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Study of TNF-α, IL-6 and Insulin Resistance in Type 2 Diabetes Mellitus at Vidharbha Region

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: DM is a metabolic condition caused by deficiencies in insulin synthesis, insulin action, or both. It is characterised by chronic hyperglycemia and glycosuria, as well as abnormalities in carbohydrate, fat, and protein metabolism. Diabetes and its complications are believed to be caused by a variety of causes. Genetics, diet, sedentary lifestyle, perinatal causes, age, and obesity are among them. The relationship and interaction of various risk factors with disease severity is still unknown, so the aim of the proposed study was to determine the possible relationship between biochemical markers glycosylated haemoglobin, lipid profile, insulin resistance, and immunological markers TNF- and IL-6, in order to suggest appropriate measures to reduce the country's diabetes burden.
Materials and Methods: A total of 300 people were chosen for the study after visiting Shalinitai Meghe hospital in Nagpur for a health check-up. The three groups were contained in this area.

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Results: Both biochemical and immunological parameters rose in managed diabetic patients and significantly increased in uncontrolled diabetic patients, according to the report, but values did not differ between groups 1.
Conclusion: Low-grade inflammation and inflammatory mediator upregulation have been suggested to play a role in T2DM etiology. TNF- and IL-6 have a positive connection with T2DM and insulin sensitivity, according to our data. These can be used as T2DM biomarkers in the early stages of the disease. To help doctors monitor and treat T2DM successfully, more research on a larger spectrum of pro and anti-inflammatory cytokines (mediators) in conjunction with other biochemical, immunoassay, and hematological markers is needed.

Keywords: Tumour necrosis factor; il-6; insulin receptor substrate; fasting blood sugar; glycated hemoglobin.

1. INTRODUCTION

Diabetes mellitus is a metabolic condition characterized by hyperglycemia and disturbances of carbohydrate, fat, and protein metabolism triggered by insulin action and secretion deficiencies or both [1]. Diabetes was on the rise in the year 2000, with 151 million people worldwide suffering from the condition. By 2010, this number is expected to rise to 239 million, with 300 million by 2025. India reportedly has the largest number of diabetic adults in the country, with an estimated 32.7 million [2]. Long-term complications of chronic hyperglycemia, a complication of diabetes; include inflammation, dysfunction, and breakdown of various organs, including the skin, kidneys, nerves, heart, and blood vessels.

Diabetics die from cardiovascular disease in 50% of cases. Diabetes claims the lives of around 5% of the world's population per year [3].

Insulin resistance is described as when insulin's biological effects on glucose disposal in skeletal muscle and suppression of endogenous glucose production, mainly in the liver, are less than predicted [4].

TNF-α, an anti-inflammatory cytokine produced by a variety of cell types, but mainly by macrophages, lymphocytes, and adipose tissue, is a pro-inflammatory cytokine [5]. In muscle skeletal in vitro and in vivo, TNF- inhibits the insulin signaling Cascade in many regulating proteins, including the insulin receptor substrate (IRS) and the Akt substrate [6]. These two studies suggest that an increase in TNF- is not a negative effect of the pathological conditions associated with insulin resistance, but rather plays a direct role in glucose metabolism. Type 2 diabetes is characterized by insufficient insulin resistance in the skeletal muscle [7].

Type 2 diabetes is linked to an increase in proinfigueatory cytokines. IL-6 has been shown to have insulin resistance and type 2 diabetes for all invading cytokines [8]. In obese patients and type 2 diabetes, plasma levels of IL-6 are higher than those of studies low. Increased blood glucose, decreased glucose tolerance, and decreased insulin sensitivity are all associated with these elevations [9]. Insulin resistance increases with the presence of IL 6. Inhibition of hepatic glycogen synthase, phosphorylated glycogen synthesis and lipolysis, and increased triglyceride release are all associated with elevated levels of IL-6 [10]. levels. As a result, it has been suggested that IL-6 plays a role as a glucoregulatory hormone [11].

Numerous studies published in recent years have suggested that chronic inflammation of an incurable disease may be an important factor in insulin resistance and type 2 diabetes. As a result, diabetes is classified as chronic inflammation [12].

2. MATERIALS AND METHODS

The research was conducted at Nagpur's Datta Meghe Medical College's Department of Biochemistry. The study included 300 people, including 100 type 2 diabetics who were uncontrolled, 100 type 2 diabetics who were under control, and 100 healthy people. All of the diabetic patients were taking oral hypoglycemic medications. From established families, age-matched stable control subjects were chosen. Before beginning the study, patients' informed consent was also obtained. In a proforma, a record of the patient's clinical history and prior investigations of their disorders is collected.

2.1 Sample Collection

Following an overnight fast, 5 ml of blood was drawn from the anticubital vein. Vacutainer with

plain, fluoride, and EDTA were used to collect the blood sample. Biochemical and immunological tests were performed on the blood sample, which included:

- **Biochemical Parameters**

1. Blood Sugar: Fasting and Post Meal
2. HbA1c
3. Lipid Profile
4. Insulin
5. Insulin resistance by HOMA–IR calculation method

- **Immunological Parameters**

6. Tumor Necrosis Factor-α
7. Interleukin-6

2.2 Biochemical Investigations

Biochemical parameters such as fasting blood sugar, total cholesterol, and triacylglycerides were measured using a fully automated analyser in a commercially available package, as directed by the manufacturer. Rai KB and Pattabiraman method TN were used to calculate glycosylated haemoglobin (1984) [13].

2.3 Separation of Serum

A blood sample was taken and incubated for 30 minutes at 37 degrees Celsius in a plain vial. The clot was removed after incubation, and the remaining sample was transferred to a centrifuge test tube. For 10 to 20 minutes, samples were spin at 3000 rpm. Supernatant was collected in a clean and dry serum test tube for study of fasting blood glucose, lipid profile, insulin, and the inflammatory markers interleukin-6 and tumour necrosis factor-α.

2.4 Immunological Investigations

A highly responsive sandwich-enzyme associated immunosorbant assay (ELISA) tool in a commercially available kit was used to estimate the immunological markers interleukin-6 and tumour necrosis factor- α (Immunotech, Backman Coulter, France).

2.5 Statistical Analysis

Data was inserted on Microsoft excel 2011 spreadsheet. All the data were expressed as a mean ± standard deviation and significance value (p) were calculated. Data analysis was performed by using statistical 'software SPSS 16.1. For all assessments, $p<0.05$ were considered statistically significant.

3. RESULTS

Table 1 indicates that diabetic patients in groups 2 and 3 had substantially higher levels of all biochemical parameters (Fasting Blood Sugar, HbA1c, Total Cholesterol, Triglyceride, HDL-c, LDL-c, and HOMA) than stable controls in group 1. As a result, all biochemical parameters in uncontrolled diabetic patients are higher than in regulated diabetic volunteers.

Table 2 indicates that the immunological parameters in diabetic patients in groups 2 and 3 are substantially higher than in the group 1 control group. As compared to the regulated diabetic group and the usual group, the uncontrolled diabetic group's TNF and IL-6 levels increased.

Table 1. Overall status of biochemical parameters in group I healthy control subjects (N=100), group II diabetic control subjects (N=100), group III diabetic uncontrolled subjects (N=100)

Sr. No	Biochemical Parameters	Healthy control subjects (N=100)	Diabetic Control subjects (N=100)	Diabetic uncontrolled subjects(N=100)
1.	Fasting Blood Sugar (FBS)	78.9±12.6	122.28±28.51	181.5±38.6
2.	Glycosylated haemoglobin (HbA1c)	00.28±00.02	00.55±00.08	00.87±00.30
3.	Total Cholesterol (TC)	160.80±21.75	190.21±42.3	273.82±52.73
4.	Triglyceride (TG)	126.8±22.4	166.44±52.9	285.58±90.65
5.	High density-lipoprotein cholesterol (HDL-c)	48.47±9.34	32.80±6.95	28.53±2.45
6.	Low density lipoprotein cholesterol (LDL-c)	93.46±28.85	123.68±52.8	165.35±45.2
7.	Insulin resistance by HOMA–IR calculation method	3.26±00.45	3.24±0.85	6.95±3.50

Table 2. Overall status of immunological parameters in group I healthy control subjects (N=100), group II diabetic control subjects (N=100), group III diabetic uncontrolled subjects (N=100)

Sr.No	immunological parameters	Healthy control subjects (N=100)	Diabetic Control subjects (N=100)	Diabetic uncontrolled subjects(N=100)
1	TNF **α**	11.31±9.52	18.12±5.45	32.5±13.1
2	IL-6	10.92±2.38	14.64±4.58	30.41±9.88

4. DISCUSSION

DM is a metabolic disorder caused by insufficient insulin secretion, activity, or both. Chronic hyperglycemia and glycosuria, as well as elevated starch, fat, and protein metabolism, are both symptoms [14].

The relationship and interaction of various risk factors with disease severity is still unknown, so the aim of the proposed study was to determine the possible relationship between biochemical markers glycosylated haemoglobin, Total cholesterol, Triacylglceride , HDL-c, LDL-c, insulin resistance, and immunological markers TNF- and IL-6, in order to recommend appropriate measures to reduce the burden of diabetes in the future.

In comparison to group I, we find a highly important rise in fasting blood sugar and glycosylated haemoglobin levels in groups II and III.

The rise in HbA1c is due to high glucose concentrations within and outside the cells, which favours spontaneous and non-enzymatic reactions between glucose and protein in intracellular and extracellular compartments, resulting in advanced glycation end products Suji G, et al. 2004 [15].

According to the findings, dyslipidemia is a well-known risk factor for cardiovascular disease Yach D, et al. 2004 [16].

In diabetes mellitus, insulin deficiency decreases the function of hepatic lipase and causes many changes in LPL Samatha P, et al. 2012 [17].

Regeneration of the long-term immune system, as well as genetic and environmental factors in chronic inflammation, promotes disease rather than self-mutilation, which contributes to the formation of type 2 diabetes, according to Riverio A, et al. 2009 [18].

Recent evidence indicates that adipose tissue produces 10-35 percent of IL-6 in resting individuals, and that this output increases as adiposity increases Mohamed-Ali V, et al. 1997 [19].

Increased IL-6 levels have been linked to increased triglyceride production, inhibition of hepatic glycogen synthase, activation of glycogen phosphorylase, and lipolysis Tsigos C, et al. 1997; Kanemaki T, et al. 1998 [20]. Because of these findings, it has been proposed that IL-6 acts as a glucoregulatory hormone.

Another proinflammatory cytokine that was found in abundance in this analysis is TNF-. It's made by a number of cell types, but macrophages and lymphocytes make the most of it. We discovered a large increase in group II and group III relative to group I after observing the TNF-calculation [21-24].

5. CONCLUSION

Low-grade inflammation and inflammatory mediator upregulation have been suggested to play a role in T2DM etiology. TNF- and IL-6 have a positive connection with T2DM and insulin sensitivity, according to our data. These can be used as T2DM biomarkers in the early stages of the disease. To help doctors monitor and treat T2DM successfully, more research on a larger spectrum of pro and anti-inflammatory cytokines (mediators) in conjunction with other biochemical, immunoassay, and hematological markers is needed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by

the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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