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Association of inflammatory markers, serum ferritin and high sensitive C reactive protein, with HbA1c and dyslipidemia in male patients of type 2 diabetes mellitus

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ABSTRACT

Background: Diabetes mellitus (DM) is a common metabolic disorder affecting insulin secretion, insulin sensitivity, or both, resulting in hyperglycemia. As it progresses, almost all systems including cardiovascular, nervous, renal, etc. are involved eventually manifesting as several health-related complications. As all the systems of the human body work in harmony with each other, development of insulin resistance, dyslipidemia, and setting in of chronic inflammatory states evidenced by the rise in inflammatory markers are interrelated. **Aims and Objectives:** The aim of this study was to establish the correlation of the inflammatory markers, serum ferritin and high sensitivity C-reactive protein (hs-CRP), with glycated hemoglobin (HbA1c) and dyslipidemia in patients of type 2 DM. **Materials and Methods:** The present cross-sectional study was carried out in the Department of Biochemistry, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand. A total of 80 male patients of type 2 DM aged between 40 and 60 years with blood HbA1c level of more than 6.5% were included in the study. **Results:** The inflammatory markers hs-CRP and ferritin were found to be positively correlated with HbA1c, triglycerides, total cholesterol, and low-density lipoprotein-cholesterol but negatively correlated with high-density lipoprotein-cholesterol. **Conclusion:** Estimation of serum ferritin and hs-CRP can detect the ongoing inflammatory and free radical-mediated damages in diabetic patients at an early period before the development of diabetic-related complications such as atherosclerosis and myocardial infarction.

Key words: Diabetes mellitus; High sensitivity C-reactive protein; Ferritin; Dyslipidemia; Glycated hemoglobin

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INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorder that shares the phenotype of hyperglycemia. Hyperglycemia in diabetes results from defects in insulin secretion, insulin action, or most commonly, both. The underlying abnormalities involved in the development of hyperglycemia vary widely.[1]

The prevalence of diabetes is increasing sharply in the developing world with India and China being the largest contributors to the world's diabetic load.

Type 2 diabetes is a prototypic multi-factorial complex disease. Environmental factors, such as a sedentary lifestyle and dietary habits, unequivocally play a role, as will become evident when the association with obesity is considered. Genetic factors are also involved in the pathogenesis.[1]

Ferritin is an ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. In humans, it acts as a buffer against iron deficiency and iron overload. Plasma ferritin is also an indirect marker of the total amount of iron stored in the body. Ferritin serves to store iron in a non-toxic form, to deposit it in a safe form,

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and to transport it to areas where it is required.[2] The presence of iron is toxic to cells as it acts as a catalyst in the formation of free radicals from reactive oxygen species through the Fenton's reaction and Haber–Weiss reaction.[3] Thus, increased body iron might result in diabetes by 3 mechanisms: (a) insulin deficiency-pancreatic islets are extremely sensitive to free radical oxidative damage; iron deposition also occurs in pancreatic interstitial cells with resultant collagen deposition and defective microcirculation (b) Insulin resistance; and (c) hepatic dysfunction.

Studies conducted elsewhere have reported the association of inflammatory markers with diabetes. Inflammation includes a large number of responses to noxious stimuli, such as fever, increased cortisol levels, leukocytosis, thrombocytosis, anemia, and muscle wasting. Inflammation is strongly related to insulin resistance, although the question of whether treatment directed at the inflammatory process could lead to benefits, such as decreasing the development of diabetes, has yet to be answered.

High levels of the acute phase reactants C-reactive protein (CRP) have been reported to correlate with insulin resistance.[4] Serum ferritin has also been reported to have a significant role in the development of insulin resistance in metabolic syndrome as well as DM.[5] However, now, there is increasing evidence that elevated body iron stores, evaluated by serum ferritin, may be associated with hypertension, dyslipidemia, elevated fasting insulin and blood glucose, and central obesity. Serum ferritin has been found to be independently associated with dyslipidemia.[6]

It has been found that significantly higher high-sensitivity CRP (hs-CRP) was present in patients with dyslipidemia than without dyslipidemia in non-diabetic subjects.[7]

Although literature on the individual effects of dyslipidemia and inflammation on diabetes is available, more data are required on the combined effects of dyslipidemia and inflammation in diabetes. Furthermore, there is a dire need for local data on diabetic population of Ranchi, Jharkhand, showing the correlation of dyslipidemia and inflammation.

The study was designed to determine whether inflammation and dyslipidemia existed in Jharkhand diabetic population and if so, to find the relationship between inflammatory markers and the various parameters of lipid profile and glycated hemoglobin (HbA1c).

Aims and objectives

- To study the levels of inflammatory markers serum ferritin and hs-CRP in the patients of type 2 DM
- To study the relation of these inflammatory markers with lipid profile and HbA1c.

MATERIALS AND METHODS

The present cross-sectional study was carried out in the Department of Biochemistry, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand. A total of 80 male patients of Type 2 DM patient aged between 40 and 60 years with blood HbA1c level of more than 6.5% were included in the study.

Age and sex-matched healthy volunteers having blood HbA1c level <5.7% were taken as control.

A signed informed consent was obtained from all the subjects or their legally responsible attendants. The protocol of the study was pre-approved by the institutional ethical committee.

The following were the exclusion criteria:

1. Type 1 DM
2. Other states associated with altered serum ferritin levels such as:
   Hemochromatosis, chronic alcoholics, chronic inflammatory conditions like systemic lupus erythematosus, rheumatoid arthritis, hepatitis, patients with repeated blood transfusions, iron deficiency anemia, recent history of blood loss, bleeding piles, recent history of major surgery, diabetic foot
3. Hypothyroidism
4. Cardiovascular disorders
5. Diabetic nephropathy
6. Anemia of any cause
7. Other complications of diabetes such as diabetic retinopathy and others
8. History of malignancy or chemotherapy
9. Those who are taking medication for painful inflammatory conditions such as osteoarthritis, gout, or taking antilipidemic drugs (Statin)
10. Female patients were excluded from this study as menstrual blood loss is a factor affecting serum ferritin level.

About 6 mL of fasting blood samples were obtained from the antecubital vein of each subject and control. 4 mL of blood samples were transferred to clean dry sterile plain vacutainers and allowed to clot for 30 min and then centrifuged to obtain serum which was then used to estimate triglycerides (TGL), total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, ferritin, and hsCRP. 2 mL of blood were transferred to EDTA vial for HbA1c estimation. Processing and biochemical analysis of blood sample was done by AU- 480 Autoanalyser and ARCHITECT Chemiluminescence Immunoassay. TGL was estimated by glycerol phosphate oxidase and peroxidase method,[3] TC and

HDL-cholesterol (HDL-C) were estimated by cholesterol oxidase and peroxidase method,[3] and LDL-cholesterol (LDL-C) was calculated using Friedewald's equation.[3] CRP was estimated by autoanalyzer Beckman Coulter AU480 latex turbidimetry.[3] Serum ferritin was estimated by ARCHITECT Ferritin assay which is a Chemiluminescent Microparticle Immunoassay for quantitative determination of ferritin in human serum.[3] HbA1c was done by particle-enhanced immunoturbidimetric method (Table 6).[3]

RESULTS

At the completion of study, the data and results obtained were statistically analyzed using SPSS and arranged in the form of tables and charts. Frequency and percentage were used to describe categorical variables such as age group and lifestyle. Independent sample t-test was done to compare numeric variable between two groups. Pearson's correlation coefficient (r) value was calculated to check linear correlation between HbA1c, lipid profile, and inflammatory markers. Difference was considered significant at $P<0.05$.

Majority of person in control group belong to the age group 40–49 years (34.3%) and 50–59 years (30%) (Table 1). Majority of persons in diabetic group belong to the age group of 50–59 years (45%).

Majority (85.7%) of the subjects of control group and diabetic group (86.3%) were Hindus (Figure 1).

Mean HbA1C, serum ferritin, and hs-CRP levels were found to be significantly higher in diabetic group as compared to control group (Table 2).

Mean serum TGL, TC, and LDL-C were found to be significantly higher in diabetic while HDL-C level was found to be significantly lower in diabetic group as compared to control group (Table 3).

Among the diabetic subjects, 58.8% were categorized as high risk, 40% as average risk, and 1.2% as low risk (Table 4).

Among the control group, 1.4% were categorized as high risk, 35.7% as average risk, and 62.9% as low risk (Table 5).

1. A significantly positive correlation was found between hs-CRP and HbA1c, TGL, TC, LDL-C. A significantly negative correlation was found between hs-CRP and HDL-C
2. A significantly positive correlation was found between serum ferritin and HbA1c, TGL, TC, and LDL-C. A significantly negative correlation was found between serum ferritin and HDL-C.

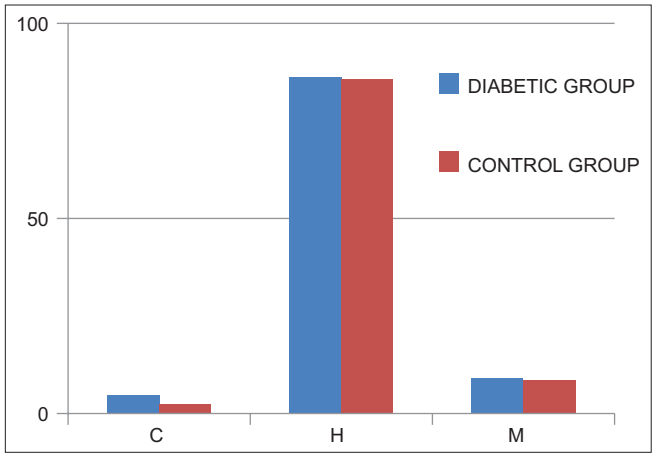

Figure 1: Religious distribution of diabetic and control group

Table 1: Age distribution of control group and diabetic group

Group	Frequency % in control group%	Frequency % in diabetic group
1) 20–29 years	5.7	00
2) 30-39 years	18.6	00
3) 40-49 years	34.3	30
4) 50-59 years	30	45
5) 60 and above	11.4	25

Table 2: Mean HbA1c %, serum ferritin, and hs-CRP in diabetic and control group

Group	Mean HbA1c %	Mean S. Ferritin (ng/mL)	Mean hsCRP (mg/L)
Diabetic	7.69	219.38	3.16
Control	5.25	83.46	1.18
P-value	< 0.001	< 0.001	< 0.001

hs-CRP: High sensitivity C-reactive protein, HbA1c: Glycated hemoglobin

Table 3: Comparison between mean value of total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride in diabetic and control group

Group	TGL (mg/dL)	T.CHOL (mg/dL)	LDL.CHOL (mg/dL)	HDL.CHO (mg/dL)
Diabetic	234.28	204.63	121.42	36.10
Control	179.36	181.82	107.82	41.68
P-value	<0.001	<0.001	<0.001	<0.001

TGL: Triglycerides, LDL: Low-density lipoprotein, HDL: High-density lipoprotein

DISCUSSION

Recently, several evidence has emerged showing a close link between metabolism and immunity. Metabolic and immune pathways have evolved into an interdependent system because they are the most basic requirements for survival. Under conditions of metabolic stress, the integration of metabolism and immunity can become deleterious. The

Table 4: Frequency distribution of CRP in diabetic subjects

hsCRP	Frequency	%
1) ≤1 mg/L	1	1.2
2) 1.0–3.0 mg/L	32	40.0
3) ≥3 mg/L	47	58.8
Total	80	100.0

hs-CRP: High sensitivity C-reactive protein

Table 5: Frequency distribution of CRP in control group

hsCRP	Frequency	%
1) ≤1 mg/L	44	62.9
2) 1.0–3.0 mg/L	25	35.7
3) ≥3 mg/L	1	1.4
Total	70	100.0

hs-CRP: High sensitivity C-reactive protein

Table 6: Correlation between inflammatory markers (hsCRP and serum ferritin) and HbA1c and lipid profile

Group	HbA1c	TGL.	T.CHOL	HDL-C	LDL-C
hsCRP	r=0.802 P<0.001	r=0.886 P<0.001	r=0.856 P<0.001	r=-0.685 P<0.001	r=0.783 P<0.001
S. ferritin	r=0.810 P<0.001	r=0.864 P<0.001	r=0.800 P<0.001	r=-0.572 P<0.001	r=0.714 P<0.001

TGL: Triglycerides, LDL-C: Low-density lipoprotein-cholesterol, HDL-C: High-density lipoprotein-cholesterol, hs-CRP: High sensitivity C-reactive protein, HbA1c: Glycated hemoglobin

anabolic pathways such as insulin signaling pathways can be suppressed in response to inflammation, whereas the catabolic pathways are favored by inflammation. Inflammation indicated by raised serum inflammatory markers has been seen associated with high level of insulin resistance.[8] Thus, the process of inflammation can initiate insulin resistance.

In this study, the mean levels of serum ferritin were higher in the diabetic group as compared to the control group. These findings support the previous studies which concluded that inflammation plays a positive role toward insulin resistance and have found that high ferritin level favors a higher incidence of type-2 diabetes.[8]

It has been seen that female with higher daily iron intake is associated with an increased risk of dyslipidemia with higher TG.[9] Hyperferritinemia is associated with increased oxidized LDL level in pre-diabetic patients suggesting the role of iron-mediated free radical stress in causing dyslipidemia.[10] Serum ferritin level is positively correlated with TG, TC, and LDL-C, waist circumference, BMI, and serum insulin level in non-diabetic population.[11] This suggests that insulin increases iron uptake causing higher body iron stores which then leads to the development of insulin resistance and dyslipidemia by several underlying mechanisms including free radical mediated damages and setting on of chronic inflammatory condition in the body.

Obesity has profound effects on the sensitivity of tissues to insulin and as a consequence on systemic glucose homeostasis. Excess intracellular non-esterified fatty acids (NEFAs) overwhelm the fatty acid oxidation pathways, leading to the accumulation of cytoplasmic intermediates such as diacylglycerol and ceramide. These "toxic" intermediates can activate serine/threonine kinases, which cause aberrant serine phosphorylation of the insulin receptor and insulin receptor substrate proteins which attenuate insulin signaling thus allowing phosphoenolpyruvate carboxykinase to "ramp-up" gluconeogenesis. Excess NEFAs also compete with glucose for substrate oxidation, leading to feedback inhibition of glycolytic enzymes, and thereby further exacerbating the existing glucose imbalance.[2]

Adipokines are a group of hormones secreted by adipocytes. Both pro-hyperglycemic adipokines (e.g., resistin, retinol-binding protein 4), and anti-hyperglycemic adipokines (leptin, adiponectin) have been identified. Leptin and adiponectin improve insulin sensitivity by directly enhancing the activity of the AMP-activated protein kinase (AMPK), an enzyme that promotes fatty acid oxidation, in the liver and skeletal muscle. Adiponectin levels are reduced in obesity, thus contributing to insulin resistance. Notably, AMPK is also the target for metformin, a commonly used oral antidiabetic medication.[2] Fall in adiponectin level is associated with dyslipidemia and its rise contributes to rise in HDL level and fall in TGL by different mechanisms involving several receptors of lipoprotein metabolism as well as lipoprotein lipase. Baseline level of adiponectin has been found to be low in cases of type 2 DM and related inversely to inflammatory markers.[12] Adiponectin reduces oxidative stress, level of inflammatory cytokines, insulin resistance, hepatic gluconeogenesis, and glycogenolysis and increases peripheral utilization of free fatty acid and glucose.[13]

Adipose tissue also secretes a variety of pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-6, and macrophage chemoattractant protein-1, the last attracting macrophages to fat deposits. Studies in experimental models have demonstrated that reducing the levels of pro-inflammatory cytokines enhances insulin sensitivity. These cytokines induce insulin resistance by increasing cellular "stress," which in turn, activates multiple signaling cascades that antagonize insulin action on peripheral tissues.[2]

Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor and transcription factor expressed

in adipose tissue and plays a seminal role in adipocyte differentiation. A class of antidiabetic medications known as thiazolidinediones acts as agonist ligands for PPARγ and improves insulin sensitivity. Activation of PPARγ promotes secretion of anti-hyperglycemic adipokines like adiponectin and shifts the deposition of NEFAs toward adipose tissue and away from liver and skeletal muscle.[2]

Several works have reviewed the association of markers of inflammation with diabetes. Acute-phase reactants, part of the innate immune system, either increase in levels or result in activation of monocytes as well as complement and other effectors systems.

However, now, there is increasing evidence that elevated body iron stores, evaluated by serum ferritin, may be associated with hypertension, dyslipidemia, elevated fasting insulin and blood glucose, and central obesity.

Low-grade inflammation plays an important role not only in the pathogenesis of DM but also has an association with dyslipidemia encountered so commonly in diabetics. The process of inflammation induces hepatic synthesis of various acute phase proteins such as hs-CRP and serum ferritin, which are believed to play a role in insulin resistance as well as atherosclerosis.

Inflammatory markers CRP and GlycA have been seen to decrease insulin secretion as well as insulin sensitivity even before the development of overt DM.[14] This may reveal the hidden link between adiposity associated with rise in CRP followed by the development of Type 2 DM.

It has been seen that inflammatory markers such as CRP, IL-6, and TNF alpha are positively correlated with HbA1c in type 2 diabetic patients showing a pathogenetic link between inflammation and development of insulin resistance.[4]

Serum ferritin level has been found to be associated with rise in TCHOL, TGL, and LDL CHOL but decrease in HDL CHOL level independent of liver and renal function status showing some link between free radical injury mediated by ferritin and lipid peroxidation eventually resulting into dyslipidemia thus initiating a cascade for development of insulin resistance.[5]

HbA1c has been found to be positively correlated with inflammatory markers in the aqueous humor of patients with diabetic cataract.[15] The detrimental effects of rise in blood sugar could be production of advanced glycated end products, causing initiation of inflammation and dyslipidemia, leading to increase in insulin resistance followed by development of diabetes related complications.

Rise in inflammatory cytokines and oxidative stress with reduced serum antioxidant capacity is associated with rise in T CHOL, LDL CHOL, TGL, and fall in HDL CHOL. These factors are also positively associated with aging, obesity, and DM.[16]

Previous study has also demonstrated positive correlation between HbA1c in diabetic patients with several inflammatory markers such as serum ferritin, CRP, and WBC, indicating the role of inflammation as a cause or as sequel of DM.[1]

Limitations of the study

The sample size was quite small and female patients were not included in the study. Confounding factors for iron overload like non-vegetarian diet and consumption of red meat were not taken into consideration in this study.

CONCLUSION

The study revealed that low-grade inflammation was present in diabetic patients and the levels of inflammatory markers are positively correlated with the levels of serum TGL, TC, and LDL-C, while the levels of HDL-C have a negative significant relation with the inflammatory markers.

Ferritin is the marker of iron overload and has a role in insulin resistance. Thus, routine screening for serum ferritin concentration in pre-diabetes patients should be done to assess the body iron stores and the risk of development of diabetic vascular complications by reactive oxygen species.

It was found that uncontrolled DM is significantly associated with dyslipidemia and increased hsCRP level and 58% of diabetic subjects were found to be at high-risk individual for cardiovascular disease.

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RK - Concept and design of the study, preparation, and drafting of manuscript; **SKK -** Coordination, data interpretation, and statistical analysis; **RS -** Data interpretation; **RjK -** Revision of manuscript; **AK -** Overall guidance in scripting the manuscript.

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