

STUDY OF TNF- α , IL-6 AND INSULIN RESISTANCE IN TYPE 2 DIABETES MELLITUS AT VIDHARBHA REGION

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.

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AIM: We conducted a case-control analysis to determine the relationship between biochemical and immunological markers in diabetic patients who visited SMHRC Nagpur for a routine checkup.

MATERIAL 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. Control subjects were healthy volunteers, control diabetic patients were in group 2, and uncontrolled diabetic subjects were in group 3. Each community consists of 100 patients. 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.

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:

Finally, in the last few years, the pathogenetic vision of diabetes mellitus has shifted, with inflammatory pathways playing a key role in the development and progression of diabetes complications. As a result of these emerging pathogenic influences, new therapeutic approaches are being considered. Modulation of inflammatory processes in the context of diabetes is a hot topic these days.

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KEYWORDS: TNF- α , IL-6, IRS, FBS, HbA1c

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 muscle 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 proinflammatory 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.¹³

MATERIAL 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-

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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.

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

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).¹⁴

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 .

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).

RESULT

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)

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Sr. No	Biochemical Parameters	Healthy control subjects (N=100)	Diabetic Control subjects (N=100)	Diabetic uncontrolled subjects(N=100)
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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

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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:- 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

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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.

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.¹⁵

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, Triacylglyceride, 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.**¹⁶

According to the findings, dyslipidemia is a well-known risk factor for cardiovascular disease **Yach D, et al. 2004.**¹⁷

In diabetes mellitus, insulin deficiency decreases the function of hepatic lipase and causes many changes in LPL **Samatha P, et al. 2012.**¹⁸

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.**¹⁹

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.**²⁰

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**²¹. 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.²²⁻²⁵

CONCLUSION:

Finally, in the last few years, the pathogenic vision of diabetes mellitus has shifted, with inflammatory pathways playing a key role in the development and progression of DM complications. As a result of these emerging pathogenic influences, new therapeutic approaches are being considered. Modulation of inflammatory processes in the context of diabetes is a hot topic these days. It's likely that in the coming years, the promise of new therapeutic strategies focused on anti-inflammatory properties that have beneficial effects on diabetic complications will be realized.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

REFERENCES:

- 1) Valdez R, Liu T, Yoon PW, Khoury MJ. Family history and prevalence of diabetes in the U.S. population. *Diabetes Care.* 2007; 30:2517-22.

Comment [SMC9]: Why the sudden change in the referencing style?

Comment [SMC10]: Poor conclusion.

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- 2) Ram Vinod Mahato, Prajwal Gyawali, Pramod P. Raut, Prashant Regmi, Khelanand P. Singh, Dipendra Raj Pandeya, Prabin Gyawali. Association between glycemic control and serum lipid profile in type- 2 diabetic patients: Glycated haemoglobin as a dual biomarker. *Biomedical Research*. 2011; 22 (3): 375-380.
- 3) Dinneen S, Gerich J, Rizza R. Carbohydrate metabolism in noninsulin-dependent diabetes mellitus. *N Engl J Med*. 1992; 327: 707–13.
- 4) Stumvoll Michael, Tataranni PA, Stefan N, Vozarova B, Bogardus C. Glucose allostasis. *Diabetes*. 2005; 52: 903-09.
- 5) Hotamisligil GS, Murry DL, Choy LN, Spiegelman B. TNF α inhibits signalling from insulin receptor. *Proc Natl Acad Sci USA*. 1994; 91:4854-4858.
- 6) Jr, B. F. P. ., & Federico R. Tewes. (2021). What attorneys should understand about Medicare set-aside allocations: How Medicare Set-Aside Allocation Is Going to Be Used to Accelerate Settlement Claims in Catastrophic Personal Injury Cases. *Clinical Medicine and Medical Research*, 2(1), 61-64. <https://doi.org/10.52845/CMMR/2021v1i1a1>
- 7) Bouzakri K, Zierath JR. MAP4K4 gene silencing in human skeletal muscle prevents tumor necrosis factor- α -induced insulin resistance. *J Biol Chem*. 2007; 282:7783–7789.
- 8) Plomgaard P, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK. Tumor necrosis factor- α induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. *Diabetes*. 2005; 54:2939–2945.
- 9) Plomgaard P, Nielsen AR, Fischer CP, Mortensen OH et al. Associations between insulin resistance and TNF- α in plasma , skeletal muscle and adipose tissue in humans with and without type II diabetes. *Diabetologia* 2007; 50:2562-2571.
- 10) Daniel, V. ., & Daniel, K. (2020). Diabetic neuropathy: new perspectives on early diagnosis and treatments. *Journal of Current Diabetes Reports*, 1(1), 12–14. <https://doi.org/10.52845/JCDR/2020v1i1a3>
- 11) Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G: Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab*. 2001; 280:E745–E751.
- 12) Tsigos C, Papanicolaou DA, Kyrou I, Defensor R, Mitsiadia CS, Chrousos GP: Dose-dependent effects of recombinant human interleukin-6 on glucose regulation. *J Clin Endocrinol Metab*. 1997; 82:4167–4170.
- 13) Joseph J. Senn, Peter J. Klover, Irena A. Nowak, and Robert A. Mooney. Interleukin-6 Induces Cellular Insulin Resistance in Hepatocytes. *Diabetes* 2002; 51:3391–3399.
- 14) Sjöholm Ake, Thomas Nystrom. Inflammation and the etiology of type-2 diabetes. *Diabetes Metab Res Rev*. 2006; 22: 4–10
- 15) Daniel, V., & Daniel, K. (2020). Perception of Nurses' Work in Psychiatric Clinic. *Clinical Medicine Insights*, 1(1), 27-33. <https://doi.org/10.52845/CMI/2020v1i1a5>
- 16) Kathryn E. Wellen and Gokhan S. Hotamisligil, Inflammation, stress, and diabetes: J. *Clin. Invest*. 2005; 115:1111–1119.
- 17) Rai KB, Pattabiraman TN. Glycosylated haemoglobin levels in iron deficiency anaemia. *Indian J Med Res*. 1986; 83: 234-6.

- 18) Smith LL, Burnet SP, Mc Neil JD. Musculoskeletal manifestations of diabetes mellitus. *Br J Sports Med.* 2003; 37: 30-35.
- 19) Suji G and Sivakami S. Glucose, glycation and ageing. *Bio gerontology.* 2004; 5: 365-373.
- 20) Yach D, Hawkes C, Gouuld CL, Hofman KJ. The Global Burden of Chronic Diseases. Overcoming the impediments to prevention and control. *JAMA.* 2004; 291: 2616-2622.
- 21) Schmitz-Peiffer C. Protein kinase C and lipid-induced insulin resistance in skeletal muscle. *Ann. N. Y. Acad. Sci.* 2002; 967:146-157.
- 22) Daniel, V., & Daniel, K. (2020). Exercises training program: It's Effect on Muscle strength and Activity of daily living among elderly people. *Nursing and Midwifery, 1*(01), 19-23. <https://doi.org/10.52845/NM/2020v1i1a5>
- 23) Hadke S, Gawali R. Analysis of Acute Neurologic Problems in Young Non Diabetic Patients with Hypertension. *JARHAS.* 2021; 2(1): 1-4
- 24) Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppel SW: Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metabo.* 1997; 82:4196-4200.
- 25) Ranjit Ambad, Roshan Kumar Jha, Dhruva Hari Chandi, Saurabh Hadke. Association of Leptin in Diabetes Mellitus and Obesity. *Research J. Pharm. and Tech.* 2020; 13(12):6295-6299.