Evaluation of Interleukin-6 Levels in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Background: *It has been suggested that inflammatory processes play an important role in the pathogenesis of type 2 diabetes. Individuals who develop type 2 diabetes show signs of low-grade inflammation years before disease onset. This low-grade inflammation has been assumed to be implicated in the pathogenetic mechanisms that cause type 2 Diabetes Mellitus. Inflammatory mediators such as the IL-6 family of cytokines have been proposed to be involved in the events that lead to type 2 diabetes. IL-6 has immunoregulatory actions been proposed to affect glucose homeostasis and metabolism directly and indirectly by action on skeletal muscle cells, adipocytes, hepatocytes, pancreatic β-cells and neuroendocrine cells. Many studies have suggested the role of IL-6 in the pathogenesis of type 2 diabetes mellitus. However, no references have been observed in Bangladesh regarding IL-6 and its association with type 2 diabetes mellitus. It thus may highlight the importance of low-grade inflammation in the pathophysiology of type 2 diabetes mellitus. The purpose of this study was to evaluate type 2 diabetes patients' levels of interleukin-6.*

Materials and methods: *A hospital-based cross-sectional observational study was carried out in the Department of Biochemistry Chittagong Medical College, Department of Endocrinology of Chittagong Medical College Hospital and Chattogram Diabetic Hospital. One hundred (100) type 2 diabetes mellitus patient was included in the study by non-probability consecutive sampling. Important variables in this study were serum IL-6, BMI, waist circumference and duration of Diabetes Mellitus.*

Results: *The mean serum IL-6 level was 10.08 ± 0.39 pg/ml in patients with type 2 Diabetes Mellitus. Serum IL-6 was positively correlated (r 0.33, $p <0.01$) with waist circumference and duration of diabetes mellitus (r 0.26, $p <0.05$). The mean IL-6 was significantly different between BMI groups ($F_{(2,97)} =5.39$, $p=0.006$) and was higher in the obese group (10.52 ± 4.00) compared to the overweight and normal BMI (6.78 ± 1.95) and overweight (8.08 ± 3.15) group.*

Conclusion: *It can be concluded that type 2 diabetes mellitus patients had increased serum levels of IL-6. It can give an insight into the possible role of sub clinical inflammation among patients with type 2 Diabetes Mellitus.*

Key words: Interleukin-6; Low-grade inflammation; Type 2 Diabetes Mellitus.

Introduction

Inflammation is considered as an important factor for the pathogenesis of type II diabetes mellitus.[1] The inability of the beta cells to counteract peripheral insulin resistance is a hall mark of type 2 diabetes, which is marked by persistent hyperglycemia brought on by both beta cell malfunction and insulin resistance.[2] A growing amount of evidence supports the idea that immuno-related mechanisms and influences play a part in the pathophysiology of type 2 diabetes, including the destruction and gradual failure of beta cells as well as peripheral insulin resistance. Studies have suggested that inflammatory mediators may not only be markers of metabolic aberrancies in type 2 diabetes but may also directly contribute to cell dysfunction and insulin resistance. These have been produced over the past ten years as a result of interest in the role of inflammation in a broad range of diseases that are not typically thought of as immune-mediated disorders, such as atherosclerosis and adiposity.[3,4] The immunoregulation and nonimmune events in the majority of cell types and tissues outside of the immune system are significantly impacted by Interleukin IL-6's pleiotropic nature.[5] Different cascades of sign alling transduction, called classicandtrans-signalling, have been identified as being responsible for the pro-inflammatory activities of IL-6. Inflammatory and immunological processes, haematopoiesis and liver and neural regeneration are all impacted by IL-6.[6,7] IL-6 has a

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pro and anti-inflammatory effect on the adaptive immune system.[8] IL-6 uses both conventional and trans-signalling pathways to exert its biological effects.[9] The complicated signal transduction mechanism of IL-6 may help explain the cytokine's pleiotropic character.[10] Despite these observations, there are no published prospective data evaluating the relationship between IL-6 and the development of type 2 diabetes mellitus. Therefore, this study evaluated whether plasma levels of IL-6 are increased or not among type 2 diabetes mellitus patients.

Materials and methods

This hospital-based cross-sectional observational study was carried out in the Outpatient Department of Endocrinology, Chittagong Medical College Hospital in collaboration with the Department of Biochemistry, Chittagong Medical College, Chattogram Diabetic Hospital. The study was conducted from July 2019 to January 2023. The study was undertaken after approval by the Ethical Review Committee of Chittagong Medical College and the concerned Departments. Informed consent was obtained from each subject.

After conducting consecutive non-probability sampling methods one hundred (100) patients (60 male and 40 female) a mean age of 53.04±8.83 of type 2 diabetes mellitus patients were enrolled in this study (Table I).

Inclusion criteria:

● Patients with Type 2 Diabetes Mellitus.

● Age > 35 yrs.

Exclusion criteria:

● Chronic liver disease
● Acute infection
● Autoimmune disease and malignancy
● Sepsis, burn
● Pregnancy.

Subject were selected from the Outpatient Department (OPD) of the Department of Endocrinology, Chittagong Medical College Hospital and Chattogram Diabetic Hospital who had come for their regular checkup for diabetes.

After taking a brief history and preliminary selection each subject and their informed verbal consent was taken. Then they were requested to report to the Department of Biochemistry, Chittagong Medical College in the morning between 8.00 and 9.00 am following an overnight (8-12 hours) fasting. When the subjects were reported, informed written consent was taken. A predesigned case record form was used to record relevant clinical, medical, demographic, and socio-economic data from subjects.

The cut-offpoint of normal IL-6 was considered by ≤5pg/ml.[17]

Serum Interleukin-6 was measured by a commercially available IL-6 kit which is an in-vitro chemiluminescence immunoassay for quantitative determination (MAGLUMI 2000). BMI and WC (Waist circumference) were measured by standard procedure. The fasting serum blood glucose was measured by glucose oxidase enzymatic kinetic method using an auto analyzer. 2HPPBS was measured by an auto-analyzer Simens dimension EXL 200.

All the data had processed and analyzed using IBM-SPSS (Statistical Package for Social Science) v 25.0 for Windows. p value < 0.05 will be considered to be statistically significant. Variables expressed as mean ± Standard Error of Means (SEM)/SD. The normality of the distribution of the data were tested by the Kolmogorov-Smirnov Test, and a p-value greater than 0.05 indicated that the observed distribution of a variable is not statistically different from the normal distribution. The pearson correlation test has been applied whenever necessary to see the statistical significance. ANOVA tests and post hoc tests were used for the analysis of mean differences between groups.

Results

Table I Baseline Anthropometric and sociodemographic parameters of the study population (Type II Diabetes Mellitus) patient(n=100)

Variables of Type II Diabetes Mellitus Patients	Mean ± Standard Deviation			Range (Min-Max)	Median
	Male	Female	Total		
Age (Years)	55.40±8.9	49.50±7.3	53.04±8.83	40-82	52.50
Height (cm)	163.05±6.7	157.28±6.9	160.74±7.3	137-186	160.50
BMI (Kg/m^2)	27.96±4.0	28.0±4.3	27.93±4.08	21-48	28
Waist circumference (cm)	100.38±7.2	95.15±11.7	100.21±8.3	75-150	100
Duration of Diabetes Mellitus (Years)	6.5±4.03	5.5±4.10	6.15±4.10	1 month to 22 years	6
Gender distribution					
Male 60			Female 40		

Table I contain the mean and standard deviation of anthropometric and sociodemographic parameters showing 60% of the study population were male and 40% were female, the mean age of men was 55.40±8.9 years and of the women 49.50±7.3 years. The mean BMI of the men was 27.96±4.0 kg/m^2 and of the women 28.0±4.3 kg/m^2. The mean waist circumference of the men was 100.38±7.2 cm and of the women 95.15±11.7 cm. In the study population, the mean age was 53.04±8.83 years. The mean BMI was 27.93±4.08 kg/m^2. The mean waist circumference was 100.21±8.3. The mean duration of diabetes mellitus was 6.15±4.10 years. The Median age of the patients was 52.50 years, the median BMI was 28 kg/m^2, the median waist circumference was 100cm and the median duration of diabetes was 6 years.

Table II Distribution of Risk factors among the study subjects (Type II Diabetes Mellitus) (n=100)

Risk factors			Percentage (%)
	Normal		4%
BMI	Overweight		14%
	Obese		82%
Waist Circumference	Male ≥ 90cm		51%
	Female ≥ 80cm		29%
Duration of Diabetes Mellitus (Years)	≥ 5yrs		65%
	< 5yrs		35%
History of Diabetes Mellitus in 1 relative		Yes	76%
		No	24%
Serum IL-6 (pg/ml)	>5pg/ml		86%
	≤5pg/ml		14%

Table II showing that 14% and 82% of type 2 Diabets Mellitus patients were overweight and obese. The frequency of waist circumference was greater in male than the female. 65% of type II Diabetes Mellitus patients had a history of more than 5 years of Diabetes. Diabetes Mellitus in 1° relative had 76%.

Table III Biochemical parameters in Type II Diabetes Mellitus patients (n=100)

Parameter (n=100)	Mean ± SEM		Median	Range
Fasting Blood Sugar (mmol/L)	11.39±0.36		10.65	7.0-20.8
2 hours Postprandial Blood glucose (mmol/L)	17.25±0.52		15.75	8.5-31.9
Serum IL-6 (pg/ml)	5pg/ml	10.90±0.37	11.03	5.31-19.86
	≤5pg/ml	4.56±0.07	4.58	4.14-4.87
	Total	10.08±0.39	9.82	4.14-19.86

Table III showing that Mean Fasting Blood sugar was 11.39±o.36 mmol /L, the mean 2 hours postprandial blood glucose was 17.25±0.52mmol/L, the mean serum IL-6 concentration was 10.08±0.39 pg/ml. The mean Il-6 value > 5 pg/ml was 10.90±0.37.

Figure 1 showing a box plot for serum Il-6 concentration among patients with type 2 Diabetes Mellitus. It showed that the minimum IL-6 value was 4.14 pg/ml, the maximum IL-6 value was 19.86 pg/ml and the median was 10.025 pg/ml. 25% of data fell below 6.82 pg/ml, and 75% of data fell below 12.5 pg/ml.
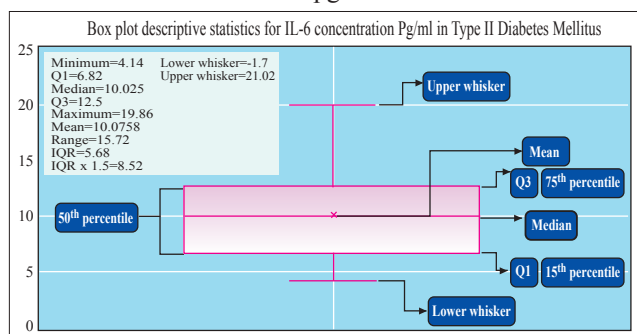

Figure 1 Descriptive analysis of serum IL-6 concentration among patients with Type 2 Diabetes Mellitus

Figure 2 contain the regression coefficient (0.0009) and scatter distribution showing that the BMI of the study population was dense between 20-30 kg/m^2 with IL-6 concentration > 5 pg/ml.
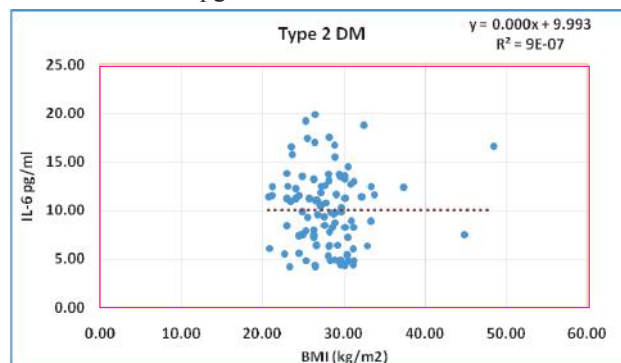

Figure 2 Scatter diagram showing the distribution of BMI with relation to IL-6 concentration among the study population

Figure 3 shows the waist circumference of the study population dense between 90- 110 cm with IL-6 concentration > 5 pg/ml.
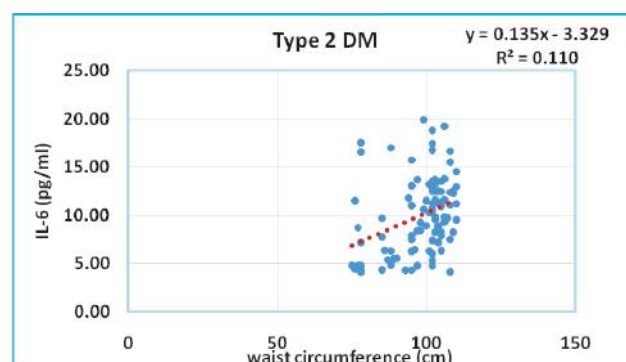

Figure 3 Scatter diagram showing the distribution of waist circumference with relation to IL-6 concentration among the study population

Figure 4 shows the mean plot has shown to have an increased serum IL-6 mean as the BMI increased.
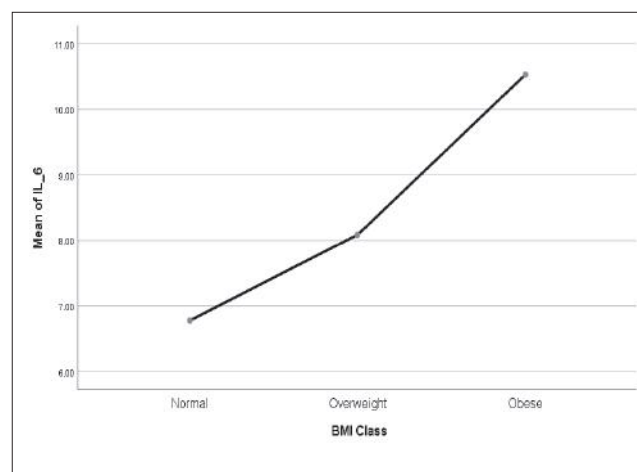

Figure 4 Mean Plot diagram for IL-6 and BMI group

Table IV Pearson's Correlation between serum IL-6 and waist circumference BMI and Duration of Diabetes among the study subjects

Variable	r	p-value
IL-6 and BMI	0.98	>0.05
IL-6 and Waist Circumference	0.33	< 0.01
IL-6 and Duration of Diabetes Mellitus	0.26	< 0.05
IL-6 and FBS	0.12	> 0.05
IL-6 and 2HPPBS	0.03	> 0.05

Table IV shows that serum IL-6 concentration was significantly positively correlated with waist circumference (r 0.33; p < 0.01) and duration of Diabetes Mellitus (r 0.26, p <0.05) among type 2 diabetes mellitus patients.

Table V One-way ANOVA test between BMI groups and serum IL-6 concentration

BMI			F	p-value
Normal	Overweight	Obese		
6.78±1.95	8.08±3.15	10.52±4.00	5.39	0.006* (< 0.05)

Post Hoc Tests					
	Mean difference	Standard error	p-value	C.I Lower Bound	C.I Upper Bound
Obese Vs Normal	3.75*	1.40	.009	.96	6.54
Obese Vs Overweight	2.44*	1.09	.029	.25	4.62

There was a statistically significant difference in IL-6 mean between BMI groups as determined by one-way ANOVA (F (2,97)=5.39, p=0.006).

Discussion

This study was designed and conducted to evaluate serum IL-6 in type II diabetes mellitus. It was observed by Van Greevenbroek et al. that excess visceral obesity, particularly excess visceral adiposity, could lead to chronic low-grade inflammation.[1] In this study type 2 diabetes mellitus patients have a mean IL-6 of 10.08±0.39 pg/ml (Table III) and the value was more than normal. Rose-John S, Laishram V et al. and Marques-Vidal P et al. also observed such kind of increased mean IL-6 in their study.[11-13]

Patients with obesity and type 2 diabetes mellitus had plasma IL-6 concentrations as high as 2-3 pg/ml.[15,16] It had shown (Figure 1) in a box plot for serum IL-6 concentration among patients with type 2 Diabetes Mellitus. It showed that the minimum IL-6 value was 4.14 pg/ml, the maximum IL-6 value was 19.86 pg/ml and the median was 10.025 pg/ml. 25% of data fell below 6.82 pg/ml, and 75% of data fell below 12.5 pg/ml. Naila A and Sattar S found that the mean IL-6 concentration in type 2 diabetes mellitus patients was 30.81±2.43.[17]

As a response to mechanical and endoplasmic reticulum stress in obesity, immune cells are recruited to the white adipose tissue. Dying adipocytes are surrounded by macrophages. In obese white adipose tissue, macrophages release inflammatory cytokines like Interleukin-6 resulting in systemic low-grade inflammation.[14] It was shown in Figure 2, that the BMI was dense between 20-30 kg/m^2 with IL-6 concentration > 5 pg/ml.

The most important observation of type 2 diabetes mellitus was identified as insulin resistance in adipose tissue.[13] The main sources of circulating IL-6 in type 2 diabetes were adipocytes and adipose tissue macrophages.[13] Research showed that long-term exposure of adipocytes with IL-6 had negative immunoregulatory effects on insulin signalling pathways.[17] This study also found that 82% of the patients with type 2 diabetes mellitus patient were obese. (Table II).

It was shown (Figure 3) that for every one-unit increase in waist circumference,plasma IL-6 concentration shall increase by 0.135 times (y = 0.1358 - 3.3298). Table IV displayed that serum IL-6 concentration was significantly and positively correlated with waist circumference (r 0.33, p < 0.01) and duration of Diabetes Mellitus (r 0.26, p <0.05), among type 2 diabetes mellitus patients. A Fischer's Least Significant Difference (LSD) test (Table V) and means plot (Figure 4) revealed that the mean IL-6 concentration was significantly increased in the overweight (8.08±3.15, p .029) kg/m^2 and Obese group (10.52±4.00, p.009) kg/m^2 compared to the normal BMI group. (6.78±1.95) kg/m^2.

Limitation

The study had a small sample size that might not reflect the generalization of the findings to the reference population.The prevalence of increased IL-6 concentration in type 2 diabetes mellitus patients might not be able to distinguish between the actual incidence and duration of type II diabetes mellitus.

Conclusion

From this study, it can be concluded that there was increased IL-6 concentration in type 2 diabetes mellitus patients. Obese individuals with type 2 diabetes mellitus had an increased level of IL-6 concentration. As the progress in the identification of the IL-6 predisposition to type 2 diabetes mellitus in the local population was limited, therefore, future research would be needed to examine the association of IL-6 with insulin resistance.

Recommendation

A prospective, multicentre study is indicated to establish the increased serum IL-6 concentration in type 2 diabetes mellitus. The association between IL-6 and insulin resistance and between Hs-CRP and Insulin resistance need to be evaluated. A feasibility study may be aimed to convey awareness regarding the measurement of inflammatory marker serum IL-6 and Hs-CRP in type 2 Diabetes Mellitus and its complications.

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