ORIGINAL ARTICLE

Levels of Inflammatory Markers and their Correlation with Dyslipidemia in Diabetics

Palvasha Waheed, Abdul Khaliq Naveed and Fareeha Farooq

ABSTRACT

Objective: To measure the levels of inflammatory markers (serum ferritin and high sensitivity C-reactive protein) and dyslipidemia in diabetics and to find a correlation between these inflammatory markers and dyslipidemia.

Study Design: Comparative study.

Place and Duration of Study: Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi, from March 2007 to February 2008.

Methodology: The study included 30 known type-2 diabetic patients randomly inducted from diabetic clinics of Rawalpindi. Healthy volunteers (n=30) having blood glucose less than 6 mmol/L were inducted as the comparison group. Fasting blood samples of diabetics and controls were analyzed for glucose, glycated hemoglobin (HbA1c), lipid profile, high sensitivity C-reactive protein (hs-CRP) and serum ferritin.

Results: The diabetic subjects had significantly higher levels of glucose, HbA1c, total cholesterol, LDL cholesterol, triglycerides, hs-CRP and ferritin as compared to normal subjects ($p<0.001$), while the level of HDL cholesterol was significantly lower in diabetics ($p<0.001$). Furthermore, a significant positive correlation was found between the inflammatory markers, hs-CRP and ferritin, and the parameters of dyslipidemia i.e. total cholesterol, LDL cholesterol and triglycerides ($p<0.001$ $r=0.72$) except for HDL cholesterol, which had an insignificant negative correlation with the inflammatory markers ($p>0.05$ $r=-0.10$).

Conclusion: Low-grade inflammation exists in Diabetes mellitus and it is positively related with dyslipidemia (except for HDL cholesterol) in diabetics.

Key words: *Diabetes mellitus. Serum ferritin. High sensitivity C-reactive protein. Dyslipidemia.*

INTRODUCTION

The main abnormality seen in type-2 Diabetes Mellitus (DM) is abnormal glucose metabolism and it is believed that the pathogenesis of type-2 DM is mainly linked to disordered lipid metabolism.[1] Abnormal lipid profile is often seen in patients with diabetes because insulin regulates several steps of lipid metabolism. Dyslipidemia in diabetics is often characterized by elevated fasting and postprandial levels of serum triglycerides, total cholesterol and LDL cholesterol and a significant decrease in the HDL cholesterol levels.[2]

Low-grade inflammation plays an important role not only in the pathogenesis of Diabetes mellitus but also has an association with dyslipidemia encountered so commonly in the 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.[3-5] Higher incidence of type-2 diabetes has been observed in subjects with high levels of serum ferritin.[6,7] Serum ferritin concentration is found to have a positive correlation with plasma triglycerides, LDL cholesterol and apolipoprotein-B concentrations, and is negatively correlated with HDL cholesterol.[8] Various studies show that elevated levels of CRP is a risk factor for the development of insulin resistance, Diabetes mellitus and cardiovascular disease.[9-11] It is believed that there is a positive correlation between CRP and lipids like total cholesterol and triglycerides and also between CRP and LDL cholesterol, whereas a negative correlation is believed to exist between CRP and HDL cholesterol.[12,13]

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

The study was aimed to determine if inflammation and dyslipidemia existed in diabetic population and if so, to find out the relationship between inflammatory markers and the various parameters of lipid profile.

METHODOLOGY

A comparative study was conducted at the Department of Biochemistry and Molecular Biology, Army Medical

Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi.

***Correspondence:** Brig. Abdul Khaliq Naveed, Professor and Head of Department of Biochemistry and Molecular Biology, Army Medical College, Abid Majeed Road, Rawalpindi. E-mail: khaliqnaveed2001@yahoo.com*

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College, Rawalpindi. Non-probability convenience sampling was carried out to enroll patients from diabetic clinics of Military Hospital and Holy Family Hospital, Rawalpindi. The study was conducted from March 2007 to February 2008. A total number of 60 samples were included in the study and divided into the diabetic group (n=30) and the control group (n=30).

Inclusion criteria were known cases of type-2 diabetics, aged between 18-65 years, with a body mass index between 19-40 kg/m^2 and a history of more than 5 years of diabetes. Age and gender matched healthy volunteers having fasting blood glucose level < 6 mmol/L were inducted in the control group.

Patients having confounding co-morbidities like chronic liver disease, cardiomyopathies etc, end-stage renal disease (creatinine clearance < 10 ml/min), pregnancy, anemia (Hb < 12 mg/dL as per WHO classification) or taking medications for painful inflammatory conditions like osteoarthritis, gout etc. were excluded from the study.

Venous blood of 8.0 ml was drawn under aseptic conditions. Plasma glucose was estimated by enzymatic colorimetric method using glucose oxidase enzyme to oxidize glucose (levels <6.0 mmol/L were considered normal).[14,15] Glycated HbA1c was determined by column chromatography with cation-exchange resins (values <6.5% considered normal).[16] Serum triglycerides were estimated by enzymatic colorimetric method using glycerol kinase to act on glycerol released after the hydrolysis of triglycerides (levels <1.7 mmol/L taken as normal).[17,18] Total cholesterol was measured by enzymatic colorimetric method using cholesterol oxidase, (values <5.16 mmol/L considered normal).[19,20] HDL cholesterol was measured by direct method, employing cholesterol oxidase in the first step and a surfactant acting on the HDL in the second step (level >1.42 mmol/L in males and >1.68 mmol/L in females were taken as normal).[21] LDL cholesterol was determined by using Polyvinyl Sulphates (PVS) method (values <2.5 mmol/L considered normal).[22] Serum ferritin was measured by Ferritin kit using a solid-phase, two-site chemiluminescent immunometric assay (males with levels between 20-250 ng/ml and females with 10-150 ng/ml were considered normal). High sensitivity CRP was measured using quantitative turbidimetric method (normal value ranged 0.2-0.7 mg/L).

Data was analyzed on SPSS version 15. Mean and Standard Deviation (SD) were used to describe numeric variables like age, glycemic status, lipid profile and inflammatory markers. Frequency and percentages were used to describe categoric variables like gender. Independent samples 't' test was used to compare numeric variables between the two groups. Pearson's correlation coefficient (r) values were calculated to check the linear correlations between lipid profile and inflammatory markers. Differences were considered significant at $p<0.05$.

RESULTS

The mean ± SD value for blood glucose in diabetics was 10.1±1.90 mmol/L and in controls, it was 4.45±0.62 mmol/L. The mean HbA1c in diabetics was 7.0±1.28 percent, while in normal controls, it was 5.41±0.63 percent.

The serum cholesterol level in diabetics (mean ± SD) was 6.93±1.88 mmol/L and in controls 3.87±0.87 mmol/L. The mean triglyceride level in diabetics was 4.39±1.21 mmol/L while it was 1.14±0.37 mmol/L in controls. The mean level of LDL cholesterol in diabetics was 3.39±0.79 mmol/L as compared to controls which was 2.32±0.80 mmol/L. HDL cholesterol was lower in diabetics with a mean ± SD value of 0.96±0.11 mmol/L and 1.10±0.18 mmol/L in controls.

Mean ± SD for serum ferritin in diabetics was 272.53±1.87 ng/ml as compared to normal controls which was 91.30±1.78 ng/ml. In diabetics, the mean ± SD of hs-CRP was 5.09±0.16 mg/L and in controls 1.0±0.26 mg/L.

A significantly positive correlation was found between serum ferritin and total cholesterol (r=0.80), triglycerides (r=0.68), and LDL cholesterol (r=0.73) in diabetics. A negative but insignificant correlation was found between serum ferritin and HDL cholesterol (r=-0.24) in diabetics (Table I).

Pearson's correlation coefficient values between hs-CRP and total cholesterol (r=0.72), triglyceride levels (r=0.58) and LDL cholesterol (r=0.72) were significantly positive in diabetics but an insignificant negative correlation was found between hs-CRP and HDL cholesterol (r=-0.10) in diabetics (Table I).

Table I: Showing correlation coefficients between inflammatory markers (serum ferritin and hs-CRP) and dyslipidemia in diabetics.

Variables		Ferritin	hs-CRP
Cholesterol	r^1	0.80 **	0.72 **
	p^2	< 0.001	< 0.001
Triglycerides	r^1	0.68 **	0.58 **
	p^2	< 0.001	0.001
LDL Cholesterol	r^1	0.73 **	0.72 **
	p^2	< 0.001	< 0.001
HDL Cholesterol	r^1	-0.24 *	-0.10 *
	p^2	> 0.05	> 0.05

r^1= Pearson's correlation coefficient; p^2= probability value.
** p>0.05 = not significant; ** p ≤ 0.001 = very highly significant*

DISCUSSION

Recently, a large amount of evidence has emerged showing a close link between metabolism and immunity. The metabolic and immune systems are the most basic requirements across the animal kingdom. Perhaps, this

is the reason why metabolic and immune pathways have evolved into an interdependent system. The integration of metabolism and immunity, which is beneficial for the body under normal conditions, can become deleterious under conditions of metabolic stress. The anabolic pathways, such as insulin signaling pathways can be suppressed in response to inflammation, whereas the catabolic pathways are favoured by inflammation.[23] 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 conclude that inflammation plays a positive role towards insulin resistance and have found that high ferritin levels favour a high incidence of type-2 diabetes.[6,7]

Pradhan *et al.* found CRP to be a risk factor for the development of insulin resistance and Diabetes mellitus.[10] In other studies, elevated levels of CRP were found to be associated with raised incidence of Diabetes mellitus.[9,11] This study supports these findings as the levels of hs-CRP were significantly higher in the diabetic group.[9-11]

The deficiency of insulin or insulin resistance is invariably associated with the release of excess Free Fatty Acids (FFA) in the postabsorptive and post-prandial states.[24,26] In this study, the levels of triglycerides, total cholesterol and LDL cholesterol were significantly higher in diabetics, whereas the level of HDL cholesterol was higher in the control group. These findings are supported by various studies.[1,2,27]

In 1997, van Jaarsveld *et al.*, found that LDL-cholesterol, apo-B and LDL particles were elevated with increasing ferritin concentrations.[28] A year later, another study conducted by Fernandez-Real *et al.*, found a positive correlation between serum ferritin concentration with plasma triglycerides and apolipoprotein-B concentrations, and a negative correlation of serum ferritin with HDL cholesterol.[8] Sung *et al.*, found levels of CRP and dyslipidemia to be interrelated with each other.[13] Recent studies have also found a positive correlation between CRP and total cholesterol as well as triglyceride levels.[12] In this study, the levels of serum ferritin and hs-CRP were found to have a significant positive association with the levels of triglycerides, total cholesterol and LDL cholesterol, while no significant correlation could be established between HDL cholesterol and inflammatory markers.

CONCLUSION

The study revealed that low-grade inflammation is present in diabetic patients and the levels of inflammatory markers are positively correlated with the levels of serum triglycerides, total cholesterol and LDL cholesterol, while the levels of HDL cholesterol do not have a significant relation with the inflammatory markers.

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