ORIGINAL ARTICLE J. Med. Sci. (Peshawar, Print) January 2013, Vol. 21, No. 1: 40-43

VASCULAR ENDOTHELIAL GROWTH FACTOR, INTERLEUKIN 6 AND LEPTIN IN THE SERUM AND VITREOUS FLUID OF TYPE 2 DIABETIC PATIENTS

Nargis Parveen[1], Irfan Zia Qureshi[2]

[1]Department of Physiology, Khyber Medical College, Peshawar - Pakistan

[2]Department of Animal Sciences, Quaid-e-Azam University, Islamabad - Pakistan

ABSTRACT

Objectives: To determine the relationship of different stages of diabetic retinopathy with the vascular endothelial growth factor (VEGF), interleukin-6 (IL-6) and leptin in the serum and vitreous fluid of type 2 diabetic patients.

Material and Methods: In this cross sectional analytical study carried out during 2010-2011 in Al-Shifa Trust Eye Hospital, Rawalpindi and three tertiary hospital of Peshawar, 86 adult type 2 diabetic patients with different grades of retinopathy (no retinopathy, non-proliferative and proliferative retinopathy) and 40 non-diabetic non-retinopathic subjects of both sexes were recruited and evaluated for serum and vitreous levels of VEGF, IL-6 and leptin.

Results: Serum VEGF, IL-6 and leptin concentrations and vitreous levels of VEGF and IL-6 were significantly and progressively greater in all grades of retinopathy as compared to normal controls. Females had comparatively more concentration of this factor than males.

Conclusion: Significantly raised levels of VEGF and IL-6 in both serum and vitreous fluid and of leptin only in the serum are found in patients with diabetic retinopathy. Knowledge about these levels may be a helpful tool to a clinician in assessing the onset and progression of retinopathy for taking preventive and therapeutic measures.

Key Words: Type 2 diabetes mellitus, Diabetic retinopathy, Vascular endothelial growth factor, Intereukin-6, Leptin.

INTRODUCTION

Diabetes mellitus (DM) is non-communicable disorder that is growing out of proportions and expectations. The world-wide prevalence of diabetes among adults (aged 20-79 years) was 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% and 439 million adults by 2030. Similarly our country Pakistan is expected to rise to 4th rank from the current 7th position with a prevalence of 7.1 million diabetic population in the next two decades[1].

Among chronic complications of type 2 DM, diabetic retinopathy (DR) which is evident in most of the patients 20 years after diagnosis has become highly prevalent in this part of the world[2,3]. DR passes through a number of pathological stages before it renders the patient blind. The most threatening abnormality is the appearance of tiny and fragile new vessels on retina which can bleed into vitreous causing visual loss. One of the mechanisms involved in the aforementioned phenomenon is considered to be the local release of hypoxia induced growth factors like platelet derived growth factor, advanced glycation end products, VEGF, IL-6 and leptin some of which also appear in general circulation[4,5].

There has been very limited data with reference to above growth factors in our local diabetic population. We conducted the current study to investigate both serum and vitreous levels of VEGF, IL-6 and leptin in relation to DR.

Address for Correspondence:
Dr. Irfan Zia Qureshi
Associate Professor
Physiology and Neuroscience,
Department of Animal Sciences,
Quaid-e-Azam University Islamabad - Pakistan
Cell: 0307-7111295
Email: irfanzia@qau.edu.pk

MATERIAL AND METHODS

This was a cross-sectional analytical study carried out over a period of one year during 2010-2011 in Al-Shifa Trust Eye Hospital, Rawalpindi and the three tertiary care hospitals of Khyber Pakhtunkhwa (KPK) Province based in Peshawar i.e Khyber Teaching Hospital, Hayatabad Medical Complex and Lady Reading Hospital. A total of 126 cases were recruited for the study among 2000 consecutive patients attending the eye departments of above mentioned health facilities with different ophthalmic problems. Both male and female subjects belonging to different districts of KPK having age more than 30 years were included in the study. Exclusion criteria were severe systemic illness, cardiac, renal or cerebral

dysfunction as well as ophthalmic conditions like cataract, severe or chronic conjunctivitis, glaucoma and previous laser therapy. Selected cases for the study were divided into four groups; diabetes with no retinopathy group serving as positive controls (CDNR, n=39), non-proliferative diabetic retinopathy group (NPDR, n=21), proliferative diabetic retinopathy group (PDR, n=26) and normal subjects ie non-diabetic non-retinopathic group serving as negative controls (NS, n=40). Informed consent was obtained from all subjects and the research had the approval of ethical committee of Pakistan Medical and Research Council.

Anthropometric and clinical data including age, sex, diabetes duration, weight, body mass index (BMI), history of present and past illnesses, systolic and diastolic blood pressures (BP) of all the subjects were obtained on direct interview and examination. Fundoscopic examination of the dilated eyes of each patient was performed with the help of indirect ophthalmoscopy substantiated by fundus flouroscine angiography and/or fundus photography using standard techniques. Non-proliferative diabetic retinopathy (NPDR) was defined as having microaneurysms, dot or blot hemorrhages, hard or soft exudates or venous beadings while proliferative diabetic retinopathy (PDR) was defined as having neovascularization or pre-retinal hemorrhages in either of the eye[6].

Five ml of blood sample obtained from each subject was preserved at -20° while 0.1-1ml of vitreous samples, obtained only from patients having diabetic retinopathy and undergoing surgery, were preserved at -30°. Meaurements of both serum and vitreous VEGF, IL-6 and leptin levels were performed through enzyme linked immunoassay using Human VEGF ELISA kit manufactured by R&D Systems, Inc (Mineapolis, MN, USA). Other laboratory tests such as fasting and random blood glucose, hemoglobin A1c (HbA1c), urea, creatinine and lipid profile were also done.

The data was recorded on a proforma designed for the purpose and statistical analysis was done using the statistical package for social sciences (SPSS version 16.0, Chicago, Illinois, USA). Group comparisons were made with one-way analysis of variance (ANOVA). Data were presented as means and standard deviation. A *p*-value of < 0.05 was considered as significant difference.

RESULTS

A total of 126 subjects were assessed. 86 subjects were known type 2 diabetics while 40 were non-diabetic non- retinopathic control (NS) subjects. Male to female ratio was 1:1.3. Mean age of the patients was 50.13±7.1 (range=30-70) years. Mean BMI was 34.72±4.96 for diabetic patients vs. 24.87±4.30 for the normal subjects with significant difference ($p<0.01$). Of 86 diabetic patients, 21 had NPDR, 26 had PDR while 39 had no retinopathy. Serum concentrations of all three factors were significantly higher in CDNR, NPDR and PDR groups as compared to NS subjects ($P < 0.001$). Highest mean values were found in PDR patients followed by NPDR, CDNR and NS groups in that order. Inter-group comparison among retinopathic patients showed that serum levels in CDNR patients were significantly lower than both NPDR and PDR patients. Evaluation for sex differences revealed somewhat higher values in the females. A positive, though not much significant, correlation of BMI, HbA1c and systolic and diastolic blood pressures with progression of retinopathy was noted as their values were found progressively higher in the upper grades. However there was no significant difference by age among different groups (Table 1). The vitreous levels obtained only for NPDR and PDR patients showed significant difference in case of IL-6 and VEGF ($p<0.001$) but not leptin ($p=0.0915$).

DISCUSSION

Metabolic control, reflected by the blood glucose level and HbA1c value, is an important indicator for the onset and progression of DR[7]. Nevertheless the precise pathogenic mechanism of DR is yet not clear and current therapeutic strategies signify the importance of better understanding of the pathogenesis for an improved management of DR. Several studies have shown association of IL-6, VEGF and Leptin with retinopathy[8,9]. We conducted the present study on non-diabetic and diabetic non-retinopathic and retinopathic patients in our own population to evaluate whether levels of these factors deviate from normal in their serum and vitreous fluids. Increased local expression of VEGF has drawn much attention relating to the pathogenesis of DR. Evidence suggests that VEGF is causally involved in the development of diabetic retinopathy and its levels have been found to be markedly increased in the vitreous and aqueous fluids of patients with PDR[10]. Although intraocular levels of VEGF have been studied extensively, systemic evaluation has not been abundantly carried out. Besides, the association of serum VEGF levels with DR has been conflicting in the literature. Most important aspect of our present study is that serum VEGF levels were found significantly elevated in NPDR and PDR patients as compared to diabetic but non-retinopathic patients ($p<.001$). Similar to ours, an earlier study by Lip *et al* and a recently concluded study by Mahdy *et al* showed that patients with DR had significantly raised VEGF plasma levels as compared to both positive and negative controls[11,12].

Regarding vitreous VEGF, our results (Table 2) are similar to the study by Brooks *et al* showing a positive correlation between intravitreal levels of VEGF and severity of DR, values being higher in PDR than

Table 1: Conventional parameters in different groups of retinopathy

Group	Age (years)	BMI (kg/m2)	HbA1c (%)	Systolic BP (mmHg)	Diastolic BP (mmHg)
NS	49.82 ± 7.30	24.87 ± 4.30	5.30 ± 0.67	125.64 ± 4.75	83.97 ± 5.40
DNR	50.47 ± 6.40	32.68 ± 4.86*a*	7.22 ± 0.74*a*	129.47 ± 7.60	86.31 ± 4.74
NPDR	49.83 ± 8.11	35.70 ± 5.23*	8.16 ± 1.59*	134.16 ± 1.28*	86.57 ± 4.96*
PDR	50.41 ± 6.57	35.80 ± 4.81*	8.31 ± 1.62*	134.37 ± 2.30*	87.08 ± 5.15*

Age: p value= 0.889;

BMI: * P < 0.001 vs NS, *a* p < 0.05 vs NPDR & PDR;

HB.A1C: * p < 0.001 vs NS, *a* p < 0.05 vs NPDR & PDR;

Systolic BP: * p < 0.001 vs NS;

Diastolic BP: * p < 0.007 vs NS

NPDR[13]. All these findings indicate that VEGF is an angiogenic factor that reflects the degree of neovascularization. Although in the present study much stronger association of VEGF with known risk factors like BMI, HbA1c and blood pressures were not observed, even though significant positive correlations were evident. High levels of VEGF therefore may possibly act as independent risk factor. Significant concentration of VEGF in the sera of NPDR and PDR patients as compared to DNR and NDC patients highlight the diagnostic significance of this factor during routine clinical examination. IL-6 is a multifunctional cytokine that indirectly causes an increase in vascular permeability and neovascularization by inducing the expression of VEGF[14]. In our current study, we showed highly significant levels of IL-6 in both serum and vitreous fluids of DR patients which is consistent with a recent study conducted by Koleva-Geogieva et al[15]. In contrast there also have been studies in which no difference was noted in the serum levels of IL-6 between less severe and more severe DR with some of them showing a high percentage of undetectable IL-6, a finding that could be attributed to ethnicity variation[16]. Research has indicated that leptin exerts a potent proangiogenic activity both *in vitro* and *in vivo* thus exerting a role in proliferative DR[17,18]. A study conducted in Turkey by Uckaya et al related higher the plasma leptin levels to the degree of advancement of DR, without showing any causal relationship between the two[17]. However, another study in the same region failed to confirm a significant relationship between plasma leptin levels and diabetic microvascular complications including DR[19]. By contrast, our present study has shown a significant increase in serum leptin levels in NPDR and PDR patients as compared to normal controls (p=<0.001) although the difference in the vitreous leptin levels between PDR and NPDR patient remained at non-significant level (p=0.915).

The present study showed significantly higher HbA1c, BMI and systolic as well as diastolic BP values in the diabetic subjects with retinopathy as compared to normal non-diabetic non-retinopathic subjects. This is in agreement to a number of studies mostly carried out in this region[20,21,22]. The interaction of these conventional parameters with the specific factors (VEGF, IL-6 and Leptin) was not studied in our study. However, a noteworthy observation is that a significant increase of systolic and diastolic BP exists in NPDR and PDR groups as compared to NDC while such a difference does not exist between NDC and DNR groups and also among diabetic retinopathic groups themselves. This could mean that once retinopathy initially starts to develop in diabetic patients, there is little contribution of rising hypertension in the progression of retinopathy. This inconsistency to the previous findings[23] may need further evaluation by appropriate research protocol.

CONCLUSION

Evaluation of serum and if feasible, vitreous samples of diabetic patients for VEGF, IL-6 and of serum samples for leptin at the time when they present with diabetes related complaints, may prove helpful tool for diagnosis and management of vision threatening retinopathy.

Acknowledgements:

The authors are thankful to colleagues at Al-Shifa Trust Eye Hospital, Rawalpindi, Khyber Teaching Hospital Peshawar, Hayatabad Medical Complex, Peshawar and Lady Reading Hospital, Peshawar for screening of patients, fundus examination and collection of vitreous samples.

REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Research and Clinical Practice 2010; 87: 4-14.

2. Girach A, Andersen LA. Diabetic macular edema: A clinical overview. International Journal Clinical Practice 2007; 61: 88-97.

3. Klein R, Klein BE, Moss SE. Epidemiology of proliferative diabetic retinopathy. Diabetes Care. 1992; 15: 1875-91.

4. Hammes HP. Pathophysiological mechanisms of diabetic angiopathy. Journal of Diabetes and Its Complications 2002; 17: Suppl 16-19.

5. Norbert D, Wangsa, Linsenmeier RA. Retinal Oxygen; Fundamental and clinical aspects. Arch Ophthalmol. 2003; 121: 547-57.

6. Viswanath K, McGavin M. Diabetic retinopathy: Clinical findings and management: Community Eye Health. 2003; 16: 21-24.

7. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent DM. N Engl J Med 1993; 329: 977-86.

8. Moan MC, Kadayifcilar S, Eldem B. Elevated intravitreal interleukin-6 levels in patients with proliferative diabetic retinopathy. Canadian journal of ophthalmology 2006; 41: 747-52.

9. Gonzalez RR, Cherfils S, Escobar M, Yoo JH, Carino C, Styer AK. Leptin signaling promotes the growth of mammary tumors and increases the expression of vascular endothelial growth factor and its receptor type two (VEGF-R2). J Biol Chem 2006; 281: 26320-28.

10. Aiello LP: Clinical implications of vascular growth factors in proliferative retinopathies. Curr Opin Ophthalmol 1997; 8: 19-31.

11. Lip PL, Belgore F, Blann AD, Hope-Ross MW, Gibson JM, Lip GY. Plasma VEGF and soluble VEGF receptor FLT-1 in proliferative retinopathy: relationship to endothelial dysfunction and laser treatment. Invest Ophthalmol Vis Sci. 2000; 41: 2115-19.

12. Mahdy RA, Nada WM. Evaluation of the role of vascular endothelial growth factor in diabetic retinopathy. Ophthalmic Res. 2011; 45: 87-91.

13. Brooks HL Jr, Caballero S Jr, Newell CK. Vitreous levels of vascular endothelial factor and stromal derived factor 1 in patients with diabetic retinopathy and cystoid macular edema before and after the intraocular injection of triamcinolone. Archive of ophthalmology 2004; 122: 1801-07.

14. Cohen T, Nahari D, Cerem LW, Neufeld G, Levi BZ. Interleukin 6 induces the expression of vascular endothelial growth factor. J Biol Chem. 1996; 271: 736-41.

15. Koleva-Georgieva DN, Sivkova NP, Terzieva D. Serum inflammatory cytokines IL-1beta, IL-6, TNF-alpha and VEGF have influence on the development of diabetic retinopathy. Folia Medica 2011; 53: 44-50.

16. Meleth AD, Agron E, Chan C, Reed GF, Arora K, Byrnes G, Csaky KG, Ferris FL, Chew EY. Serum Inflammatory Markers in Diabetic Retinopathy. Invest Ophthalmol Vis Sci. 2005; 46: 4295-301.

17. Uckaya G, Ozata M, Bayraktar Z, Erten V, Bingol N, Ozdemir IC. Is leptin associated with diabetic retinopathy? Diabetes Care 2000; 23(3): 371-76.

18. Jin X, Fukuda N, Su J, Takagi H, Lai Y, Lin Z, et al. Effects of leptin on endothelial function with OB-Rb gene transfer in Zucker fatty rats. Atherosclerosis 2003; 169: 225-33.

19. Sari R, Balci MK, Apaydin C. The relationship between plasma leptin levels and chronic complication in patients with type 2 diabetes mellitus. Metabolic Syndrome Related Disorders 2010; 8: 499-503.

20. Aamir AH, Rahman S, Ali SS, Jadoon MZ. Pattern of microvascular complications and associated comorbidities amongst diabetic patients at a tertiary care hospital. J Postgrad Med Inst 2005; 19: 400-06.

21. Arbab TM, Hanif S, Iqbal S, Mirza MA. Hypertension as risk factor in diabetic retinopathy in type-2 diabetes. Pak J Ophthalmol 2008; 24: 201-03.

22. Hosseini SM, Maracy MR, Amini M, Baradaran HR. A risk score development for diabetic retinopathy screening in Isfahan-Iran. Journal of Research in Medical Sciences 2009; 14: 105-110.

23. Gillow JT, Gibson JM, Dodson PM. Hypertension and diabetic retinopathy, what's the story? Br J Ophthalmol 1999; 83: 1083-87.

The Journal of Medical Sciences, Peshawar is indexed with WHO IMEMR (World Health Organisation Index Medicus for Eastern Mediterranean Region) and can be accessed at the following URL.

http://www.who.int/EMRJorList/details.aspx?docn=4468