Effect on Certain Inflammatory Markers in Uncontrolled Diabetic Patients of North-West Indian

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ABSTRACT

Background: Diabetes is the chronic disorder resulting from a number of factors in which an absolute or relative deficiency of insulin or its function occurs. It has emerged as a major public health problem in India. The risk of coronary artery disease (CAD) among Indian diabetic population is 2-4 times higher than that of non-diabetic subjects. The inflammatory markers such as C-reactive proteins (CRPs), fibrinogen, interleukin-4, homocysteine have been recognized as an independent risk factor for CAD. **Aim:** The aim was to evaluate the inflammatory markers for early detection of CAD in diabetics. **Materials and Methods:** In the present study, 50 diabetic patients (both sexes) with raised glycosylated hemoglobin in the age range of 30-60 years and equal number of age and gender matched normal healthy subjects (control) were recruited. The levels of plasma CRP, fibrinogen, homocysteine along with various lipoproteins were evaluated in uncontrolled diabetic patients. **Results:** The levels of total cholesterol, triglycerides, very-low-density lipoprotein (LDL)-cholesterol and LDL-cholesterol significantly increased while high-density lipoprotein-cholesterol levels were significantly decreased in serum of diabetic patients in comparison to normal healthy control subjects. A significant increase in the CRP ($P < 0.001$), fibrinogen ($P < 0.001$), and homocysteine ($P < 0.01$) levels in diabetic patients with respect to control subjects was observed. **Conclusion:** This study therefore suggests the importance of assessing inflammatory markers along with other routine investigations in diabetic patients in addition to primary and secondary preventives measures to migrate the devastating consequences of diabetes leading to CAD. This strategy may help to identify and monitor high-risk diabetic for any cardiovascular event thereby reducing the economic burden and improving the quality of life.

Keywords: Cardiovascular diseases, C-reactive proteins, fibrinogen, glycosylated hemoglobin, homocysteine, lipoproteins, Type-2 diabetes

INTRODUCTION

Diabetes is a group of disorder characterized by hyperglycemia resulting from defects in insulin secretion or insulin action.[1] Cardiovascular complications are the leading cause of mortality and morbidity in diabetic patients of both developed nations and developing countries particularly in the younger population.[2] Framingham heart study demonstrated a direct association between diabetes and heart failure.[1] Prevention of coronary artery diseases (CADs) can be approached in many ways, including healthy promotion campaign, specific protection strategies, lifestyle modification programs, early detection, and control of risk factors and constant vigilance of emerging risk factors. The concept of cardiovascular risk factors arose from the framing heart study, a landmark study in cardiovascular disease epidemiology. Prospective cardiovascular Munster, simple scoring scheme identified risk factors such as age, smoking, hypertension, hyperlipidemia, diabetes, obesity, etc.[3,4] Despite these classic risk factors, a continued focus on newer factors is warranted as they may further improve our ability to predict future risk and manage cardiovascular diseases (CVDs), when they are included along with the classic risk factors. The study of these new risk factors is important since the ability to accurately predict the CVD risk of specific individual based on his/her conventional risk factor profile is limited.[5-7]

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Literature reports also revealed that CAD progresses because of activation of neurohormones and proinflammatory cytokines following an initial cardiac insult or injury or a mutation of the genetic program and overexpression of these biologically active molecules exerts toxic effects on the heart and circulation.[8,9] The role of the immune system activation in CAD has received considerable interest in the last decade. It is now becoming increasingly apparent that inflammatory mediators play a crucial role in the development of CAD and several strategies to counterbalance various aspects of the inflammatory response are considered. One of the possible targets includes pro- and anti-inflammatory cytokines and their receptors. The concept that biological markers may accurately predict the outcome of CAD patients is an attractive one. Several reports have indicated that elevated blood levels of inflammatory markers are associated with an adverse prognosis of CAD.[10,11] Therefore, the present study was undertaken with the aim to evaluate the inflammatory markers such as fibrinogen, C-reactive protein (CRP), and homocysteine in uncontrolled diabetic patients to assess CAD risk in North-West Indians.

MATERIALS AND METHODS

Subjects

The present study was conducted in the Department of Biochemistry, Government Medical College Amritsar on 50 Type-2 diabetic patients (with raised glycosylated hemoglobin [HbA1c]) of both sexes (36 male and 14 females) in the age range of 30-60 years (Figures 1 and 2) attending various wards of Medicine Department, Sri Guru Nanak Dev Hospital Amritsar were recruited in this study after taking informed consent from them. Patients having renal failure, chronic liver disease, malignancy, acute or chronic inflammatory or infectious diseases such as infection or sepsis, rheumatoid arthritis, connective tissue diseases, and other autoimmune diseases known to cause elevation in high sensitivity CRP, fibrinogen and homocysteine levels were excluded from this study. The control group of the study consists of the equal number of age and sex matched normal healthy subjects. All subjects were vegetarian, non-smoker and non-alcoholic, and there was no positive family history of CAD in these subjects.

Study design

This study was a case–control cross-sectional prospective study. This study protocol was approved by The Ethical Committee of the Government Medical College Amritsar. A detailed history, physical, and systemic examination, including measurement of height, weight, heart rate, blood

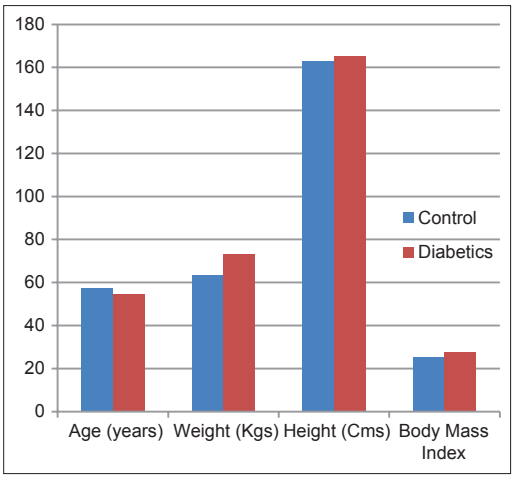

Figure 1. Anthropometric measurements of normal healthy controls subjects (n = 50) and Type-2 diabetic patients (n = 50). Values in figure are expressed as mean ± standard deviation.

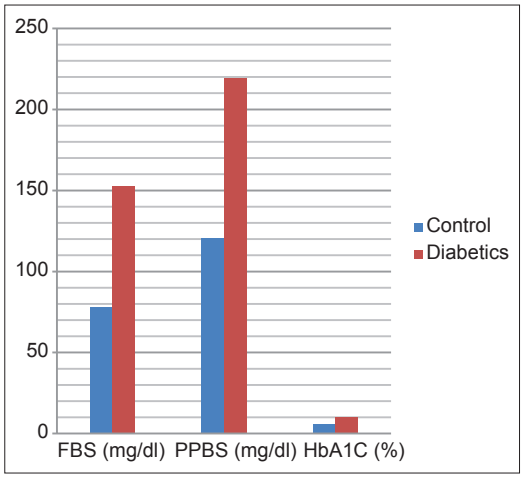

Figure 2. Alterations in fasting blood glucose, post-prandial blood glucose and glycosylated hemoglobin levels in normal healthy controls subjects (n = 50) and Type-2 diabetic patients (n = 50). Values in figure are expressed as mean ± standard deviation.

pressure, and body mass index (BMI) was carried out in every subject who entered the study as per a pre-designed proforma for assessing the signs of chronic heart failure and also the presence of any exclusion criteria.

Blood sampling

A volume of 5 ml of peripheral venous blood was collected by vein puncture using a dry, disposable syringe between 8 and 9 AM after an overnight fast from both groups (control and Type-2 diabetic patients). Blood was collected in sterile tubes containing a mixture of potassium oxalate and sodium fluoride in the ratio of 3:1 and was centrifuged at 3000 rpm for 15 min. The plasma so, obtained was used for the estimation of glucose, fibrinogen and CRP levels. 2 ml of a blood sample was collected separately in ethylenediaminetetraacetic acid

vial for the analysis of HbA1c. Samples were stored at 4°C and used for various biochemical assays.

Anthropometric measurements

The examination of body weight was done by taking weight in kilogram (kg) and height was measured in centimeters. The BMI was calculated from the formula: BMI = weight in kg/(height in meters)^2 (Figure 1).

Biochemical assays

1. Glucose: Fasting and post-prandial blood glucose levels were estimated using orthotoluidine method of Hyvavria and Nikkila, 1962.[12]
2. HbA1c: HbA1c was analyzed by using kit supplied by Transasia Biomedical Private Limited, Mumbai (India) based on ion-exchange resin method in which a hemolyzed preparation of the whole blood is mixed continuously for 5 min with a weak binding cation exchanges resin. During this time, non-HbA1c binds to the resin. After the mixing period a filter is used to separate the supernatant containing the glycohemoglobin from the resin. The glycohemoglobin percent is determined by measuring the absorption at 415 nm of the glycohemoglobin fraction and the total hemoglobin fraction. The ratio of the two absorbances gives the percentage glycohemoglobin.
3. Total cholesterol: Serum total cholesterol level was assayed by the method of Allain *et al.* 1974.[13]
4. Triglyceride (TG): Serum TG level was estimated by using the method of McGowan *et al.*, 1983.[14]
5. High-density lipoprotein (HDL)-cholesterol: HDL-cholesterol was estimated by the method of Grillo and Izzo, 1985.[15]
6. Low-density lipoprotein (LDL)-cholesterol: Serum LDL-cholesterol was estimated from the primary measurements by using the empirical equation of Friedewald *et al.* 1972.[16]
   LDL-cholesterol = Total cholesterol − (HDL-cholesterol−TG/5).
7. Plasma Fibrinogen: Fibrinogen content in plasma was measured by tyrosine method as given in Varley *et al.*, 1980.[17] The fibrin clot is formed in the diluted plasma by the addition of calcium ions. The clot then separated from the solution and washed free of other proteins. The clot is dissolved in alkali, and the protein is determined with the FolinCiocalteu reagent. Blue color developed was measured at 680 nm. Results were ex-pressed as mg/dl of fibrinogen.
8. CRP: The hs-CRP estimation was performed by using UBI Magiwel CRP-quantitative AD-401 kit, a solid phase enzyme linked immunosorbent assay as per instructions of the manufacturer supplied with the kit.
9. Homocysteine: The serum homocysteine level was analyzed by enzymatic immunoassay (EIA) method by axis-shield homocysteine EIA kits. Quantification limit of this assay (CV < 20%) is 1.0 μmol/L. Briefly, in this method, protein-bound Hcy is reduced to free Hcy and then reacts with serine catalyzed by cystathionine beta synthase to form L-cystathionine. Cystathionin is cleavaged to homocysteine, pyruvate and ammonia by cystathionine beta lyase. Pyruvate is converted to lactate with nicotinamide adenine dinucleotide (NADH) as coenzyme by lactate dehydrogenase. Concentration of homocysteine is directly proportional to the rate of NADH conversion to NAD.

Statistical analysis

The data were analyzed using a SPSS-17 program and expressed as mean ± standard deviation continuous variables with normal distribution. The Student's *t*-test was used to test the significance of differences between the mean values diabetic patients and normal health subjects (control) groups. The differences were considered significant at $P < 0.053$.

RESULTS AND DISCUSSION

A significant increase was observed in the serum total cholesterol, TG and LDL-cholesterol and very-LDL-cholesterol level from 182.00 ± 15.00 mg/dl to 217.00 ± 12.29 mg/dl (by 19.23% with respect to control subjects), 136.00 ± 13.00 mg/dl to 192.00 ± 11.24 (41.18% with respect to control subjects) and 160.20 ± 12.22 mg/dl to 218.40 ± 13.27 (36.33% with respect to control subjects) and 27.20 ± 4.60 mg/dl to 38.40 ± 6.80 (41.18% with respect to control subjects) respectively in Type-2 diabetic patients, whereas the level of serum HDL-cholesterol was significantly decreased ($P < 0.01$) from 49.00 ± 5.60 mg/dl to 37.00 ± 4.80 in Group 2 patients (Figure 3). The existence of a causal relationship between altered levels of lipids and lipoproteins and CAD is well-established fact.[18,19] The United States Lipid Research Clinical trial has shown that cholesterol makes most significant individual contribution to risk of CAD.[20,21] Our findings of alteration in serum total cholesterol, LDL-cholesterol, TG and HDL-cholesterol levels in the Type-2 diabetic patients are in agreement with the reports.[22-24] that hyperlipidemia is one the risk factor for the onset of CAD.

CRP is a critical component of the immune system, a complex set of proteins that our bodies make when faced with a major infection or trauma. Inflammation, the key regulator of CRP synthesis plays a pivotal role in

atherothrombotic CVD. There is a powerful predictive association between raised serum CRP values & the outcome of acute coronary syndromes, and remarkably between even modestly increased CRP production and future atherothrombotic events in otherwise healthy individuals.[1,25,26] In the present study, a significant increase ($P < 0.001$) in CRP levels was seen in the Type-2 diabetic patients with respect to normal healthy control subjects (Figure 4), suggests that CRP may contribute to the pathogenesis and complications of CVD in uncontrolled diabetic subjects.

In Type-2 diabetic patients, we found a significant increase ($P < 0.001$) in fibrinogen level by 39.98% with respect to control subjects that is from 311.15 ± 17.32 mg/dl to 453.54 ± 22.71 mg/dl (Figure 5). Fibrinogen is a circulating glycoprotein that acts at the final step in the coagulation response to vascular and tissue injury. There are several mechanisms by which fibrinogen may increase the cardio-vascular risk. First, it binds specifically to activated platelets via glycoprotein IIb/IIIa, contributing to plate aggregation. Second, increased fibrinogen levels promote fibrin formation. Third, it is a major contributor to plasma viscosity. Finally, fibrinogen is an acute phase reactant that is increased in inflammatory states.[27,28] A significant increase of fibrinogen level in the present study suggesting that fibrinogen is directly associated with CAD and also indicates that fibrinogen may play an important role in plaque rupture and thrombosis in response to inflammation.

A significant increase ($P < 0.001$) in homocysteine levels were observed in the uncontrolled Type-2 diabetic patients by 138.776% (from 5.03 ± 0.34 mMg/dl to 12.01 ± 1.41 mM/dl with respect to non-diabetic subjects control healthy subjects (Figure 4). Significant increase in plasma concentration of homocysteine, associated with the increased incidences of atherosclerosis and other CVD.[29,30] The increase in homocysteine levels might induce atherothrombosis through the formation of homocysteine thiolactate, a byproduct of oxidation of homocysteine. Homocysteine thiolactate combines with LDL to form foam cells.[31,32] The LDL rich foam cells embed themselves in the vascular endothelium and be-come fatty streak, which is the beginning of an atherosclerotic plaque. Homocysteine thiolactate could also impair the oxidative phosphorylation and enhancement of the proliferation and fibrosis of smooth muscle cells.[33,34] Homocysteine may also induce atherosclerosis by affecting endothelial-derived relaxing factor that is nitric oxide (NO). NO combined with homocysteine in the presence of oxygen to form s-nitroso homocysteine, which inhibits sulfhydryl dependent

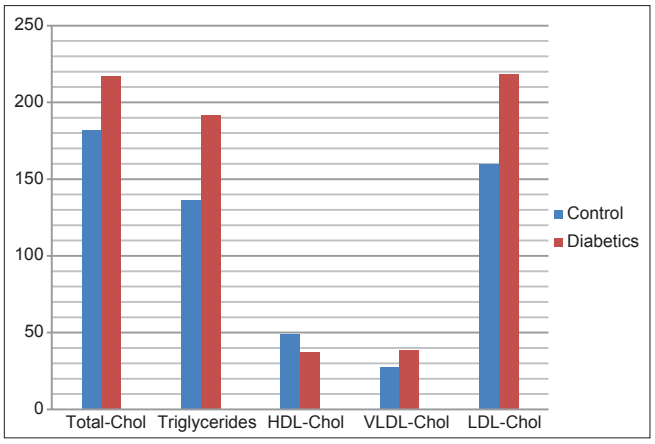

Figure 3. Alterations in various lipoprotein levels in normal healthy controls subjects ($n = 50$) and Type-2 diabetic patients ($n = 50$). Values in figure are expressed as mean ± standard deviation.

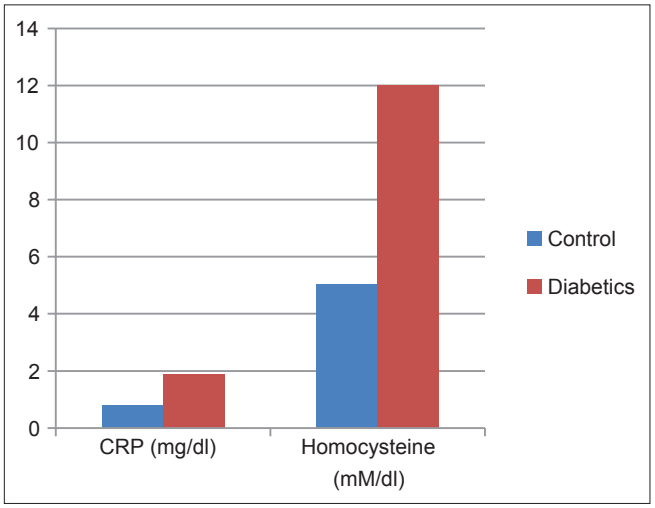

Figure 4. Alterations in C- reactive proteins and homocysteine levels in normal healthy controls ($n = 50$) and Type-2 diabetic patients ($n = 50$). Values in figure are expressed as mean ± standard deviation.

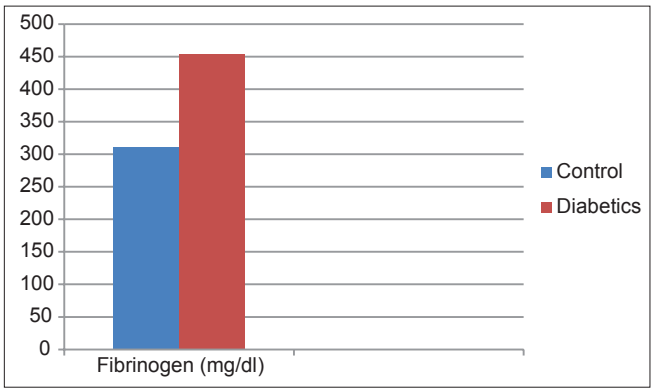

Figure 5. Alterations in fibrinogen levels in normal healthy controls ($n = 50$) and Type-2 diabetic patients ($n = 50$). Values in figure are expressed as mean ± standard deviation.

generation of hydrogen peroxide. The bioavailability of NO is decreased due to endothelial cell injury. This dysfunctional

endothelial may be due to generation of oxygen radicals produced by homocysteine. Homocysteine may also reduce the anti-oxidant status, which could injure endothelial cells.[35] Therefore, a significant increase ($P < 0.001$, 49.86%) of homocysteine level in Type-2 diabetics (Figure 4) in the present work could be responsible for the pathogenesis of CAD in diabetes patients.

CONCLUSION

All the aforementioned observations suggested that the inflammatory markers be monitored regularly for assessing the CVD risk. Diabetics or healthy population having dyslipidemia with regard to triglyceridemia (TG > 150 mg/dl) and/or having elevated levels of LDL-cholesterol may be encouraged to assess inflammatory markers such as CRP, fibrinogen, and homocysteine levels to monitor the risk of CVD or diabetes. This strategy might be helpful to identify and monitor high-risk diabetic subjects for any cardiovascular event, thereby could improve the quality of life and reduce the economic burden.

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