ORIGINAL ARTICLE

Inflammatory Markers and Their Significance in Glycemic Control among Type 2 Diabetes Patients

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ABSTRACT

Objective: To compare serum C-reactive protein and Ferritin levels between type 2 diabetes mellitus patients and healthy individuals and also to assess their association with HbA_1clevels.

Study Design: Comparative cross-sectional study

Place and Duration of Study: This study was carried out from March 2024 to October 2024 at the Department of Pathology, Fauji Foundation Hospital, Rawalpindi.

Materials and Methods: Total 300 participants were divided into diabetic and non-diabetic groups. Diabetic group included 195 known type 2 diabetes patients having diabetes for at least 5 years. Non-diabetic group included 105 apparently healthy subjects. Patients having type-1 Diabetes, hemochromatosis, acute or chronic infection/inflammation, hypertension, pregnancy, anaemia, hemoglobinopathy, recent blood loss, blood transfusion/donation or those taking iron supplements were excluded. All demographic and clinical details were noted followed by blood sample collection and Laboratory analysis for serum Ferritin, CRP, fasting plasma glucose and plasma HbA_1c. Results were statistically analyzed on SPSS 22.

Results: The study comprised of 300 participants who were stratified into diabetic and non-diabetic groups. Elevated levels of serum Ferritin and CRP were observed in diabetic patients compared to healthy subjects; serum Ferritin level 165(98.50-190.00) ng/ml vs. 85.00(55.20-105.25) ng/ml (p = 0.036) and serum CRP level 8.50(5.70-11.10) mg/l vs. 2.80(2.30-4.00) mg/l (p <0.001) respectively. Significant positive correlation was also noted between these inflammatory markers and plasma HbA_1c; for serum CRP, r=0.464, p <0.001 and for serum Ferritin, r=0.231, p =0.001.

Conclusion: Our study revealed significantly elevated levels of serum CRP and Ferritin in type 2 Diabetes patients as compared to healthy subjects. Serum CRP and Ferritin are positively correlated with HbA_1c in patients with type 2 diabetes. These findings support the hypothesis that inflammatory markers may reflect glycemic control status in type 2 diabetes patients.

Key Words: *CRP, Ferritin, HbA_1c, Inflammation, Type 2 Diabetes.*

Introduction

Diabetes Mellitus (DM) is a disorder of multi-factorial involvement including genetic and environmental factors that cause either suppressed insulin secretion, action or both; ultimately resulting in hyperglycemia.[1] Pakistan holds 3rd rank in the world regarding prevalence of diabetes following China and India and IDF report has documented that 26.7% adult population in Pakistan is already having diabetes.[1,2] In recent years, a link has been suggested between inflammatory markers and glycemic control in type 2 diabetes (T2D) patients.[3,4] Elevated inflammatory markers like C-reactive protein (CRP) and Ferritin have been attributed to cause insulin resistance, poor glycemic control and other associated complications in diabetic patients.[5,6] Due to the potential implications of these inflammatory markers on T2D management and complications, the current study was planned to evaluate the association between inflammatory markers and glycemic marker in our diabetic population.

CRP is secreted by hepatocytes in response to cytokines released during an infective or inflammatory condition playing an important role in

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defense system of host.[3] Several viral, noninfectious inflammatory states and malignancies also lead to increased serum CRP levels. CRP values rise quickly in two hours and attain a peak at 48 hours, making it an early and reliable marker for monitoring disease.[7] Elevated CRP levels have been documented to be present in T2D patients with poor glycemic control and also have been found to be correlated with plasma HbA_{1c} level in these subjects.[3,5] It is postulated that CRP leads to phosphorylation of serine at insulin receptor, resulting in impaired ability of the receptor to activate phosphatidyl inositol 3-kinase, ending up in the development of insulin resistance and poor glycemic control.[3]

Serum Ferritin level is routinely used to reflect the iron stores in individuals but it also serves as an important acute phase reactant indicating inflammation. High serum Ferritin levels have been associated with hyperglycemia in T2D patients and have also been linked to increased frequency of chronic complications of T2D such as retinopathy, nephropathy, and vascular dysfunction.[4,8] It is suggested that hyperglycemia leads to increased glycation of hemoglobin in red cells releasing free iron from them. Free iron then initiates redox reactions leading to free radical synthesis that damages β-cells of pancreas and further augments the inflammatory response.[6] Damage to β-cells contributes to decreased insulin secretion and abnormally high blood glucose levels. It is also documented that high Ferritin may influence the expression of glucose transporters (GLUT4) in muscles and adipose tissue. This may influence signaling pathways related to glucose uptake and may disturb GLUT4 translocation to the cellular membrane with decreased entry of glucose into the cells and hyperglycemia.[9]

Available literature suggests an important role of inflammatory markers in glucose regulation among T2D patients, although their cause and effect relationship is still not fully known. In a research carried out in Pakistan, influence of serum Ferritin on glycemic control was studied in T2D patients and significantly elevated Ferritin levels were found to be associated with poor glycemic control 19. International literature from India, Ethiopia and Egypt also revealed markedly high serum Ferritin and CRP levels in T2D patients when compared to their healthy counterparts [3, 4, 5]. Kant and colleagues and Elimam and colleagues also studied correlation of inflammatory markers Ferritin and CRP with HbA1c and documented a positive correlation between these markers, indicating the involvement of inflammation in pathogenesis of diabetes.[3,5] A Quasi-experimental study conducted in Iran investigated serum Ferritin levels in T2D patients with poor glycemic control and reported significant decrease in Ferritin levels by controlling hyperglycemia after the study.[10]

In the light of growing evidence associating inflammation to the metabolic regulation in T2D and scarcity of local literature on this subject, this study was aimed to estimate serum Ferritin and CRP levels in T2D patients and to determine the their association with HbA_1c. We hypothesized that elevated serum CRP and Ferritin levels occur in T2D patients and are correlated with high HbA1c levels in them. Our study underscores the potential of utilizing these inflammatory biomarkers as indicators of metabolic dysregulation and disease severity in T2D patients.

Materials and Methods

This comparative cross-sectional study was conducted at Pathology Department of Fauji Foundation Hospital Rawalpindi from March 2024 to October 2024 after getting approval from Institutional Ethical Review Committee (No. 701/RC/FFH/RWP). The 300 participants included in this study were recruited from medical outpatient department through non-probability consecutive sampling. We stratified the study participants into two groups based on whether they were diagnosed with type 2 diabetes or not. “Diabetic group” comprised of 195 T2D patients who were already diagnosed by the physician, fulfilling American Diabetes Association (ADA) criteria [11] and had T2D for minimum 05 years. Second group labeled as “non-diabetic group” included 105 age and sex-matched healthy subjects. Sample size was estimated through WHO sample size calculator with type 2 diabetes prevalence in Pakistan 26.7%, confidence level 95% and margin of error 5% [1].

Exclusion criteria included age of the subjects < 18 years or >65 years, duration of T2D less than 5 years and medical evidence or history of any of the following condition: type-1 Diabetes mellitus,

hemochromatosis, acute or chronic infection/inflammation, hypertension, pregnancy, recent blood loss, intake of iron supplements or anti-inflammatory drugs, history of blood transfusion or donation during past three months, history of any hemoglobinopathy or presence of anemia.

After getting written informed consent of the subjects, demographic and clinical details were recorded on a structured questionnaire. Blood samples were then collected using EDTA tube for HbA_1c and blood complete picture (CP) while blood specimen in gel separator tube was used for performing serum Ferritin and CRP. Blood for fasting plasma glucose (FPG) was collected in sodium fluoride tube. Blood CP was performed on fully automated hematology analyzer (Beckman Coulter). Plasma HbA_{1c} was performed by immuno-turbidimetric technique on fully automated chemistry analyzer (Atellica CH by Siemens, Germany). FPG was also measured on same chemistry analyzer by hexokinase method.

Samples collected in gel separator tube were initially placed at room temperature, followed by centrifugation at 3000 rpm for serum separation. Serum was utilized for estimating Ferritin by Chemiluminescence immunoassay (Atellica IM by Siemens, Germany) and for quantitative estimation of CRP by enzyme-linked immunosorbent assay (MicroLISA by Amgenix International, USA; sensitivity: 0.01 mg/l, linearity: 160 mg/l). Intra-assay precision was also checked by randomly selecting few samples and running them along with their duplicates for calculating their mean, standard deviation and percent coefficient of variation (CV %). All samples were analyzed along with the routine Laboratory tests after checking daily internal quality control results. Laboratory technicians were blinded and were unaware of the groups assigned to the samples.

Diagnosis of patients with T2D was already done by the Physician according to ADA guidelines having either plasma HbA_1c level > 6.5%, FPG >7.0 mmol/l, post-prandial blood glucose > 11.0 mmol/L or 2hr blood glucose level > 11.0 mmol/L after 75g OGTT [11]. Based on ADA guidelines, FPG level ≥ 5.6 mmol/l in healthy subjects and ≥ 7.0 mmol/l in T2D patients was considered elevated while HbA_1c level ≥ 5.7% in healthy individuals and ≥ 6.5% in diabetic patients was considered elevated [11]. According to WHO guidelines, serum Ferritin level > 200ng/ml was considered elevated for males while serum Ferritin > 150ng/ml was taken elevated for females [12]. Serum CRP level > 6.0 mg/L was considered elevated [13]. Hemoglobin (Hb) level <12g/dl in females or <13 g/dl in male participants was defined as anemia according to WHO [14].

Data were analyzed on SPSS version 22. Data was assessed for distribution by Kolmogorov-Smirnov Test of Normality. Median and inter-quartile ranges were calculated for variables with non-parametric distribution. For categorical variables, frequency and percentage (%) was calculated. Categorical variables were compared across the study groups by Chi square test. Continuous biochemical and clinical parameters were compared between the study groups by Mann Whitney U test. Correlation of serum CRP and Ferritin with HbA_1c was determined by Spearman correlation. The probability value (p) was significant when it was less than 0.05.

Results

There were 300 participants in this study which were divided into two groups: diabetic and non-diabetic. Diabetic group comprised of 195 known patients with T2D while non-diabetic group included 105 non-diabetic healthy subjects. Median age of the study participants was 55 years. In diabetic group, 136(69.7%) were females and 59 (30.3%) were male subjects while in non-diabetic group, 73(69.5%) were female and 32(30.5%) were male. Since we included age and sex-matched subjects in both groups, no significant difference was noted in age and gender between the two study groups as shown in Table I.

In our study, 90(46.7%) diabetic patients had 5-10 years of disease duration, followed by 57(29.2%) subjects having 10-15 years duration, 24(12.3%) patients were diabetic for more than 20 years and 23(11.8%) were diagnosed with diabetes for 15-20 years. Among the participants, 105 (53.8 %) diabetic patients exhibited poor glycemic control as reflected by raised plasma HbA_1c levels while only 7 (6.7%) non-diabetic subjects had abnormal HbA_1c level (p<0.001). Similarly 111(56.9%) diabetic patients had raised fasting plasma glucose levels while only 11(10.5%) non-diabetic subjects had abnormal FPG levels (p<0.001). Serum CRP and serum Ferritin levels

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which indicate inflammation were also markedly elevated in diabetic group as compared to non-diabetic group. CRP was high in 53 (27.2%) diabetics while serum Ferritin was raised in 26 (13.3%) diabetic patients in this study (p<0.001). Comparison of frequencies of these variables by Chi square test is shown in Figure 1.

Fasting plasma glucose and plasma HbA_1c levels were significantly elevated among T2D patients than in healthy subjects in our study (median values 8.70 mmol/l vs. 4.60 mmol/l for FPG and 7.5% vs. 5.1% for HbA_1c respectively). On comparing median values of fasting plasma glucose and HbA_1c by Mann Whitney U test, significant difference was observed between diabetic and non-diabetic groups (Table II).

Upon investigating the levels of inflammatory markers in study groups, higher levels of serum Ferritin and CRP were noted in subjects having T2D compared to the non-diabetic healthy individuals (median levels of 165 nmol/l vs. 85 nmol/l for serum Ferritin and 8.50 mg/l vs. 2.80 mg/l for serum CRP respectively). Comparison of median values of these parameters by Man Whitney U test also showed significant difference between the two groups (Table II).

In order to determine the occurrence of any linear relationship between HbA_1c and inflammatory markers in T2D patients, Spearman correlation coefficient test was applied. It was revealed that among diabetic patients, a weak but statistically significant positive correlation was found between serum Ferritin and HbA_1c ($r = 0.231$, $p = 0.001$) while a moderate correlation was observed between serum CRP and HbA_1c ($r = 0.464$, $p < 0.001$), as displayed in Table III.

Discussion

Diabetes mellitus has become a very common health issue nowadays and the number of affected individuals is exponentially increasing with each passing year. Inflammatory markers have been attributed to be involved in the pathogenesis of T2D, since they may lead to decreased insulin sensitivity, poor glycemic control and associated complications.[5] Our study estimated serum Ferritin and CRP levels in T2D patients and age-matched healthy subjects. We also determined correlation of these inflammatory markers with HbA_1c in T2D patients.

In the current study, serum CRP level was significantly higher in T2D patients than in healthy

Table I: Comparison of Age and Gender Distribution between the Study groups (n=300)

Variables		Diabetic group N= 195	Non-diabetic group N= 105	*p*-value
Age (years) Median(IQR)		55.00 (51.00-67.00)	55.00 (50.00-64.00)	0.121
Gender n (%)	Male	59 (30.3%)	32 (30.5%)	0.487
	Female	136 (69.7%)	73 (69.5%)	

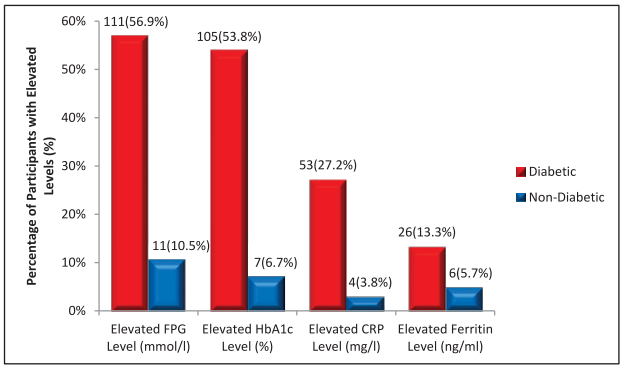

Figure 1: Frequency of Elevated Glycemic and Inflammatory Biomarkers in Diabetic vs. Non-Diabetic Groups.

Table-II: Comparison of Median and Inter-quartile Range (IQR) of Biochemical Parameters between study groups (n=300)

Variables	Study Groups		*p*-Value
	Diabetic (n=195) Median(IQR)	Non-Diabetic (n=105) Median(IQR)	
Fasting Plasma Glucose (mmol/l)	8.70 (6.50 - 13.00)	4.60 (4.20 - 4.90)	<0.001*
Plasma HbA_1c (%)	7.50 (7.00 - 9.30)	5.10 (4.80 - 5.40)	<0.001*
Serum Ferritin (ng/ml)	165.00 (98.50-190.00)	85.00 (55.20 - 105.25)	0.036*
Serum CRP (mg/l)	8.50 (5.70 - 11.10)	2.80 (2.30 - 4.00)	<0.001*

*p <0.05: significant

Table-III: Correlation of serum Ferritin and CRP Levels with plasma HbA_1c in T2D patients.

	Plasma HbA_1c	
	r	*p*-Value
Serum Ferritin	0.231	0.001
Serum CRP	0.464	<0.001

r = Spearman correlation coefficient; 0.00-0.19: very weak, 0.20-0.39: weak, 0.40-0.59: moderate, ≥ 0.60: strong

subjects. Similar to our finding, significantly higher CRP levels in T2D patients have been documented in several other studies.[5,13,15,16] Patne, Hisalkar and Dubey determined the association between CRP and HbA_1c in T2D patients and noted significantly elevated CRP levels in them.[17] This can be due to the fact that CRP can impart indirect effect on insulin sensitivity and its production from beta cells of pancreas by altering immune response through augmenting systemic inflammation.[13] A study by Lima and colleagues, however, contradicted our finding as they did not report a significant difference of serum CRP levels between diabetic and healthy individuals.[18] This discrepancy can be due to the difference in ethnicity or inclusion of diabetic patients with variable duration of disease which can cause variable degree of inflammation.

Significantly elevated serum Ferritin levels were also noted in T2D group than in non-diabetic group in our study. Consistent to our results, a research carried out in Pakistan also reported significantly elevated Ferritin levels in T2D patients with poor glycemic control.[19] International literature also revealed markedly high serum Ferritin levels in T2D patients compared to their healthy counterparts in studies conducted across India, Ethiopia and Egypt.[3,4,5]

A strong positive correlation between CRP and HbA_1c was found in diabetic patients in the current research. Similar relationship has been noted between CRP and glycated hemoglobin in studies conducted across the globe. A research conducted in Menoufia University, Egypt observed a positive correlation between CRP and HbA_1c in diabetics which is consistent with our results.[5] Likewise, studies done by Gautam and colleagues in Nepal and another study by Patne and colleagues in India assessed the relationship between inflammatory and glycemic control markers among T2D population and concluded a positive correlation between serum CRP and HbA_1c in these subjects.[13,17] This significant positive correlation indicates that increase in CRP level may be associated with elevated HbA_1c level and hence poor glycemic control in T2D patients. Therefore serum CRP can be postulated to serve as a useful tool in determining glycemic control and possible risk of complications in diabetic population. However, further studies are needed to validate this idea.

Serum Ferritin exhibited a significant positive correlation with HbA_1c levels among T2D patients in our study indicating that increased serum Ferritin is associated with increased plasma HbA1c level and hence poor glycemic control in these individuals. Supporting our results, Bayih et al and Elimam H et al also reported significant positive correlation between serum Ferritin and HbA_1c in their studies conducted in Ethiopia and Egypt respectively.[4,5] Similarly, Shubham J, et al. conducted a study in India to determine serum Ferritin level and its correlation with HbA1c in diabetic individuals and concluded a significant positive correlation between them.[8] This correlation can be attributed to the synthesis of advanced glycosylation end products (AGEs) which can occur due to hyperglycemia, since hyperglycemia leads to the release of free iron and other elements that have toxic potential associated with oxidative stress and inflammation [5]. Our finding contradicts the results reported by Al Argan and colleagues, who determined association between Ferritin and HbA_1c in Saudi population. In their study, they observed significantly raised serum Ferritin levels in diabetic patients similar to our study but contrary to us, they did not find any significant correlation between serum Ferritin and HbA_1c.[20] This difference can be attributed to the different ethnic origins, difference in duration of diabetes having different degree of inflammation or variable anti-diabetic regime given to the patients in both studies.

The findings of our study, consistent with the existing literature, suggest an association of inflammatory markers serum Ferritin and CRP with HbA_1c among T2D patients. These findings highlight the important role of monitoring inflammatory markers in diabetic patients. Elevated levels of inflammatory markers may be employed as indicators of poor glycemic control in these subjects. This may also help in monitoring diabetes worsening and progression towards its complications. Our findings also provide an insight for treating physicians and pharmaceuticals to consider inflammatory markers as a possible therapeutic target for better glycemic control in diabetic population. More prospective researches in this aspect are still needed to explore the underlying causative mechanisms and to validate the potential role of inflammation in glycemic control and disease outcomes in diabetic population.

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There were few limitations of our study, majority of the participants in this study were of female gender because our hospital mainly caters for the families of the ex-service men. Secondly, adjustment for the key confounding factors especially body mass index and metabolic syndrome could not be done in our study. Further prospective, multi-centered studies comprising of both genders along with adjustment of key confounding factors and including larger panel of inflammatory markers are needed across the national and international level to strengthen this idea.

Conclusion

Significantly elevated levels of serum Ferritin and CRP occur in type 2 Diabetes patients as compared to healthy subjects. Furthermore, a significant positive correlation exists between inflammatory markers; serum Ferritin and CRP, and glycemic marker plasma HbA_1c in T2D patients. These findings suggest an important role of inflammation in glycemic control among diabetics.

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Disclaimer

The abstract has not been previously presented or published in any conference. The manuscript was not part of a research, PhD or thesis project, or any other relevant information.

Conflict of Interest

Authors declare no conflict of any financial, personal or professional interest.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.