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Panacea Journal of Medical Sciences

Journal homepage: http://www.pjms.in/

Original Research Article

Evaluation of interleukin-6 and C - reactive protein as a inflammatory mediators in type 2 diabetic subjects in Indian population of Bhopal

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ARTICLE INFO

Article history:
Received 26-05-2021
Accepted 18-12-2021
Available online 17-08-2022

Keywords:
Type 2 Diabetes Mellitus
IL6
HbA1C
Glycemic Control
CRP
Inflammation
Metaflammation

ABSTRACT

Background: India leads the world with the largest number of Diabetic subjects, hence can be called as Diabetic Capital of World. Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder resulting from insulin insufficiency or function. The researches in past decade have revealed a critical link between metabolic disorders and inflammation which leads to a concept called metaflammation. The linkage of inflammation and type 2 diabetes mellitus (T2DM) has been extensively investigated for over a decade. The main objective of the study is to assess inflammatory markers in T2DM subjects by measuring cytokines and acute phase proteins and its comparison with healthy controls.

Materials and Methods: Total 100 subjects were studied which were divided into two groups of 100 of Diabetic cases and 100 Healthy controls after defining proper inclusion and exclusion criteria. Gender wise distribution was also done. FPG, 2H-PG, HbA1C and CRP were estimated on fully automated analyzers while IL-6 was estimated by ELISA.

Results: The result had shown that cases have significantly elevated IL-6 and CRP when compared to age and sex matched healthy controls with $p<0.0001$. FPG, 2H-PG and HbA1c was also significantly elevated in cases as compared to healthy subjects. We had also compared inflammatory and glycemic markers on the basis of gender.

Conclusion: We can conclude that in developing countries like India estimation of inflammatory markers along with glycemic markers can predict secondary complications of disease.

1. Introduction

Diabetes mellitus has been considered as silent epidemic because of most common health problem worldwide.[1] This makes diabetes as most important cause of healthcare expenditure, mortality, morbidity, and lost economic growth.[2–4] About 415 million peoples are suffering from diabetes and this number is expected to be 640 million by 2040.[5] India with maximum number of cases is considered as diabetes capital of the world.[6] Diabetes is a complex metabolic disease with hyperglycemia as a characteristic feature due to disturbance in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion or its impaired action or may be both. T2DM is now being redefined as a immune disorder due to its notable role of inflammation in its pathogenesis.[7] As such inflammation is protective mechanism of the body but in chronic conditions as diabetes, this mechanism becomes important for progression of disease. Metaflammation is a form of low grade systemic and persistent inflammation due to consumption of excess nutrients and energy.[8,9] Cytokines are pre-dominant inflammatory markers in

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https://doi.org/10.18231/j.pjms.2022.064

various diseases including T2DM. IL-6 is pleiotropic cytokine affecting glucose metabolism via adipocytes and pancreatic B cell. C-reactive protein (CRP) which is synthesized by hepatocytes in response to pro-inflammatory cytokines during inflammation or infectious reactions. Hence it is considered as indicator of low grade systemic inflammation.[10–13]

Through many research studies it has been hypothesized that T2DM is a disease of subclinical chronic inflammation. In case of T2DM IL-6 and CRP are independent predictor of disease and complication.[14] It has been evident that hypertrophied adipocytes are involved in inflammation. Proinflammatory cytokines such as IL-6 are released from adipocytes which promote insulin resistance and elevation of acute phase proteins like CRP.[15] Hence we designed a hospital based case control study to evaluate role of CRP and IL-6 in pathogenesis of T2DM.

2. Materials and Methods

This study was conducted in Peoples College of Medical Sciences and Research Centre Bhopal and associated People's Hospital Bhopal. Total 200 subjects included in this study were divided into 2 groups:

1. Group I: included 100 normal healthy individuals, who were in the age group 25- 70 years, of either sex and without any family history of diabetes mellitus.
2. Group II: included 100 diagnosed patients of type 2 DM in the same age group i.e., 25-70 years.
3. Subjects were also classified on the basis of gender, 140 Males and 60 Females were there in Control- Case group.

2.1. Inclusion criteria

Type-2 DM diagnosed on the basis of the ADA 2015 guidelines was included in the study.

2.2. Criteria for diabetes diagnosis

1. A1C ≥6.5%* Perform in lab using NGSP-certified method and standardized to DCCT assay.
2. FPG ≥126 mg/dL (7.0 mmol**/L)*** Fasting defined as no caloric intake for ≥8 hours.
3. 2-hour PG ≥200 mg/dL (11.1 mmol/L) during OGTT (75-g)* Performed as described by the WHO, using glucose load containing the equlivalent of 75g anhydrous glucose dissolved in water.
4. Random PG ≥200 mg/dL (11.1 mmol**/L)** In persons with symptoms of hyperglycemia or hyperglycemic crisis.[3]

2.3. Exclusion criteria

Type 1 DM, congestive heart failure, tuberculosis, gout, rheumatoid arthritis, renal failure and those who were on hypoglycaemic drugs and on insulin therapy were excluded from the study.

2.4. Biochemical analysis

A total of 6 ml blood sample was collected from all subjects for estimation of glycemic (FPG, 2H-PG and HbA1c) and inflammatory markers (IL-6 and CRP). 2 ml blood is collected in EDTA, Fluoride and Plain vial for HbA1c, plasma glucose, IL-6 and CRP respectively. Plasma Glucose, CRP and HbA1C, were analysed on Roche Cobas C311 while IL-6 was performed on ELISA.

Test	Bulb	Method
Glycosylated hemoglobin (HbA1C)	EDTA	Turbidimetric Inhibition Immunoassay method[16]
C-Reactive Protein	Plain Vial/Serum Separating Tube	Particle enhances Turbidimetric assay[17]
Plasma Glucose	Fluoride Vial	Hexokinase[18]
IL-6	Plain Vial/Serum Separating Tube	ELISA[19]

Statistical analysis of data: All data were expressed as Mean ± SD. Statistical analysis was done using unpaired students-t-test. A level of p value <0.05 was used to indicate statistical significance in all analyses.

3. Result

The comparison of 100 controls with 100 cases has been shown in following tables:

4. Discussion

The exponential rise in the prevalence of diabetes and its complications has been of great concern to health care provider worldwide. Diabetes is a metabolic pro-inflammatory disorder with increased level of circulating cytokines suggestive of inflammation in its eitiology.[20] This altered immune response and associated inflammation is characterized by elevated inflammatory markers like IL-6 and CRP in diabetic subjects. The result of our study shows that IL-6 and CRP were elevated in diabetic cases as compared with healthy controls. FPG, 2H-PG and HbA1c were significantly elevated in diabetic cases when compared with healthy controls. (Table 1)**.** One of the possible mechanisms for elevated inflammatory markers could be activation of signalling cascade due to hyperglycemia. Elevated plasma glucose (metabolic storm) induces IL-1β from beta cells of pancreatic islets via activation of NF-κB (amplification). This activation causes recruitment of various pro-inflammatory cytokines and

Table 1: This table shows comparison of biochemical parameters in controls and cases.

S.No.	Parameters	Controls	Cases	P value
	No. of subjects	100	100	-
1.	FPG	98.26±12.7	187.5±23.29	<0.0001
2.	2H-PG	152.3±16.1	302.8±32.87	<0.0001
3.	HbA1c	4.18±0.89	9.34±2.80	<0.0001
4.	CRP	3.80±1.04	15.66±1.92	<0.0001
5.	IL-6	2.35±0.53	6.49±1.03	<0.0001

The results were statistically significant as p value <0.001

Table 2: This table shows comparison of biochemical parameters in healthy male controls and diabetic male cases.

S.No.	Parameters	Controls male	Cases male	P value
	No. of subjects	70	70	-
1.	FPG	98.27±8.64	187.85±23.80	<0.0001
2.	2H-PG	152.27±5.34	302.2±33.3	<0.0001
3.	HbA1c	4.26±0.77	9.14±2.81	<0.0001
4.	CRP	3.80±1.00	15.52±1.98	<0.0001
5.	IL-6	2.37±0.488	6.66±1.08	<0.0001

The results were statistically significant as p value <0.001

Table 3: This table shows comparison of biochemical parameters in healthy female controls and diabetic female cases.

S.No.	Parameters	Controls Female	Cases Female	P value
	No of subjects	30	30	-
1.	FPG	98.26±7.19	186.92±22.45	<0.0001
2.	2H-PG	152.39±6.25	304.4±32.18	<0.0001
3.	HbA1c	4.01±0.84	9.80±2.78	<0.0001
4.	CRP	3.81±0.96	16.00±1.78	<0.0001
5.	IL-6	2.30±0.50	6.10±0.78	<0.0001

The results were statistically significant as p value <0.001

chemokines such as IL-6, which further induces deleterious effects in pancreatic cells producing insulin. IL-6 is a central mediator for acute phase reactions and increases production of hepatic CRP. It also impairs insulin signalling pathway in hepatocytes by activating JAK2/STAT3 pathway. Potential mechanism of action of CRP activity is inducing pro-inflammatory markers, adhesion molecules, and other signalling molecules or endothelial nitric oxide.[21–24]

The other mechanism which can induce inflammation in diabetes is that due to hyperglycemia there has been increased concentration of advanced end glycation products which further activates macrophages, increased oxidative stress and upregulate production of IL-6 and finally CRP.[25] The hallmark of T2DM is inability to control plasma glucose due to impairment in insulin secretion or its action or maybe both, this is main reason for elevated plasma glucose levels in diabetic subjects in our study. HbA1c is another gold standard marker of glycemic control. Glycation is the non-enzymatic addition of sugar to amino groups of hemoglobin protein. Any fluctuations in blood glucose level will change level of glycated Hb, but once glycation occurs the level remains relatively stable for approx three months. This is due to life span of RBC's. In type 2 diabetics the concentration of glycated Hb predicts progression of diabetic complications.[26,27]

We have also compared FPG, 2H-PG and HbA1C on the basis of Gender. Control males were compared with cases males (Table 2), and female controls were compared to female cases (Table 3) comparison were statistically significant (p<0.005). Similarly inflammatory markers were also compared based on gender and results were significantly elevated in diabetic cases as compared to controls.

The study conducted by Smitha Upadhyay et al. on adiponectin and IL6 as a mediator of inflammation in T2DM population included 162 individuals. ELISA estimated adiponectin and IL-6. The study concluded that there was a decrease in Adiponectin and an increase in IL-6 in T2DM.[28] Ahmad al shukailiet al. studied the analysis of inflammatory mediates in T2DM in a survey that included 57 diabetic cases and 30 healthy controls. CRP, HbA1c, and IL-6 were detected. A significant association has been seen in the immune mediators and T2D.[29]

Coppola et al**.** conducted a research study on 156 diabetic patients and 156 age-sex matched healthy controls. This study concluded that CRP was elevated in people with diabetes as compared to controls. They explained that hyperglycemia leads to the formation of advanced end

glycation products and activates macrophages and increases oxidative stress. All these factors upregulate the production of IL-6 and finally production of acute phase proteins.[30] This study was in agreement with our study.

The study conducted by Prof. K Goswami[31] to estimate HbA1c among 204 subjects showed similar results as our study. They also demonstrated correlation of HbA1c and estimated average glucose and found that on increasing Blood Sugar level, HbA1c % also increases. Miza Asif Baig concluded in his study that HbA1c can be used effectively for diagnosis of Type 2 Diabetes mellitus and also as a predictive marker for complication of diabetes.[32]

5. Conclusion

Our study revealed that inflammatory markers are elevated in T2DM cases and are most reliable marker and screening tool that can predict complication of diabetes. IL-6 is an pro-inflammatory marker which is elevated in low grade systemic inflammation disease, it also leads to elevate certain other acute phase proteins like CRP in case of hyperglycemia. Inflammatory and glycemic control markers when detected together the impact was substantially greater. These results support the role of hyperglycemia in development of inflammation and resistance in Type 2 Diabetes Mellitus. Early Detection of hyperglycemia and Blood Glycemic Control can prevent complication and further decrease morbidity and mortality.

6. Conflict of Interest

The authors declare that they have no conflict of interest.

7. Source of Funding

None.

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Cite this article: Hisalkar PJ, Patne A, Dubey A. Evaluation of interleukin-6 and C - reactive protein as a inflammatory mediators in type 2 diabetic subjects in Indian population of Bhopal. *Panacea J Med Sci* 2022;12(2):340-344.