

Original article:

Serum level of il-6 in patients of type-ii diabetes mellitus with and without retinopathy: a comparative study

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Abstract

Aim: Pakistan has 6.9 million people with diabetes mellitus (DM) that will be doubled by 2025. A study was designed to determine serum levels of IL-6 in type 2 diabetes mellitus (T2DM) patients. **Methods:** It was a cross-sectional case-control study of 212 subjects. Group-I included 30 subjects without DM, Group-II had 30 T2DM without retinopathy and Group-III had 152 T2DM with retinopathy. IL-6 was determined by ELISA technique. Data was analysed using SPSS 17.0. **Results:** More females were in Group-II (83%) and Group-III (66%) compared to Group-I (30%). Higher age was in Group-II (49yrs) and Group-III (50yrs) compared to Group-I (34yrs). Mean duration of disease (in years) was more in Group-III (10.51) than Group-II (7.76). Highest mean level of IL-6 was in Group-II, followed by Group-I and Group-III. On comparison, gender, age, duration of disease and the level of IL-6, there was a significant difference while there was no significant difference between percentages of HbA1c. The logistic regression model suggested low levels of IL-6 in patients of diabetic retinopathy was an independent predictor of retinopathy in patients with T2DM. **Conclusion:** Serum level of IL-6 was low in patients of diabetic retinopathy as compared to patients with T2DM without retinopathy.

Keywords: Cytokine, diabetes mellitus; HbA1c, ELISA; IL-6; inflammation; retinopathy; hyperglycemia

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Background

Diabetes mellitus (DM) may lead to many complications i.e. diabetic retinopathy, nephropathy etc. Well documented reasons for these complications are age of the patient, their obesity, hyperglycemia, disease duration, etc¹. About 6.9 million people in Pakistan have DM and it is expected that this figure may reach 11.5 million by the year 2025². The more common form of DM is type-2 diabetes mellitus (T2DM). Comparatively more young individuals and kids even younger than 8 years of age are being presenting with

T2DM. It is suggested that the rise in obesity which can be associated with changes in diet and lifestyle could be the reasons for this rapid rise in T2DM. While focusing on developing countries, due to sharp increase in the prevalence of T2DM, this disease could be blamed among the 60% of non-communicable diseases in that region³.

It is surprising that the problems of overweight and obesity are less in Asia while comparing to Western world, but Asians have high prevalence of DM. Many reasons such as children's obesity especially central, and insulin resistance with low muscle mass

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that have been linked with the increased risk of these young kids with T2DM⁴. If hyperglycemia persists for a long time it may affect most of the organs in the body and retina is one of them. It has been suggested that during DM there are hyper reactive platelets which are attracted towards damaged vessels. This interaction leads to formation of micro-thrombus and sometimes these small vessels are blocked⁵. These are the various events which has been suspected as the reason for diabetes retinopathy (DR).

DR is a shocking and depressing stage of DM and if appropriate and drastic measures will not be taken, the number of subjects who could develop DR may be doubled in a span of 30 years. In both the types of DM i.e. type 1 and 2, derangement in the immune system have been linked with their complications. It has been suggested that healthy subjects who have increased level of inflammatory markers are at risk to suffer T2DM. It has been documented that subjects with increased WBC count and high level of cytokines such as IL-6 may develop T2DM in the next 20-years. About 30% of the newly diagnosed T2DM subjects can develop DR. In these subjects good control over their glucose level is said to be an important factor that has been linked with their microvascular complications^{6,7}. It is strongly recommended to take extra measures not only to identify, and prevent this disease but to treat DR as well before the start of vision loss [6]. Keeping these facts in mind, this study was carried out to determine and compare level of serum IL-6 in subjects of T2DM with DR and with those diabetes who did not have DR.

Material and Methods

This comparative, cross-sectional study was performed in the Department of Immunology, University of Health Sciences (UHS) Lahore, Pakistan. The study was approved by the Ethical Review Committee and Advanced Studies & Research Board of UHS. It included 212 subjects presented during January 2014 to September 2015; Group-I had 30 healthy controls, Group-II had 30 diabetes patients without DR and Group-III had 152 diabetes patients with retinopathy. Patients had diabetes between 5 - 25 years. HbA1c and duration of diabetes of Group-II and Group-III was noted. Eye examination and diagnosis of retinopathy was made by the consultant ophthalmologist. Patients between 20–75 years of either sex were selected. Subjects of nephropathy, or having an infection in the last two weeks and of chronic infection like TB were excluded. After informed consent, 3ml of blood sample was

collected from each patient in EDTA containing gel vacutainer, serum was separated, and stored at -20^o C. IL-6 was detected by ELISA (KOMA BIOTECH INC, KOREA). The manufacturer of the kit claimed no cross reactivity. Data was analysed using SPSS 17.0, Mean \pm SD, frequencies and percentages are presented for qualitative variables, while tables and figures are presented for both qualitative and quantitative variables. One way ANOVA was applied for group mean differences, Post Hoc Tukey test for group means differs, and Chi-Square test for associations between qualitative variables. A *p*-value of ≤ 0.05 was considered statistically significant.

Results

The demographic data of the studied population are shown in Table-1. More females were in Group-II (83%) and in Group-III (66%) compared to Group-I (30%) ($p < 0.0001$ in each). On comparison, gender distribution there was a significant difference among the three groups ($p = 0.0029$), between Group-I and Group-II ($p < 0.0001$) and between Group-I and Group-III ($p < 0.0001$) and there was no significant difference between Group-II and Group-III. High age (in years) was observed in Group-II (49) and in Group-III (50) compared to Group-I (34) ($p < 0.0001$ in each). On comparison, age there was a significant difference among the three groups, between Group-I and Group-II and between Group-I and Group-III ($p < 0.0001$ in each) and there was no significant difference between Group-II and Group-III. Regarding the duration of disease (in years), longer mean duration of disease was found in Group-III (10.51) than Group-II (7.76). On comparison, duration of diabetes there was a significant difference between the two groups ($p = 0.0073$). On comparison, HbA1c between the two groups there was no significant difference (Table 2).

The highest mean level of IL-6 was found in Group-II, followed by Group-I and Group-III. On comparison, there was a significant difference in the three groups, between Group-I and Group-III and between Group-II and Group-III ($p < 0.0001$ in each) and there was no significant difference between Group-I and Group-II (Table 2).

The logistic regression model was applied to determine associations among various variables. There was a significant difference in the level of IL-6 between Group-II and Group-III ($p = 0.009$) while age and level of IL-6 were significant predictors between Group-I and Group-II ($p < 0.0001$, 0.0054) respectively (Table 3).

Discussion

Demographic data of the study is presented in Table-1. The findings of this study are in concordance with various studies such as Akram et al (2011)⁷, Chhutto et al (2009)⁸, and Ahmadani et al (2008)⁹. Since the numbers of females were more in both the groups, therefore it could be a reason for the non-significant findings of the study. However, Qidwai et al (2010)² documented that diabetes is more prevalent in males which is not in agreement, whereas more females had impaired glucose tolerance. The same findings have been reflected in the literature that diabetes is more common in females. On comparison of the current study did not suggest significant difference between Group-II and Group-III which might be due to inclusion of diabetic patients in both the groups, however it suggests that other factors also play a role in the development of DR (Silverman et al, 1995)¹⁰. Other researchers also reported similar findings i.e. Chhutto et al (2009)⁸ and Ahmadani et al (2008)⁹. Whereas another researcher's findings are not in concordance i.e. Akram et al (2011)⁷ which might be due to the difference in their study group as they included subjects of 40 years of age as lower limit however they did not mention the upper limit of age. Another researcher (Zhanget al 2010)¹¹ included diabetic patients of 58-62 years of age therefore their findings were also different. Since the duration of diabetes was more in Group-III as compared to Group-II, therefore on comparison a significant difference was observed ($p=0.0073$) which is similar to the suggestions of Ahmadani et al (2008)⁹ and Zhang et al (2010)¹¹. Possible reason could be that duration

of disease contributed towards DR whereas Jamal-u-Dinet al (2006)¹² performed the study on newly diagnosed diabetic patients and probably therefore detected low mean \pm SD of age. On comparison of HbA1c there was no significant difference between the two groups. Results of Ahmadani et al (2008)⁹ are in concordance but the findings of Zhanget al (2010)¹¹ are not in agreement as they could document significant difference in HbA1c. A crucial observation in the current study was that level of HbA1c in both the groups suggested poor diabetes control. Since diabetic patients for this study were included from public hospital and most of them had poor socio-economic background which could be a probable reason for this disparity. Furthermore these patients were not educated (Seeman et al, 2008)¹³. Low serum level of IL-6 was detected in DR which could be attributed to the laser treatment and other medicines which these patients were using as such medications have been suggested to decrease inflammatory changes in the eye¹⁴. There are studies suggesting that aggressive therapy of DR can reduce level of IL-6 as Dongancy et al. (2002) determined high level of IL-6 in diabetes patients who have poor control over their diabetes and Esposito et al. (2002) suggested that level of IL-6 of diabetes patients are reduced once their level of glucose comes in the normal range^{16, 17}. Further an interesting finding in the current study was high level of IL-6 in healthy subjects that could point out towards a defensive aspect of IL-6 which has been validated by experiments in non-obese diabetic mouse. These mice have high level of IL-6 expression and hence these animals have better survival time while

Tables

Table 1: Demographic data of the subjects

Variables		Group-I	Group-II	Group-III
Male n (%)		21 (70)	05 (16.66)	51 (33.55)
Female n (%)		09 (30)	25 (83.33)	101 (66.44)
Age (yrs) Mean \pm SD		34.66 \pm 8.78	49.46 \pm 9.94	50.88 \pm 8.90
HbA1c (%)		NA*	5.9 – 12.6	5.5 – 15.4
Duration of diabetes	< 10 years n (%)	NA*	25 (11.79)	84 (39.62)
	>10 years n (%)	NA*	05 (2.35)	68 (32.07)

*NA= not applicable

comparing them with mice that has comparatively normal expression of IL-6gene¹⁷. Therefore IL-6 polymorphism had been claimed as a shielding mechanism in case of retinopathy and nephropathy of T1DM¹⁸.

Conclusion

Serum level of IL-6 was low in patients of DR compared to patients with T2DM without retinopathy and it could be an independent predictor of retinopathy in patients with T2DM.

Limitations of the study

In the current study only the role of IL-6 in DR has been evaluated. Studies should be carried out to assess the other cytokines and functions of immune cells in diabetic retinopathy.

Conflict of interest: None of the researcher has conflict of interest in the products used.

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Table 2. Comparisons of different variables in different groups

Variable		Group-I (n=30)	Group-II (n=30)	Group-III (n=152)	p-value
Gender	Male (n, %)	21 (70)	5 (16.6)	51 (33.55)	0.0029* ¹
	Female (n, %)	9 (30)	25 (83.33)	101 (66.44)	<0.0001* ² <0.0001* ³ 0.0868 ⁴
Age (yrs)Mean ± SD		34.66 ± 8.78	49.46 ± 9.94	50.88 ± 8.90	<0.0001* ¹ <0.0001* ² <0.001* ³ 0.4365 ⁴
Duration (Mean ± SD)		NA	7.76 ± 4.14	10.51 ± 5.24	0.0073* ⁴
HbA1C (Mean ± SD)		NA	8.54 ± 2.06	8.83 ± 2.35	0.6044 ⁴
IL-6 (Mean ± SD) (pg/ml)		1331.98 ± 306.41	1341.78 ± 294.74	718.66 ± 614.02	<0.0001* ¹ <0.4255 ² <0.0001* ³ <0.0001* ⁴

*Statistically significant, NA=not applicable, ¹Comparison among three groups, ²Comparison between group-I and group-II, ³Comparison between group-I and group-III, ⁴ Comparison between group-II and group-III

Table 3. Logistic Regression Model

For Group-II and Group-III					
Variable	Degree of Freedom (DF)	Estimate	Standard Error	Chi-Square	p-value
Age	1	0.0004	0.0008	0.21	0.644
Duration	1	0.0019	0.0015	1.62	0.203
HbA1C	1	0.0006	0.0034	0.04	0.847
IL-6	1	-0.0000	0.0000	6.74	0.009*
For Group-I and Group-III					
Age	1	0.0174	42.93	0.0027	<0.0001*
IL-6	1	-0.0001	7.74	0.0000	0.0054*

*Statistically significant

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