



Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com



Original Article

Assessment of fibrinogen, high sensitive C-reactive protein (hsCRP) and homocysteine in type 2 diabetes mellitus under treatment

Siva Prasad Palem^{*a}, Philips* Abraham^{*b}, Sachu* Philip^{*c}

^a Research Scholar, Department of Biochemistry, Vinayaka Mission's University, Salem

^b Associate professor, Department of Biochemistry, Vinayaka Mission's University, Salem

^c Professor, Department of Biochemistry, Vivekananda Dental College for women, Tiruchengodu, Namakkal.

ARTICLE INFO

Keywords:

Type 2 diabetes mellitus (T2DM)
high sensitive C-reactive protein (hsCRP)
Homocysteine (HCY)
cardiovascular disease (CVD).

ABSTRACT

Introduction: Incidence of vascular complications is found to be high in type 2 diabetes mellitus patients. Conventional risk factors were not able to explain this high prevalence of CAD. Since inflammation is considered as one of the major reasons for vascular complications, markers like plasma fibrinogen, hsCRP and homocysteine may be used to analyze the risk. **Aim & Objective:** To assess the cardiovascular risk by measuring fibrinogen, hsCRP and homocysteine in T2DM subjects under treatment. **Materials & Methods:** 40 T2DM subjects under treatment and 40 healthy sex matched subjects of 40-60yrs were included in the study. Plasma Fibrinogen, serum hsCRP and HCY were measured by kit method. **Result & discussion:** In our study, plasma fibrinogen level (697.44 ± 84.23) and hsCRP level (1.79 ± 1.31) were significantly increased in T2DM subjects when compared to controls (156.75 ± 103.19 , 0.55 ± 0.44) with p value less than 0.05. **Conclusion:** Increased plasma fibrinogen and hsCRP were observed in T2DM subjects compared to healthy subjects. No significant difference was observed in HCY levels among the study groups. Evaluation of these parameters along with regular blood sugar may be helpful for early prediction of cardiovascular disease.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple causes which is characterized by hyperglycemia in the context of insulin resistance and relative insulin deficiency. Studies have predicted that about 439 millions of people will be affected by Diabetes mellitus by 2030 worldwide [1] American heart association has designated diabetes mellitus as a major risk factor for CVD [2]. It has been observed chance of mortality due to coronary artery disease has increased 5 times by the presence of type 2 Diabetes mellitus [3] one of the major reasons for this several fold increase is due to the presence of micro and macro vascular complications. Inflammation has also been clinically recognized as one of the major factor for vascular disease in T2DM [4]. The novel risk inflammatory markers hsCRP and fibrinogen can partly predict the incidence of cardiovascular disease in patients with T2DM [3].

Elevated fibrinogen level that contributes to the increase in blood viscosity is considered as a risk factor for later onset of complication in diabetes. Fibrinogen is associated with other acute-

phase reactants in the emerging view of subclinical inflammation and risk for type 2 diabetes [5]. CRP a systemic inflammatory marker is emerging as an independent risk factor for cardiovascular disease [6]. Studies have shown strong association of CRP and adiposity in both diabetic and non diabetic subjects. These suggests that factors stimulating diabetes like obesity and insulin resistance are interconnected in a pro-inflammatory state that may be mediated by cytokines and subsequently cause elevated levels of CRP. Hence elevated CRP concentrations have been shown to predict an increased risk of diabetes [7].

Homocysteine has been considered as an independent risk factor for CVD. High level of homocysteine promotes the formation of oxidative products such as homocysteine disulfides as well as homocysteine thiolactone, which can damage endothelial cells in turn, promote the development of thrombosis and atherosclerosis [8].

Anti-diabetic treatment has different effect on cardiovascular risk factors. Steven E et al have shown that metformin treatment on diabetic subjects moderately decreases CRP [7] and increases homocysteine levels. Long term effect of anti-diabetic treatment on cardiovascular risk factors is still unclear.

* Corresponding Author : **Mr. Siva Prasad P**

Research Scholar,
Department of Biochemistry, VMKV Medical College,
Vinayaka Mission's University, Salem-636308, Tamil Nadu, India
Email ID: sp.biocom@yahoo.co.in

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The studies have shown that glipizide, metformin and rosiglitazone have significant effects on traditional and non-traditional risk factors of T2DM [2]. Hence, current study was designed to estimate the effect of anti-diabetic treatment on fibrinogen, hsCRP and Homocysteine level in T2DM subjects.

2. Material and Methods

Subject selection: 40T2DM subjects of 40-60yrs of age were selected from outpatient department of Govt. Hospital, Salem who were undergoing regular treatment for minimum of 6 months to 15 years .40 healthy subjects of age and sex matched were taken as control.

Sample collection: Blood sample were collected from the patients after obtaining informed consent and the sample were separated as plasma and serum, and stored at -200C. Plasma was used to assess fibrinogen and serum for lipid profile, hsCRP and homocysteine estimation.

Biochemical analysis: Estimation of cholesterol was done by enzymatic 'CHOP-PAP' method, triglyceride (TG) by enzymatic GPO-POD method and high density lipoprotein cholesterol (HDL-C) by direct enzymatic colorimetric method. LDL-C and VLDL-C were calculated using the Friedewald's formula (Friedewald's W T, et.al.,1972). Plasma fibrinogen and serum hsCRP was measured by immunoturbidometric method and homocysteine by UV 2 kinetic method. Statistical analysis was done by student's t test. The difference in mean values of two groups was statistically significant at 'p' value less than 0.05.

3. Result

40 T2DM subjects without complications in the age of 40 to 60 years and 40 healthy controls of the same age were included in our study. Table I depicts the level of lipid parameters, fibrinogen, hs-CRP and Homocysteine in Diabetic group and healthy subjects.

Data are expressed as (mean + S.D). Statistical analysis was done by t test.

*Statistically significant between two groups at $p < 0.05$

In our study, no significant difference in the levels of serum total cholesterol and HDL-cholesterol was found in T2DM when compared to healthy subjects. But the levels of TG, VLDL and LDL cholesterol was found to be significantly high in the diabetic patients when compared to controls. The plasma fibrinogen (697.44±84.23) and hsCRP (1.79±1.31) levels were significantly high in T2DM subjects compared to control (156.75±103.19, (0.55±0.44). But no significant difference was observed in homocysteine level between T2DM subjects (16.83±7.70) and control (16.10±5.41), 'p' value is <0.05.

Table 1: Biochemical Parameters of study subject

PARAMETER	CONTROL (MEAN±SD)	TYPE-2 DM (MEAN±SD)	'p' values
AGE	48.93±10.55	50.75±10.46	0.439
TC	184.18±37.79	192.6±43.38	0.357
TGL	121.15±72.72	151.75±62.38	0.009*
HDL	40.83±9.52	40.83±8.21	0.758
LDL	119.13±34.13	181.7±45.72	0.000*
VLDL	24.23±14.54	30.3±12.48	0.010*
FIBRINOGEN	156.75±103.19	697.44±84.23	0.000*
hsCRP	0.55±0.44	1.79±1.31	0.000*
HCY	16.10±5.41	16.83±7.70	0.283

4. Discussion

It is well known that atherosclerotic vascular complications is responsible for majority of diabetes related morbidity and mortality. Since fatty streak formation is the major reason for hardening of blood vessel, attention was mainly given on the levels of Lipid parameters to predict cardiovascular risk. But later studies have revealed that these conventional risk factors were not able to explain high incidence of CVD in T2DM [9]. In our studies levels of Total cholesterol and anti-atherogenic HDL cholesterol in T2DM was not found to be significantly different from control subjects. The levels of TG, VLDL and LDL cholesterol were found to be high in diabetic patients. This observation can be attributed to the role of Insulin resistance on clearance of these parameters through lipoprotein lipase and Cholesterol ester transfer protein [10].

Emerging evidences reveal that inflammatory mediators are involved in every phase of atherogenesis and related complications- from the early stages of fatty streak formation to eventual growth and rupture of atherosclerotic plaque [11]. So Inflammatory markers or mediators can be used as a better indicator to assess the vascular complication [3]

Fibrinogen has been considered as an important acute phase protein and a powerful independent risk factor for cardiovascular disease in T2DM. Increased synthesis of fibrinogen in type 2 diabetes with the absence of microalbuminuria has been demonstrated in previous studies [12]. Increased plasma fibrinogen associated with body weight was also observed in various studies [5]. In the present study we have found an increased fibrinogen level in diabetic patients under treatment compared to healthy control. Previous studies have shown that increased fibrinogen production may be due to insulin resistance and increased fibrinogen degradation products may stimulate fibrinogen synthesis in the liver and causes a subclinical inflammatory state [12].

Several atherosclerotic lesions contain large amounts of fibrin. It has been found that fibrin in the intima triggers cell proliferation, cell migration synthesis of collagen and enhance permeability and vascular tone. Thus Fibrinogen participates in the formation of atherosclerotic plaque during the first stages of CAD, suggesting that it is a causative factor rather than a result [13].

CRP has been one of the traditional acute phase reactant and sensitive marker of inflammation and tissue damage [7]. In our study the CRP level was significantly high in T2DM subjects on treatment compared with control. type 2 DM has been considered as an inflammatory disease and inflammatory process seems to play an important role in the development of diabetes and its late complications [14, 15]. Steven E et al have shown strong positive correlation between CRP and insulin resistance [7]. This might be the reason for the significantly high level of acute phase inflammatory marker CRP observed in diabetic subjects when compared to control groups. Nickolaos et al has reported atherosclerosis as a chronic low-grade inflammatory disease with a continuous low-grade production of pro-inflammatory mediators by T lymphocytes and macrophages: TNF- α , IL-1, and IL-6. These cytokines escape from the plaque into the circulation. This results in an increased production in the liver of the inflammatory proteins CRP, serum amyloid A, and fibrinogen [13]. This involvement of inflammatory process and pro-inflammatory mediators might be another reason behind the significantly high ($p < 0.05$) level of CRP in patients with diabetes when compared to control subjects. Steven M et al have found an elevated level of CRP in association with both the development of type 2 diabetes and an increased risk of CVD [16] and also studies predicted that elevation of CRP concentration has been associated with increasing risk of diabetes [17, 18].

CRP can increase the production of super oxide dismutase and decrease the production of NO by the up regulation of Lectin-like oxidized LDL receptor-1 (LOX-1). Thus CRP can cause endothelial dysfunction. These evidences proved that CRP is not only an inflammatory marker but also an atherogenic molecule [19].

Moreover Sachu et al has shown the existence of positive correlation between level of CRP and severity of CAD which substantiated the significance of CRP as a predictor of CAD over traditional risk factors [20]. Both population-based and prospective studies support that systemic inflammation marker like CRP may integrate with risk of CVD [21].

Hyperhomocysteinaemia has been considered as an independent risk factor for the development of macrovascular disease [22, 23]. Homocysteine levels are influenced by insulin concentration and also by anti-diabetic treatment (insulin, metformin & glitazones). Recent studies have shown that elevated homocysteine levels are seen in diabetes only when renal function deteriorates [23].

In our study no significant difference was observed in homocysteine levels in T2DM compared with control. Folic acid and vitamin B12 supplementation might be the reason for the decrease in the level of homocysteine in T2DM, because homocysteine gets converted to methionine in presence of B12 and folic acid. Earlier studies have shown moderate increase in homocysteine level in association with decreased folic acid and B12 levels in T2 DM subjects [23]. Previous studies supports, a strong association between homocysteine level and early CVD events in type 2 diabetes [24].

5. Conclusion

Increased Fibrinogen and hsCRP levels were seen in T2DM subjects under treatment. But no significant difference in the levels of the conventional risk factors was observed in T2DM subjects. Hyperfibrinogenemia and elevated level of inflammatory marker hsCRP are considered as risk factor for cardiovascular disease in T2DM. Hence, addition of these parameters along with regular blood glucose evaluation and conventional factors may be helpful to reduce the risk and early prediction of CVD. Selection criteria limits the sample size of our study finding, since only those patients without complications, and those who were on lipid lowering and anti-oxidant drugs were only included. Further studies in a large population and prospective follow up based on duration of anti-diabetic treatment should elucidate the macro and micro complications of T2DM.

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