NOVEL INFLAMMATORY MARKERS OF DIABETES MELLITUS AND CORONARY HEART DISEASE

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ABSTRACT

Background: Coronary heart disease is one of the life threatening cardiovascular complication of type 2 diabetes mellitus. C-reactive protein (CRP) and fibrinogen are considered as basic systemic inflammatory biomarkers. Aim of the present study is to find out the alterations in CRP and fibrinogen levels in type 2 diabetic patients with and without coronary heart disease (CHD) belonging to Khyber Pakhtunkhwa and to find any possible relation of these inflammatory markers with glycosylated hemoglobin and lipid profile.

Methodology: This is a comparative study. 100 patients of Group A having diabetes mellitus without CHD and 100 patients of Group B having diabetes mellitus with CHD were randomly enrolled from Khyber Teaching Hospital and Hayatabad Medical Complex, Peshawar. Serum CRP was detected by ELISA and fibrinogen was measured through clotting method. Data was analyzed using statistical software SPSS 19.

Results: The mean hs-CRP and fibrinogen values were significantly raised in patients having type 2 diabetes mellitus with CHD than type 2 diabetic patients without CHD ($P < 0.05$). Similarly; fasting blood sugar (FBS) and glycosylated hemoglobin (HbAlc) were also significantly higher with $P < 0.05$ in diabetic patients with coronary heart disease than diabetic patients without coronary heart disease. CRP showed a significant positive association with FBS and HbAlc ($P<0.05$). Fibrinogen level showed a strong positive association with triglycerides ($P<0.001$), total cholesterol and BMI ($P < 0.05$).

Conclusion: It can be concluded from the results that hs-CRP and fibrinogen levels are higher in type 2 diabetic patients with coronary heart disease as compared to those without CHD.. Moreover, CRP and fibrinogen are associated with poor glycemic control and deranged lipid profile in the studied population.

KEYWORDS: Type 2 diabetes mellitus, Coronary heart disease, hs-CRP, Fibrinogen, HbAlc and Lipid profile

INTRODUCTION

Diabetes mellitus is one of the commonest metabolic disorders characterized by chronic hyperglycemia due to relative or absolute deficiency of insulin secretion or its actions or both. It comprises of abnormalities in carbohydrate, protein and fat metabolism[1-3]. Diabetes mellitus is increasing globally with the prevalence of about 10% in adult Pakistani population. If proper measures are not taken, it is expected to affect 333 million people worldwide by 2025 and much of this epidemic increase would occur in the developing countries like Pakistan[4,5].

Coronary heart disease (CHD) affects type 2 diabetic subjects disproportionately as compared to non-diabetic subjects. CHD will become the leading cause of morbidity and mortality in type 2 diabetic population by the year 2020[6]. Endothelial dysfunction, inflammation and increased thrombotic tendency are suggested to be the main hallmarks in the development of coronary heart disease in type 2 diabetes mellitus[7-9].

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Classical risk factors such as cigarette smoking, obesity, hypertension and dyslipidemia cannot fully explain the increased incidence of CHD in T2DM which has led researchers to identify some new inflammatory biomarkers. High sensitivity C-reactive protein (hs-CRP) is the first acute phase reactant protein and is synthesized by the liver. It is a sensitive systemic marker of inflammation, tissue damage and various malignancies[10,11]. Elevated level of serum CRP is considered a strong indicator of CHD in type 2 DM due to its role in atherosclerosis[12,13] which includes:

- Enhancing the release of tissue factor from macrophages
- Activating the complement system
- Binding with LDL-C and VLDL-C leading to their aggregation

The association between CHD and homeostatic mechanism makes it necessary to investigate defects in coagulation cascade. Fibrinogen a 340 KDa (kilo Dalton) molecule secreted by hepatocytes is an acute phase reactant and acts as a natural substrate for thrombin[14]. Fibrinogen plays a key role in the processes of inflammation, hemostasis, tissue repair and angiogenesis. It mediates coagulation cascade and inflammation by: (i) formation of fibrin by thrombin (ii) modulating adhesion of neutrophils, platelets and endothelial cells to fibrino-

gen at the site of injury (iii) attracting different proteins and cells e.g fibroblastic growth factor 2 and vascular endothelial cell growth factor at the site of inflammation[15]. It is incorporated in the lesion and converted into fibrin and fibrinogen degradation products which bind with LDL, leading to production of atherosclerotic plaque. High circulating levels of fibrinogen are reported in type 2 diabetic patients with insulin resistance[16-18].

Circulating levels of CRP and fibrinogen can be brought down through lifestyle changes such as regular exercise, weight loss, cessation of tobacco smoking and alcohol use. This leads to a possibility that serial measurement and modification of blood CRP and fibrinogen may help in the prediction and prevention of coronary heart disease in high risk type 2 diabetic subjects[19].

In Khyber Pakhtunkhwa there is a lack of literature regarding CRP and fibrinogen, the two emerging non-classical cardiovascular risk factors, in type 2 diabetic subjects with and without coronary heart disease. The aim of the present study is to investigate the levels of these inflammatory markers in the diabetic subjects of KPK.

AIMS AND OBJECTIVES

1. To determine serum CRP and fibrinogen levels in type 2 diabetic patients with and without CHD
2. To compare the level of these inflammatory markers with glycemic control in type 2 diabetics with and without CHD.
3. To find any possible relationship of these inflammatory markers with glycosylated hemoglobin and lipid profile in the studied subjects.

MATERIALS AND METHODS

A cross-sectional/ analytical study was performed among the outdoor and indoor patients of Khyber Teaching Hospital (KTH) and Hayatabad Medical Complex (HMC), Peshawar. Biochemical analysis was carried out in the Research Laboratory of Biochemistry Department, Khyber Medical College Peshawar. Study population was divided into two groups. Group A comprised of 100 patients having type 2 diabetes mellitus for at least 4 years and Group B comprised of 100 type 2 diabetic patients with CHD, who had first attack of myocardial infarction in the last 10 days. Patients with thyroid disorders, liver dysfunction, inflammatory diseases and those using lipid lowering drugs and oral contraceptive pills were not included in the study. The study was approved by Institutional Ethical Research Board (IERD) of Khyber Medical College, Peshawar.

Venous blood was obtained after an overnight fasting under aseptic techniques for measuring fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG), High density lipoprotein cholesterol (HDL-C), glycosylated hemoglobin (HbAlc), hs-CRP and plasma fibrinogen.

FBS and lipid profile were estimated by enzymatic colorimetric method on semi auto chemistry analyzer Metrolab 1600 DR on the kits provided by Eli Tech diagnostics of France.

Glycosylated Hemoglobin was determined by Ion exchange resin colorimetric method using kit provided by Human Diagnostic of Germany. Serum C-reactive protein was detected by kit provided by BioCheck USA on Elisa Reader Bio Tek ELX-800.

Plasma fibrinogen was measured by clotting method using kit of FIBRI-PRESET Diagnostic Stage S.A.S of France.

Statistical analysis

Data was analyzed with SPSS version 19. Results were expressed as mean ± SD (standard deviation). Comparison of variables between the groups was done using student's t test. Results having p value <0.05 were considered significant. Association of hs –CRP and fibrinogen with different variables was found using Pearson correlation coefficient r.

RESULTS

Table 1.1 shows the demographic, clinical and biochemical characteristics of group A (diabetics) and group B (diabetics with coronary heart disease). Group A contained 100 participants with mean age of 56.49± 5.78. Similarly Group B contained 100 participants with mean age of 58.72± 5.09. Group B shows slight increase in age with a significant P value < 0.05. FBS level was significantly higher in patients of Group B than Group A with a P value of < 0.05. Group B also showed poor glycemic control as compared to Group A with significant P value of < 0.05.

High levels of hs-CRP and fibrinogen values were also seen in Group B patients as compared to Group A with a significant p value of < 0.05.

Table 1.2 shows that the level of serum CRP is significantly ($P<0.05$) raised in the patients of group B with both poorly controlled (>7%) and fairly controlled (≤7%) HbA1c level sub group as compared to the group A patients.

Table 1.3 shows that the level of serum fibrinogen is high but not with significant P value in group B than group A with poorly controlled (>7%) HbA1c while it is significantly ($P<0.05$) raised in group B with fairly controlled (≤7%) HbAlc as compared to group A patients.

Table 1.4 shows correlation of hs-CRP and fibrinogen with different parameters in studied population. There is significant ($P<0.05$) positive association of hs-CRP with FBS and HbAlc in both groups. On looking for association of fibrinogen with BMI and triglycerides, a strong significant ($P<0.01$) positive association was

Table 1.1 Demographic, clinical and biochemical characteristics of the two groups

Parameters	Group-A	Group-B	P-value
	Mean±SD	Mean±SD	
Age (yrs)	56.49+5.78	58.72+5.09	<0.05
SBP (mmHg)	156.75+21.07	153.30+22.92	NS
DBP (mmHg)	93.15+10.43	93.70+11.62	NS
BMI (kg/m2)	28.03+3.28	28.30+3.18	NS
FBS (mg/dL)	175.61+59.13	213.80+91.62	<0.05
HbA1C (%)	8.308+3.03	10.166+3.452	<0.05
TC (mg/dL)	272.06+121.15	279.61+151.82	NS
TG (mg/dL)	290.02+203.23	324.37+142.60	NS
HDL-C (mg/dL)	44.32+15.24	40.67+16.53	NS
LDL-C(mg/dL)	171.58+125.13	176.09+154.09	NS
CRP (mg/L)	12.67+6.16	18.43+4.22	<0.05
Fibrinogen (mg/dL)	413.44+139.75	478.63+165.97	<0.05

Note: significant P value =<0.05, NS= non-significant

Table 1.2 Comparison of serum hs-CRP levels in both groups

HbA1C	Group-A	Group-B	P-value
	Mean+SD	Mean+SD	
Poorly glycemic controlled >7 %	13.48±5.88	18.09±3.69	< 0.05
Fairly glycemic controlled ≤ 7%	11.54±6.43	19.39±5.43	< 0.05

Table 1.3 Comparison of serum fibrinogen levels in both groups

HbA1C	Group-A	Group-B	P-value
	Mean+SD	Mean+SD	
Poorly glycemic controlled >7 %	430.74±144.29	480.85±165.31	NS
Fairly glycemic controlled ≤ 7%	389.55±131.17	472.31±170.97	<0.05

Table 1.4 Correlation of CRP and fibrinogen with different parameters in studied groups

Parameters	Group-A	Group-B
	CRP	Fibrinogen
Age (yrs)	.053	-.015
SBP (mmHg)	.033	.008
DBP(mmHg)	.078	.121
BMI (kg/m2)	.103	.187**
FBS (mg/dL)	.154*	.050
HbA1C (%)	.154*	.105
TC (mg/dl)	.125	.140*
TG (mg/dl)	.003	.250**
HDL-C (mg/dl)	-.101	-.067
LDL-C(mg/dl)	.129	.088

*(P<0.05) * *(P<0.01)

obtained. Total cholesterol also showed a significant ($P<0.05$) positive association with fibrinogen. A non-significant negative association of both CRP and fibrinogen with HDL-C is observed in the studied groups.

DISCUSSION

Our results show high levels of hs-CRP in patients having type 2 diabetes mellitus with CHD as compared to patients having type 2 diabetes mellitus without CHD. These results are in tune with the reports presented by Mohan et al[20], Haffner[21] and Leipold et al[22].

Amanullah et al[23] compared CRP levels between type 2 diabetic patients and normal healthy control. They observed markedly elevated levels of CRP in type 2 diabetic subjects with strong association with HbA1c ($p < 0.05$). Jabeen et al[24] studied glycemic control and high sensitivity C-reactive protein in pathogenesis of vascular complications in type-2 diabetic patients. They observed high CRP levels in their study group ($P< 0.05$) and confirmed a positive association of HbA1c and FBG with $r= 0.993$ and $p=0.0001$. These observations were not consistent with Layer et al[3] who did not find any significant difference in CRP values after they compared normal subjects with diabetic population.

In this study, plasma fibrinogen level was significantly high ($P< 0.05$) in type 2 diabetic patients with coronary heart disease. Hong et al[25] showed a strong association between raised plasma fibrinogen level and increased risk and severity of CHD in type 2 diabetic patients. They based this observation on the relationship between increased fibrinogen level and long term glycolipid abnormalities as well as presence of long standing low grade inflammation leading to atherosclerotic plaques. Same results were also reported by Acevedo et al.[26] and EPIC –Norfolk study[27].

Madhu et al[28] also reported the same observations after comparing three groups containing control and diabetic patients with and without CHD. Progressively high level ($P <0.05$) of fibrinogen was found in control, diabetic patients without CHD and diabetic patients with CHD.

Bembdi et al[29] reported high levels of plasma fibrinogen ($P< 0.05$) in type 2 diabetic patients than normal healthy controls.

In this study higher HbA1c has been observed in type 2 diabetic patients having CHD. Diabetes mellitus affects glucose metabolism involving multiple metabolic cascades such as hexosamine pathway, advanced glycation end products, polyol pathway and protein kinase C[30]. Hyperglycemia enhances atherosclerosis by glycating proteins making them more susceptible for oxidation, especially LDL-C. The Chennai-urban population study has reported the positive relationship of hyperglycemic status and development of CHD in type 2 diabetes mellitus[31].

The positive association of hs-CRP with FBS and HbAlc can be explained by the fact that inflammation is the main hallmark state of insulin resistance which is observed in obesity and type 2 diabetes mellitus[32]. There are two mechanisms which lead to inflammation. First, chronic over-nutrition and intake of glucose produce a state of oxidative stress and pro-inflammatory state and second, release of TNF-α (tumor necrosis factor α) and IL-6 (interlenkin-6) suppress the transduction of insulin signals and inhibit its action[33]. Our results are in tune with Dehgan et al[34] and Meshram et al[35] who also confirmed linear association of hs-CRP with HbAlc. On the contrary, Karthryn et al[36] observed that levels of inflammatory markers remain high inspite of achieving good glycemic control. We observed that fibrinogen level was higher in patients with poor glycemic control ($HbA1c > 7$) as compared to those having fair control ($HbA1c \leq 7$) in both groups however we could not establish a significant relationship between the glycemic status and fibrinogen level.

In this study we have seen a positive association of fibrinogen with BMI, triglycerides (TG) and total cholesterol (TC). Linear trends of fibrinogen with age, total cholesterol, triglycerides and body mass index was reported by Popoola et al[37], Ang et al[38], Strc J et al[39] and Kanna et al[40].

The limitations of this study include a relatively small sample size which may have led to non-significant results, and the inclusion of the participants of specific age group (40 and above). Results may be different for individuals of different age groups having different life styles and activity levels. However, the randomized design of the present study is its strength.

CONCLUSION

It can be said that raised levels of two important inflammatory biomarkers; hs-CRP and fibrinogen contribute towards imbalance of glycolipid metabolism in type 2 diabetic subjects with and without coronary heart disease. Therefore these inflammatory markers may prove helpful to identify the diabetic patients who are prone to develop coronary heart disease.

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