# LEVELS OF SERUM INTERLEUKIN IL-6 IN PATIENTS WITH ADVANCED DIABETIC RETINOPATHY

*Nargis Parveen,[a] Ashfaq Ahmad Shah Bukhari,[a] Qaiser Khan,[b] Khayam Khan[c]*

## ABSTRACT

**Introduction:** The pro-inflammatory Interleukin IL-6 is reportedly raised in serum and vitreous humor of patients with diabetic retinopathy; possibly IL-6 plays a role in the pathogenesis of retinal neo-vascularization in these patients. The present study was conducted to determine levels of serum IL-6 in patients with advanced Diabetic Retinopathy.

**Materials & Methods:** A comparative study was conducted from June to November 2010 in the Outpatients Departments and Inpatients of three major hospitals of Peshawar and one Eye Trust hospital from Rawalpindi, Pakistan, in which 47 patients of diabetic retinopathy were compared with 45 diabetics without retinopathy and 39 normal healthy subjects. Retinopathic groups were designated as diabetic non-proliferative retinopathy (DNPDR, n=21) and diabetic proliferative retinopathic (DPDR, n=26). Serum IL-6 determination was done by ELISA. Data were analyzed by SPSS version 14.0.

**Results**: Serum IL-6 concentration was significantly greater in DNPDR (122.99 ± 40.76 pg/ml) & DPDR (190.42 ± 84.73 pg/ml) patients than CDNR (69.73 ± 16.47 pg/ml) patients ($p<0.001$).

**Conclusion:** Serum IL-6 levels were significantly raised in association with diabetic retinopathy, particularly proliferative retinopathy; levels were highest in females with proliferative retinopathy.

**Key words:** Diabetes Mellitus; Diabetic Retinopathy; Interleukin-6; Proliferative Vitreoretinopathy.

***Author Designation & Affiliation***

[a] Department of Physiology, Rehman Medical College, Peshawar.

[b] Newham University Hospital, Bart Health NHS Trust, London, UK.

[c] Department of Anesthesia, Khyber Teaching Hospital, Peshawar.

## INTRODUCTION

Diabetes mellitus (DM) is a global health issue, and its prevalence is expected to increase to 366 million worldwide by 2030.[1]

With the increasing prevalence of type 2 diabetes mellitus in the community, diabetic retinopathy–related visual impairment has become a major health problem; however, the pathogenesis of diabetic retinopathy (DR) is not well understood. Longer duration of diabetes, hypertension, poor metabolic control, nephropathy, high blood cholesterol, smoking, age, sex, and genetic disposition are risk factors for the development of DR, but no one has been able to fully explain the development of this diabetic complication.[2] In addition, the process by which DM without retinopathy progresses to proliferative diabetic retinopathy is a chronic course, and the change in the levels of intraocular fluid cytokines is a dynamic process.[3]

The hallmark of proliferative diabetic retinopathy is neovascularization characterized by proliferation of newly formed fragile blood vessels from the optic nerve head, neovascularization on the disc or along the retinal venules elsewhere. These blood vessels proliferate through internal limiting membrane of the retina along the surface of and into the vitreous body. Newly formed fragile vessels break easily due to any stress with ensuing visual loss due to vitreous hemorrhage. Subsequently, florid fibrous tissue formation can result in severe retinal distortion and detachment.[4]

Many groups reported the positive role of Proinflammatory cytokines in the development of diabetic retinopathy (DR).[5-8] It has been reported that proinflammatory cytokines such as IL-6 are elevated in proliferative diabetic retinopathy (PDR).[9-11]

IL-6 is raised in serum and vitreous in patients with diabetic retinopathy,[12] and is implicated in the pathogenesis of retinal neo-vascularization.[13,14] Corticosteroids, estrogens

and androgens are among the inhibitors of IL-6 expression.[15]

## MATERIALS & METHODS

The present comparative study was carried out from June to November 2010 in the Outpatients Departments and Inpatients of three major hospitals of Peshawar (Khyber Teaching Hospital, Hayatabad Medical Complex and Lady Reading Hospital), and Al-Shifa Eye Trust Hospital located in Rawalpindi, Pakistan.

In this study, 47 patients with confirmed diabetes and retinopathy were selected through convenience sampling without considerations of ethnic or regional backgrounds; they were further designated as diabetic non-proliferative retinopathy (DNPDR) and diabetic proliferative retinopathy (DPDR) groups. Inclusion criteria were marked diabetes, adult onset of the disease and visual symptoms. Exclusion criteria were systemic diseases, kidney or heart disease, liver malfunction and respiratory or gastrointestinal disorders; exceptions were patients who had hypertension related with diabetes. For purpose of comparison, the two control groups were diabetic non-retinopathy (CDNR, n = 45) and normal healthy subjects (NS, n = 39) respectively. Written informed consents were obtained from all subjects. Detailed history was followed by standard physical examination including the measurement of blood pressure, testing of visual acuity and fundus examination. All examinations of patients were carried out in the presence of a qualified diabetologist and ophthalmologist. Venous blood was aspirated for the serum separation of IL-6.

Performa was designed to record anthropomorphic data, clinical case histories and investigations as well as laboratory findings. Data were entered and analyzed by SPSS computer program version 14.0 (Chicago, Illinois, USA). Group comparisons were tested by Chi Square and Student's T tests, keeping $p \leq 0.05$ as significant.

### *Serum IL-6 Determination*

#### *Principle*

The Immunotech™ IL-6 enzyme immunoassays (IM1120, IM11120) are intended for quantitation of human IL-6 in plasma, serum or culture supernatants. Samples and calibrators were incubated in a microtiter plate coated with the first monoclonal antibody anti IL-6, in the presence of the second anti-IL-6 monoclonal anti body linked to acetylcholinesterase (ACE). After incubation, the wells were washed and the bound enzymatic activity detected by addition of a chromogenic substrate. The intensity of the coloration was proportional to the IL-6 concentration in the sample or calibrator.

#### *Calibration Curve*

A quadratic mode curve fit with absorbance taken on vertical axis and the IL-6 concentration of the calibrators taken on the horizontal axis (0-1000 pg/ml) was drawn.

#### *Procedure*

Lyophilized components were solubilized; after 10 minutes wait, components were mixed gently to avoid foaming; 50 ml of the wash solution (20x) were dissolved in 950 ml of distilled water. The lyophilized conjugate was reconstituted with the volume of distilled water stated on the vial label. Calibrator or sample of 100 µl and 100 µl conjugate were added per well, incubated for 2 hrs at 18-25$^0$C with frequent shaking. Substrate (200 µl) was added and incubated for 30 minutes at 18-25$^0$C followed by Stop solution of 50 µl. Absorbance was read at 450 nm.

#### *Specificity*

The assay measures natural or recombinant, human IL-6 no cross-reactivity or interface with other cytokines or cytokine receptors is known.

#### *Precision*

Intra-assay precision was determined by assaying sera nine times. Coefficient of variance (CVs) ranged between 1.6 and 6.8%. Inter-assay

precision was determined by assaying sera five times. CVs ranged between 7.9 and 14.6%.

***Accuracy***

***a. Dilution Test***

Sera containing IL-6 were diluted to 1:8 using the IL-6 diluent 2. The observed recovery was between 95 & 109%.

***b. Recovery Test***

IL-6 was added at different concentrations using different samples. The observed recovery average was ranged between 97 and 105%.

## RESULTS

Out of 47 diabetic patients, (26 males and 21 females), 21(44.7%) were of DNPR group and 26(55.3%) of DPDR group. Serum IL-6 concentration was significantly greater in DNPDR and DPDR patients than CDNR patients and normal subjects with significantly elevated values found in DPDR patients having a median value of 219.0 pg/ml, ranging from 71.33-380.33 pg/ml ($P < 0.001$). No difference was found between CNDR patients and normal subjects as regards serum IL-6 concentration (Table 1).

**Table 1**: **Serum level of IL-6 in different groups of subjects**

| S # | Groups | IL-6 levels (Mean ± SD; pg/ml) | | Overall pg/ml | Range pg/ml | p value |
|---|---|---|---|---|---|---|
| | | Males | Females | | | |
| 1. | Normal (n=39) | 57.72± 9.37 | 58.79±13.57 | 58.25±11.47 | 37.80-88.47 | NS |
| 2. | CDNR (n=39) | 63.91±16.22 | 75.55±16.73 | 69.73±16.47 | 39.71-101.28 | |
| 3. | DNPDR (n=21) | 126.83±45.36 | 119.16±36.16 | 122.99±40.76 | 67.33- 214.33 | <0.001 |
| 4. | DPDR (n=26) | 181.30± 80.25 | 199.54±89.21 | 190.42±84.73 | 71.33-380.33 | |

p value is highly significant between the control and patient groups. NS = Not Significant.

## DISCUSSION

The present study showed that serum IL-6 levels increased in patients with advancement of retinopathy. Globally an estimated 45 million people around the world are blind, most due to diabetic retinopathy. The number is expected to rise to 76 million by 2020 if some positive steps towards diagnosis and treatment are not taken. Despite advancement of ophthalmological care, diabetic retinopathy remains a major cause of preventable blindness.

It is to be noted that all of the patients had the disease duration of 5-20 years and all were receiving medications against diabetes, which included metformin, thiazolidones, glyburide and others in combination or alone according to the disease status. Also, they were taking medicines for hypertension and nephropathic complications. It is therefore alarming that the levels of cytokines have been found elevated. As regard the PDR the levels of IL-6 were more markedly raised in females; this is also shown by other studies.[16,17] Beside genetic factors, females seem to be a victim of retinopathy and other diabetic complications due to factors like ignorance, gender preferences etc. playing a major role in the alarming rise of blindness in females' specifically in the Khyber Pakhtunkhwa Province of Pakistan.

Intensive treatment of patients can bring the conventional parameters within normal range in the initial stages of diabetes; however, damage done to retina cannot be reversed in long standing history of diabetes, a situation which is evident in the present study.[4,18]

Rise is serum IL-6 was observed presently, making IL-6 another very significant marker for diagnosing or understanding at least the status of

the disease. Although there are as such no reports on clinical trials of anti-IL-6, application of anti-IL-6 may help in control of retinopathy changes. Notably, that IL-6 acts as an inducer of Vascular Endothelial Growth Factor (VEGF).[19] Thus IL-6 levels along with VEGF in the serum may indicate the process of neovascularization and ultimate sever damage to the retina.[20]

It is pertinent that the IL-6 level show marked elevations despite controlled blood sugar and other parameters. This indicates that conventional diabetes therapies have been unable to stop worsening of retinal changes and blood vessel growth. Fundus examination and angiography indicated severe retinal changes especially in the PDR patients. Currently application of ranibizumab[21-23] and photocoagulation[24] are effective therapies however application of anti-IL-6 antibodies may play important roles against progressing retinopathic conditions.[25-28]

## CONCLUSION

The present study revealed raised IL-6 levels in diabetic patients with retinopathy; comparing NPDR with PDR, higher concentrations were observed in serum of PDR patients, particularly in females.

## REFERENCES


1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–53.
2. Schram MT, Chaturvedi N, Schalkwijk C, Giorgino F, Ebeling P, Fuller JH, Stehouwer CD EURODIAB Prospective Complications Study Vascular risk factors and markers of endothelial function as determinants of inflammatory markers in type 1 diabetes: the EURODIAB Prospective Complications Study. Diabetes Care 2003;26:2165–73.
3. Cheung CM, Vania M, Ang M, Chee SP, Li J. Comparison of aqueous humor cytokine and chemokine levels in diabetic patients with and without retinopathy. Mol Vis. 2012;18:830–7.
4. Frank RN. Diabetic retinopathy. N Engl J Med 2004 Jan 1;350(1):48–58.
5. Ozturk BT, Bozkurt B, Kerimoglu H, Okka M, Kamis U, Gunduz K. Effect of serum cytokines and VEGF levels on diabetic retinopathy and macular thickness. Mol Vis. 2009;15:1906–14.
6. Yoshimura T, Sonoda KH, Sugahara M, Mochizuki Y, Enaida H, Oshima Y, Ueno A, Hata Y, Yoshida H, Ishibashi T. Comprehensive analysis of inflammatory immune mediators in vitreoretinal diseases. PLoS ONE. 2009;4:8158.
7. Funk M, Schmidinger G, Maar N, Bolz M, Benesch T, Zlabinger GJ, Schmidt-Erfurth UM. Angiogenic and inflammatory markers in the intraocular fluid of eyes with diabetic macular edema and influence of therapy with bevacizumab. Retina. 2010;30:1412–9.
8. Wakabayashi Y, Usui Y, Okunuki Y, Kezuka T, Takeuchi M, Iwasaki T, Ohno A, Goto H. Increases of vitreous monocyte chemotactic protein 1 and interleukin 8 levels in patients with concurrent hypertension and diabetic retinopathy. Retina. 2011;31:1951–7.
9. Hernández C, Segura RM, Fonollosa A, Carrasco E, Francisco G, Simo R. Interleukin-8, monocyte chemoattractant protein-1 and IL-10 in the vitreous fluid of patients with proliferative diabetic retinopathy.Diabet Med. 2005;22:719–22.
10. Funatsu H, Noma H, Mimura T, Eguchi S, Hori S. Association of vitreous inflammatory factors with diabetic macular edema. Ophthalmology. 2009;116:73–9.
11. Forooghian F, Kertes PJ, Eng KT, Agron E, Chew EY. Alterations in the intraocular cytokine milieu after intravitreal bevacizumab. Invest Ophthalmol Vis Sci. 2010;51:2388–92.
12. Dogan Y, Akarsu S, Ustundag B, Yilmazm E, Gurgose MK. Serum IL-1β, IL-2 and IL-6 in insulin-dependent diabetic children. Mediators Inflamm. 2006;1:59206.
13. Ohara K, Funatsu H, Kitano S., Hori S, Yamashita H. The role of cytokines in the pathogenesis of diabetic retinopathy. Nihon. Ganka Gakkai Zasshi. 2001;105(4): 213–7.

14. Grant MB, Afzal A, Spoerri P, Pan H, Shaw LC, Mames RN. The role of growth factors in the pathogenesis of diabetic retinopathy. Expert Opin Investig. Drugs 2004;13(10):1275–93.
15. Dor Y, Porat R, Keshet E. Vascular endothelial growth factor and vascular adjustments to perturbations in oxygen homeostasis. Am J Physiol Cell Physiol. 2001;280(6):C1367–74.
16. Basit A, Hydrie MZI, Hakeem R, Ahmedani MY, Waseem M. Glycemic control, hypertension and chronic complications in type 2 diabetic subjects attending a tertiary care centre. J Ayub Med Coll. 2005;17(2):1–6.
17. Hallman DM, Klein BE, Huber JC, Klein R, Gonzalez VH, Hanis CL. Familial aggregation of severity of diabetic retinopathy in Mexican Americans from Starr County, Texas. Diabetes Care. 2005 May;28(5):1163-8.
18. Schiffelers RM, Fens MH, van Blijswijk JM, Bink DI, Storm G. Targeting the retinal microcirculation to treat diabetic sight problems. Expert Opin Ther Targets. 2007;11(11):1493–502.
19. Omori K, Naruishi K, Nishimura F, Yamada-Naruishi H, Takashiba S. High glucose enhances interleukin-6-induced vascular endothelial growth factor 165 expression via activation of gp130-mediated p44/42 MAPK-CCAAT/enhancer binding protein signaling in gingival fibroblasts. J Biol Chem. 2004;279(8):6643–49.
20. Funatsu H, Yamashita H, Noma H, Mimura T, Yamashita T, Hori S. Increased levels of vascular endothelial growth factor and interleukin-6 in the aqueous humor of diabetics with macular edema. Am J Ophthalmol. 2002;133(1):70–7.
21. Arevalo JF, Garcia-Amaris RA. Intravitreal bevacizumab for diabetic retinopathy. Curr Diabetes Rev. 2009;5(1):39–46.
22. Rodriguez-Fontal M, Alfaro V, Kerrison JB, Jablon EP. Ranibizumab for diabetic retinopathy. Curr Diabetes Rev. 2009;5(1):47–51.
23. Elman MJ, Aiello LP, Beck RW, Bressler NM, Bressler SB, Edwards AR. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. Ophthalmology. 2010;117(6):1064–77.
24. Chew EY, Ferris FL III, Csaky KG, Murphy RP, Agron E, Thompson DJ, Reed GF, Schachat AP. The long-term effects of laser photocoagulation treatment in patients with diabetic retinopathy: The early treatment diabetic retinopathy follow-up study. Ophthalmology. 2003;110:1683–89.
25. Mchiffelers RM, Fens MH, Blijswijk JM, Bink DI, Storm G. Targeting the retinal microcirculation to treat diabetic sight problems. Expert Opin Ther Targets.2007;11:1493–1502.
26. Andreoli CM, Miller JW. Anti-vascular endothelial growth factor therapy for ocular neovascular disease. Curr Opin Ophthalmol. 2007;18:502–8.
27. Nicholson BP, Schachat AP. A review of clinical trials of anti-VEGF agents for diabetic retinopathy. Albrecht von Graæes Archiv für Ophthalmologie. 2010;248(7):915–30.
28. Lacono P, Parodi MB, Bandello F. Antivascular endothelial growth factor in diabetic retinopathy. Dev Ophthalmol. 2010;46:39–53.

## Corresponding Author

Dr. Ashfaq Ahmad Shah Bukhari, Assistant Professor of Physiology, Rehman Medical College, Peshawar.
Email: ashfaq.bukhari@rmi.edu.pk

Submitted for Publication: May 29, 2015.
The authors have no conflict of interest. All authors contributed substantially to the planning of research, questionnaire design, data collection, data analysis and write-up of the article.

This article may be cited as:
Parveen N, Bukhari AAS, Khan Q, Khan K. Levels of serum Interleukin IL-6 in patients with advanced diabetic retinopathy. JRMI. 2015;1(1):4-8.