevalent microvascular complication of diabetes is the primary cause of visual impairment worldwide.[4] The prevalence of DR is 28.5% in United States.[5] The prevalence of DR is quoted as 10.6% in Pakistan.[6]

Diabetic retinopathy is staged into proliferative and non-proliferative types on the basis of ophthalmoscopically visible findings. The non-proliferative stage is characterized by lipid exudates, retinal hemorrhages, vascular tortuosity and micro aneurysms, while in proliferative stage there is neovascularization. Another additional categorization is Diabetic Macular Edema (DME), in which there is fluid accumulation into the neural retina[4].

As far as pathophysiology is concerned, inflammation is the major contributor of development of DR. Studies show that most of the inflammatory cytokines are increased in serum and ocular fluids in patients with DR.[7,8]

The instability of blood vessel wall is dependent mainly on two very important factors i.e. the endothelial cells and mural cells. Abnormal interaction between endothelial cells and mural cells can lead to alarming changes leading to microangiopathy and angiogenesis. The exact mechanism involving these changes is due to the release of certain growth factors like Vascular Endothelial Growth Factor (VEGF), Advanced Glycation End Products (AGEs), Platelet Derived Growth Factor (PDGF), adipose tissue-derived protein Leptin and a cytokine Interleukin-6 (IL-6). Due to hyperglycemia, the level of VEGF in vitreous is correlated to retinopathy. VEGF is responsible for angiogenesis, considered as a strong

characteristic of DR.[9] In vitro studies show that leptin regulates release of cytokines. Leptin causes proliferation and anti-apoptotic activity and angiogenesis.[10] The present study was designed to investigate samples of diabetic retinopathic and non-retinopathic patients to further delineate the association of predictor variables with retinopathy.

The objective was to measure and compare the vitreous and serum levels of IL-6, Leptin and VEGF in patients of non-proliferative and proliferative diabetic retinopathy, in normal subjects and in diabetics without retinopathy.

MATERIALS & METHODS

This was a comparative study conducted in 3 main hospitals of Peshawar including Hayatabad Medical Complex, Khyber Teaching Hospital and Lady Reading Hospital; further sampling was done from Al-Shifa Eye Trust Hospital, Rawalpindi city. A total of 274 diabetic patients were taken and detailed clinical information was noted on a structured Performa. Patients with systemic disorders, kidney or heart diseases, liver dysfunction respiratory and GI disorders were excluded. The study was completed in six months after obtaining ethical approval from Pakistan Medical Research Council (PMRC) and National Institute of Health (NIH), Islamabad. Lab analysis was done in Institute of Radiotherapy and Nuclear Medicine (IRNUM), Peshawar. Vascular endothelial growth factor concentration in both serum and vitreous samples were measured through a standard solid phase sandwich enzyme linked-immunosorbent assay (ELISA), with commercial kit of Assay Pro (Belgium). Serum and vitreous Interleukin-6 concentration were measured through a standard solid phase sandwich enzyme linked-immunosorbent assay (ELISA) using a commercial kit of Immunotech SAS (France). Leptin concentration of both serum and vitreous samples were measured through a standard solid phase sandwich enzyme linked-immunosorbent assay (ELISA) using a commercial kit of Immunotech SAS (France). Data were analyzed using SPSS version 17. Mean and standard deviation were calculated for numerical variables. Student's T-test was used for comparison of differences in mean values; p value ≤0.05 was considered significant.

RESULTS

Out of 274 diabetic patients, 31 were diabetic non-retinopathic (DNR) which were taken as positive controls. Another group of 32 normal healthy subjects (NS) were taken as negative control. A total of 88 diabetics had non-proliferative retinopathy (NPDR), and 155 diabetics had proliferative retinopathy (PDR).

Table 1 shows that serum IL-6 levels were significantly greater in PDR and NPDR patients as compared to DNR patients and normal subjects (p<0.001). Serum leptin concentrations were also significantly greater in PDR and NPDR patients as compared to DNR patients and normal subjects (p<0.001). Serum VEGF values were elevated in DNR, NPDR and PDR patients as compared to normal subjects (p<0.001).

Table 2 shows that IL-6 concentration in the vitreous fluid to be significantly greater in PDR patients as compared to NPDR patients (p<0.001).Vitreous leptin concentrations did not differ significantly between NPDR and PDR patients (p=0.915). VEGF concentration in the vitreous fluid was significantly greater in PDR patients when compared with NPDR patients (p<0.001).

Table 1: Serum levels of parameters for patients and normal subjects.

Parameter	Mean ± SD	p value
Serum IL-6 (*pg/ml*)		
NS	55.30 ±09.41	
DNR	66.15 ±15.10*	< 0.001
NPDR	117.55 ± 38.10*	
PDR	180.30 ± 79.20*	
Serum leptin (*ng/ml*)		
NS	10.14 ±4.10	
DNR	13.50 ± 6.13*	< 0.001
NPDR	25.50 ± 09.02*	
PDR	30.60 ± 09.66*	
Serum VEGF (*pg/ml*)		
NS	25.09 ±4.66	
DNR	89.77 ±26.90*	< 0.001
NPDR	193.50 ± 77.14*	
PDR	235.09 ± 77.60*	

Serum IL-6 * P < 0.001 vs normal; Serum leptin * P < 0.001 vs normal; Serum VEGF * P < 0.001 vs normal

Table 2: Vitreous levels for parameters for patients with proliferative and non-proliferative retinopathy.

Parameter	Mean ± SD	p value
Vitreous IL-6 (*pg/ml*)		
NPDR	610.35 ± 200.45*	< 0.001
PDR	1009.21 ± 399.76	
Vitreous leptin (*ng/ml*)		
NPDR	47.61 ±16.72*	0.915
PDR	48.59 ±16.97	
Vitreous VEGF (*pg/ml*)		
NPDR	435.41 ± 177.15*	< 0.001
PDR	989.59 ± 277.40	

Vitreous IL-6 * P < 0.001 between groups; Vitreous leptin ns between groups; Vitreous VEGF * P < 0.001 between groups

DISCUSSION

This study was conducted to find the levels of IL-6, VEGF and Leptin in type II diabetics. It was found that serum levels of the mentioned parameters were high in these subjects as compared to normal and the levels increased with degree of Retinopathy.

A study conducted in patients with DR reported that inflammatory cytokines and VEGF levels were markedly increased in PDR.[11] In a similar study vitreous concentration of IL-6 and VEGF were measured by Chernykh et al;[12] this study also revealed increased concentration of the above mentioned parameters in PDR. Serum level of VEGF were also shown to be elevated in patients of PDR in another study.[13] A study conducted by Wu H et al[11] also showed that IL-6 level was elevated in vitreous samples of NPDR, PDR as compared to control. In the present study, we found similar results that vitreous levels of IL-6 and VEGF were elevated significantly. The IL-6 concentration was found to correlate with severity of macular edema in a study conducted by Shimizu et al.[14] In another study,[15] serum concentration of IL-6 was shown to be high, as is shown in this study. Serum and vitreous levels of leptin were significantly elevated in patients with PDR[16] but in our study leptin levels in vitreous had no significant difference between NPDR and PDR.

A study from Iran showed elevated blood levels of leptin in retinopathy patients.[17] which was comparable to the finding of this study. Cermon et al[18] conducted a study in diabetic Iranian

population showed that serum and vitreous concentration of leptin and VEGF were elevated in DR patients. At present there are no valid biomarkers which can monitor the severity of DR but various studies[19,20] indicate that concentration of IL-6 and VEGF increases with the severity of the disease which was similar to our findings.

Studies have shown that the use of anti-inflammatory therapy such as minocycline or salicylates in DR patients has been beneficial for the prevention of irreversible vascular and neural changes,[21,22] which further endorse the findings of the present study.

CONCLUSION

The serum and vitreous levels of VEGF, IL-6 and leptin are higher in patients with diabetic retinopathy and the level of these parameters increases with the severity of the disease.

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