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EVALUATION OF INFLAMMATORY MARKERS (C REACTIVE PROTEIN & INTERLEUKIN 4) IN TYPE 2 DIABETES MELLITUS

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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. **Aim and objective:** To assess inflammatory markers in T2DM subjects by measuring cytokines and acute phase proteins and its comparison with healthy controls. **Materials and Methods:** Total 122 subjects were studied which were divided into two groups of 100 of Diabetic cases and 122 Healthy controls after defining proper inclusion and exclusion criteria. Gender wise distribution was also done. HbA1C and CRP were estimated on fully automated analyzers while IL-4 was estimated by ELISA. **Results:** The result had shown that cases have significantly elevated IL-4 and CRP when compared to age and sex matched healthy controls with $p<0.0001$. SBS, 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.

Keywords: T2DM, inflammatory, CRP, IL-4

Introduction

Diabetes mellitus, also known as diabetes, is a serious, long-term (or "chronic") condition in which blood glucose levels rise due to the body's inability to produce any or enough of the hormone insulin or to effectively use the insulin it does produce.[1]

Diabetes is a serious lifestyle illness with a rising prevalence in the world. As the prevalence of diabetes rises in these nations, Asia accounts for more than 60% of the world's diabetic population. Similar to this, it is predicted that by 2030, there will be 438 million more persons aged 20 to 70 who have type 2 diabetes than there were in 2010.[2] According to estimates, there will be 536.6 million people with diabetes

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worldwide in 2021, and that number will rise to 783.2 million by 2045, or 12.2 percent. Diabetes prevalence was comparable between genders and was highest among people aged 75 to 79. In 2021, the prevalence was predicted to be greater in urban (12.1%) than rural (8.3%) areas and high (11.1%) than low-income countries (5.5 percent). In comparison to high- (12.2%) and low-income (11.9%) countries, middle-income countries are predicted to experience the largest relative increase in diabetes prevalence between 2021 and 2045 (21.1%). The cost of treating diabetes-related illnesses worldwide was estimated at 966 billion USD in 2021 and is expected to rise to 1,054 billion USD by 2045.[3]

Diabetes is a potentially epidemic health problem that is quickly spreading throughout low- and middle-income nations like India. According to projections, India will have 69.9 million cases of diabetes by 2025, the great majority of which would go untreated. This is primarily caused by food changes and inadequate or no physical activity, which alters the physiological environment and causes overweight or obesity as well as diabetes .[4]

Levels of CRP are directly correlated with the presence and elimination of inflammatory agents. [5] CRP's role as a diagnostic and prognostic biomarker has been established for acute infectionsas well as for various chronic conditions, such as T2DM, atherosclerosis, hepatitis C and different types of cancer. Accumulating evidence suggests a connection between the activation of the complement system and the pathogenesis of T2DM. [6] There is evidence of CRP involvement in proliferation and apoptotic processes through activation of Fc receptors and the consequent production of proinflammatory mediators and proapoptotic cytokines. In addition, growing evidence show CRP is not only an inflammatory marker as its level is proven to be elevated in T2DM cases. [6]

Interleukin 4 (IL-4) is known to promote insulin signaling in adipocytes. Recently, we demonstrate that IL-4 boosts insulin-induced energy deposits by enhancing glucose uptake and lipogenesis in hepatocytes. While IL-4 is reported as a myoblast recruitment factor which promotes muscle differentiation (myogenesis) and regeneration, the effects of IL-4 on energy metabolism in muscle, the largest insulin-targeting organ, remain obscure. Therefore, regulation of IL-4 to myogenesis and glucose metabolism in the undifferentiated C2C12 myoblasts and terminal differentiated myocytes were examined in the present study. [7] Hence we designed a hospital based case control study to evaluate role of CRP and IL-6 in pathogenesis of T2DM.

Materials and Methods

Materials and Methods

The study was conducted in IMCH and RC, Department of Biochemistry. Ethical clearances was obtained from the Institutional Ethical Committee and written informed consent was taken, before carrying out the study.

Sample size

A total of 122 subjects was recruited, 61 severe diabetes mellitus patients of cases group in Department of Biochemistry, Index Medical College & Research Centre, and 61 healthy controls.

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INCLUSIONCRITERIA

- Subjects diagnosed with type 2 diabetes mellitus patient (according to ADA)
- Subject with age 25-60years.
- Subject has signed the informed consent form.

EXCLUSION CRITERIA

- Conditions where phlebotomy is contraindicated
- Subjects are suffering from chronic illness
- Subjects are suffering from any acute illness
- Liver disease
- Renal disease
- Pancreatic disease
- Using drugs

Statistics Analysis

Mean ± SD were calculated for all the parameters to examine and were differentiated by Student's t-test using SPSS 23. P-values considered significant were as follows: – $P < 0.05$– a Significant and $P > 0.001$ –a highly Significant.

Result

The comparison of 122 controls with 122 cases has been shown in following tables:

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

Parameter	Controls (Mean±SD) (n=122)	Diabetes Mellitus (Mean±SD) (n=100)	p-value
SERUM HbA1C%	5.51± 1.07	6.49± .59	0.0001
SERUM FBS (mg/dl)	84.09 ±8.76	122.09 ± 8.98	0.0001
SERUM CRP (µg/dl)	11.33 ±4.78	13.26 ±5.76	0.0463
SERUM INTROLEUKINE-4	2.07 ±0.28	2.22 ± 0.37	0.0129

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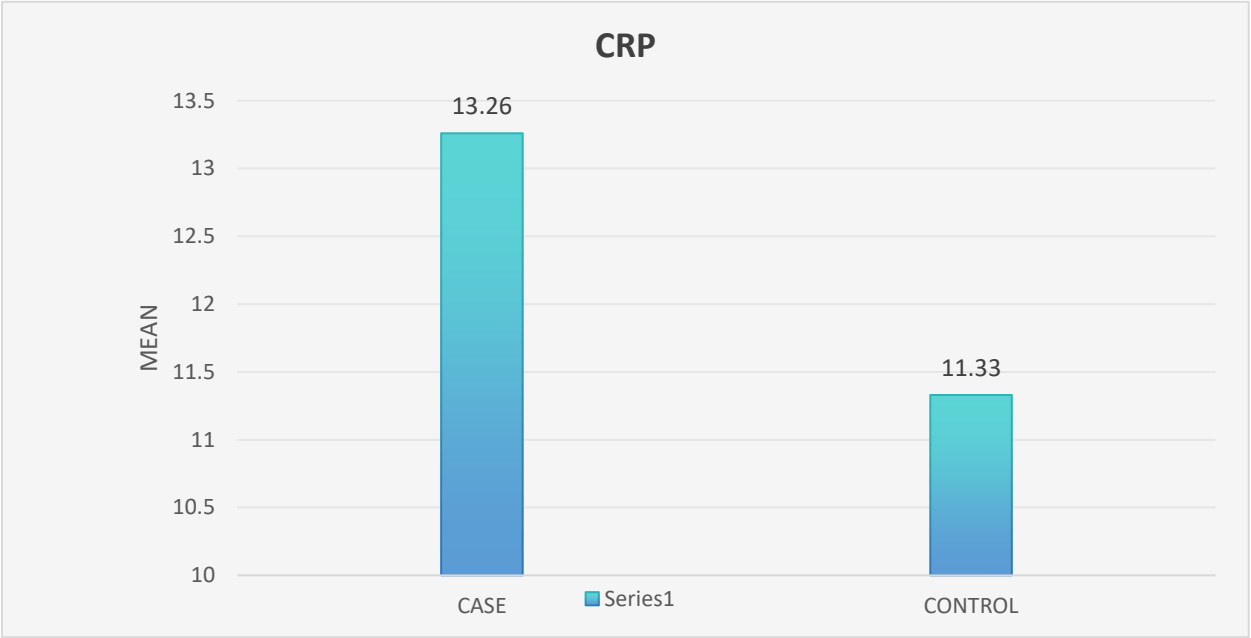

Figure-1: Comparison of Serum CRP in controls and cases

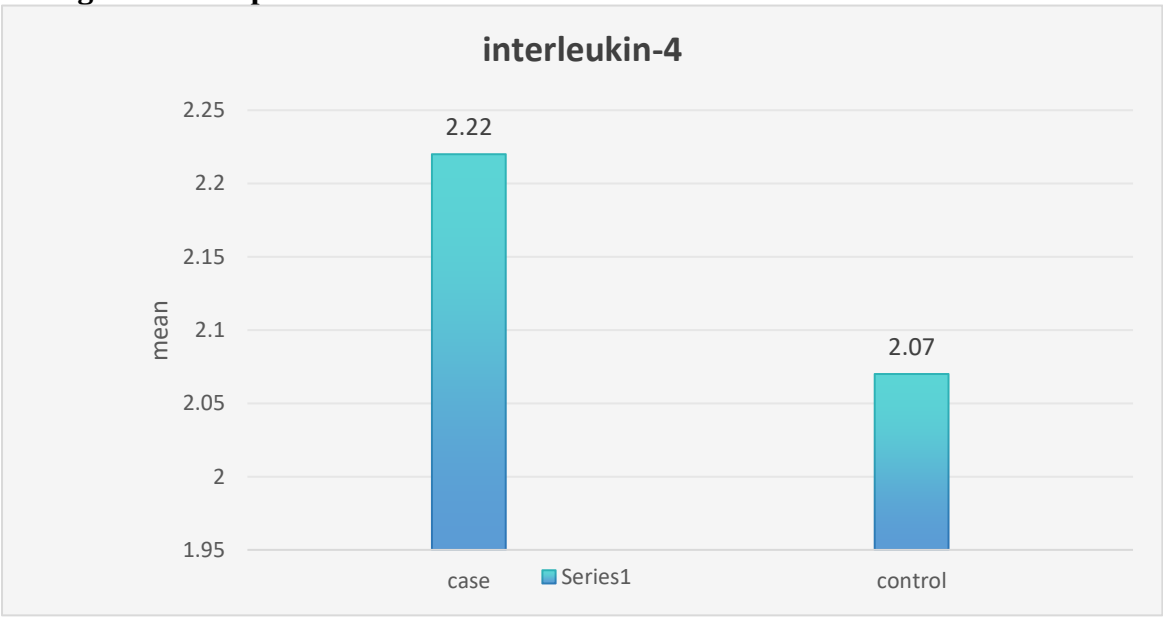

Figure-2: Comparison of Serum interleukin-4 in controls and cases

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 proinflammatory disorder with increased level of circulating cytokines suggestive of inflammation in its eitiology. [4] 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-4 and CRP were elevated in diabetic cases as compared with healthy controls. In the present study, we significantly low in serum interleukin-4 level in Type 2 diabetes mellitus patients. Our

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study showed a decrease in serum level of interleukin-4 (2.22±.37) in cases as compared with the controls group which was (2.07±.28). This finding shows the level of serum interleukin-4 was significant between cases as compared to controls.

The variance in study design may explain the differences in CRP levels in diabetic animals. Although alloxan induces diabetes through a different mechanism (oxidative stress through Fenton reaction) than streptozotocin (alkylation of DNA) [8], higher doses of alloxan applied to male and female rats induce the same effects on diabetes development and alterations of CRP levels in circulation, as streptozotocin. [8-10]

In the present study, we evaluated the status of serum CRP level in Type 2 diabetes mellitus patients. Our study showed an increase in serum level of CRP (13.26±5.76) in cases as compared with the controls group which was (11.33±4.78). This finding shows the level of serum CRP was significant between cases as compared to controls. Although the clinical relationship between diabetes and increased level of CRP is well established, the molecular mechanisms by which CRP potentially induces diabetes are yet to be clarified. Particular progress has been made in investigating multiple therapeutic approaches targeting different inflammatory factors [11]. Treatment of T2DM patients with IL-1 receptor blocker [12-13] or IL-1β antibodies [14] reduced levels of IL-6 and CRP as markers of systemic inflammation, with simultaneous improvement of glycemia and insulin secretion. [14]

The development and outcome of DM are complex and multifactorial. Focusing only on each gene or polymorphism provides a limited understanding of the same. Hence, we attempted to detect other potential genes related to DM using the online STRING server. The other ten most probable genes were obtained from the network. Among them, six genes belonged to the interleukin family and three were in the front. Four genes were related to the chemokine (C–X–C motif) ligand family. For example, the first related gene is IL-6R, which is the receptor of the IL-6 gene. Qi et al. reported that the IL6R rs8192284 variant was significantly associated with plasma CRP level and could predict diabetes risk [129]

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-4 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

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