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Study of C-Reactive Protein and Alkaline Phosphatase among Type 2 Diabetes Mellitus patients

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ABSTRACT

Introduction: - Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. In patients with Type 2 diabetes, low grade inflammation is reflected by increased plasma levels of several biomarkers such as C-reactive protein (CRP). Alkaline phosphatase (ALP) is a generally accepted clinical marker of hepatic or bone disease. It had been showed that elevated ALP acted as a prognostic indicator of decreased survival in diabetic patients with acute myocardial infarction (MI), possibly in association with decreased renal function in these patients.

Materials and methods: This study was conducted in the Department of Biochemistry at Tertiary Care Teaching Hospital over a period of 6 months. A 5 ml of venous blood was drawn from each volunteer using a disposable vacutainer system in fasting condition. Post prandial (2 hour) sample collected in fluoride vacutainer for PP2BS estimation. Analysis of sample Fasting and post prandial (2 hour) blood sugar (FBS & PP2BS) estimated by glucose oxidase-peroxidase (GOD-POD) enzymatic end point method. Glycated hemoglobin (HbA1c) concentration was measured by High Performance Liquid Chromatography (HPLC) method. Serum ALP activity was determined by carboxy substrate kinetic method. Serum hsCRP level is measured by immunoturbidimetric method.

Results: Characteristics like age and sex were not differing between groups. We did not find any significant difference in serum alanine transaminase (ALT) and aspartate transaminase (AST) concentration in group II compared to group I. But serum alkaline phosphatase (ALP) concentration is significantly increased between groups (p value is <0.05 is considered significant). In our study shows that in group I Mean serum ALP (129.43±13.43) and hsCRP (1.29±0.31) when compared to group II serum ALP (153.53±25.53) and hsCRP (4.73±0.63).

Conclusion: The present study suggests that serum ALP and hsCRP concentration is significantly increased in type 2 diabetes mellitus. There is a significant positive correlation between serum ALP activity and hsCRP. Serum ALP level and hsCRP concentration was independently and positively correlated with FBS, PP2BS and HbA1c (markers of glycemic control). All these finding suggesting a link between CVD, inflammation and glycemic control in patient with type 2 diabetes mellitus.
Keywords: Diabetes Mellitus, Alkaline phosphatase, C-reactive protein.

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. [1]

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. [2] The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. [3] Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. [4] Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia. [5]

CRP is a pentameric protein synthesized by the liver, whose level rises in response to inflammation. CRP is an acute-phase reactant protein that is primarily induced by the IL-6 action on the gene responsible for the transcription of CRP during the acute phase of an inflammatory/infectious process. [6] CRP has both proinflammatory and anti-inflammatory properties. It plays a role in the recognition and clearance of foreign pathogens and damaged cells by binding to phosphocholine, phospholipids, histone, chromatin, and fibronectin. [7] Type 2 diabetes is an inflammatory atherothrombotic condition associated with a high prevalence of cardiovascular disease. In patients with type 2 diabetes, low grade inflammation is reflected by increased plasma levels of several biomarkers of inflammation such as C-reactive protein (CRP). [8]

Alkaline phosphatase (ALP) is a generally accepted clinical marker of hepatic or bone disease. [9] It had been showed that elevated ALP acted as a prognostic indicator of decreased survival in diabetic patients with acute myocardial infarction (MI), possibly in association to decreased renal function in these patients. [10] Moreover, in a nest case–control study, ALP in type 2 diabetes seemed to be associated with CVD risk and stroke incidence in men, but with borderline

significance. [11] However, only a few previous prospective studies have been carried out to evaluate the relation of ALP and incident diabetes, and reported inconsistent results. [12] The aim of this study was evaluation the association of CRP with biochemical markers ALP in patients with DM2.

Materials and Methods

This study was conducted in the Department of Biochemistry at Tertiary Care Teaching Hospital over a period of 6 months.

Inclusion criteria

Group I – Control group (n=110): This group consisted of age and sex matched healthy subjects. They were taken from general population who came for routine checkup.

Group II – Type 2 DM patients with poor glycemic control (n=110) this group consisted of patients with type 2 DM with duration more than 5 years, HbA1c Level >7%. They were on life style modifications, oral hypoglycemic drugs, insulin or combination of all three and associated with one or more micro vascular or macro vascular complication of diabetes mellitus for e.g. diabetic retinopathy, diabetic neuropathy.

Exclusion criteria

The patients with type 1 diabetes mellitus, high (>120g/d) alcohol consumption, with known liver or gastrointestinal diseases, with liver enzyme concentrations higher than three times the upper limit, on corticosteroids, methotrexate, amiodarone, tamoxifen or other hepatotoxic drugs, any chronic infection like tuberculosis, sarcoidosis etc. hemolytic anaemia, hemoglobin variants were excluded from this study.

Blood sample collection

A 5 ml of venous blood was drawn from each volunteer using a disposable vacutainer system in fasting condition (Plain, EDTA and Fluoride). Post prandial (2 hour) sample collected in fluoride vacutainer for PP2BS estimation. Serum or plasma separated within half an hour and stored at 2-8°C temperature till analysis was done. Analysis of sample Fasting and post prandial (2 hour) blood sugar (FBS & PP2BS) estimated by glucose oxidase-peroxidase (GOD-POD) enzymatic end point method. Glycated hemoglobin (HbA1c) concentration was measured by High Performance Liquid Chromatography (HPLC) method. Serum ALP activity was determined by carboxy substrate kinetic method. Serum hsCRP level is measured by immunoturbidimetric method. All other biochemical investigation includes serum liver enzymes, lipids, and other biochemical blood measurements were determined using standard laboratory procedures on semi autoanalyser Erba CHEM7.

Ethical Considerations : Sample was collected after taking written/oral consent from the subjects. This project has been approved by the ethical committee of the institute.

Statistical analysis

The data collected during the current study were recorded and analysed statistically to determine the significance of different parameters by using Graph Pad Instant Statistical software. Results are expressed as mean ± SD. The values between groups are compared using Quick cal test. P value of <0.05 is considered as statistically significant.

RESULTS

In table 1, Characteristics like age, sex, were not differing between groups. We did not find significant difference in serum alanine transaminase (ALT) and aspartate transaminase (AST) concentration in group II compared to group I.

Table 1: Distribution of Gender between two Groups

Gender	Group I N=110 (%)	Group II N=110 (%)
Male	71 (64.5%)	78 (70.9%)
Female	49 (44.5%)	42 (38.1%)

Table 2: Distribution of biochemical parameters between two Groups

Parameters	Group I Mean±SD	Group II Mean±SD
Serum ALP concentration (IU/L)	129.43±13.43	153.53±25.53
Serum hsCRP concentration (mg/L)	1.29±0.31	4.73±0.63
HbA1c (%)	5.01±0.43	7.39±0.45
FBS (mg/dl)	69.63±7.54	135.54±14.54
PPBS (mg/dl)	124.43±11.43	169.54±16.43
Total cholesterol (mg/dl)	161.43±16.65	201.43±23.54
Triglycerides total (mg/dl)	139.56±11.75	167.43±12.43
ALT (U/L)	23.43±3.54	22.49±6.78
AST (U/L)	24.54±4.54	23.54±4.53

Table 3: Values of serum ALP and hsCRP concentration between study groups I and group II.

Study groups	Group I	Group II	p value
ALP (IU/L)	129.43±13.43	153.53±25.53	<0.05
Hs CRP (mg/l)	1.29±0.31	4.73±0.63	<0.0001

In table 3, our study shows that in group I Mean serum ALP (129.43±13.43) and hsCRP (1.29±0.31) when compared to group II serum ALP (153.53±25.53) and hsCRP (4.73±0.63). But serum alkaline phosphatase (ALP) concentration is significantly increased between groups (p-value is <0.05 is considered significant).

Table 4: Pearson's correlation analysis between serum ALP and hsCRP and glycemic control.

	Correlation coefficient r value	**Two tailed p value**
Serum ALP with hsCRP	0.31	<0.0001
Serum ALP with HbA1c	0.73	<0.0001
Serum ALP with FBS	0.37	<0.0001
Serum hsCRP with ALP	0.29	<0.0001
Serum hsCRP with HbA1c	0.27	<0.0001
Serum hsCRP with FBS	0.37	<0.0001

DISCUSSION

The term diabetes mellitus describes diseases of abnormal carbohydrate metabolism that are characterized by hyperglycemia. It is associated with a relative or absolute impairment in insulin secretion, along with varying degrees of peripheral resistance to the action of insulin. Every few years, the diabetes community revaluates the current recommendations for the classification, diagnosis, and screening of diabetes, reflecting new information from research and clinical practice. [14]

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome. [15]

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes. [16]

Previous studies have linked serum ALP levels and the risk of diabetes, but reported controversial results. In a case–control study, Malo MS reported that high intestinal alkaline phosphatase (IAP) levels appeared to be protective against diabetes irrespective of obesity. [17] However, Nannipieri M. (n=1441), Nakanishi N. (n=3260), and Hanley AJ. (n=906) found that there was no significant association between ALP and incident diabetes. Moreover, a study conducted in Taiwan, including 132,377 non-diabetic individuals, showed that higher ALP level was significantly related to increased risk of diabetes. Of note, this study did not consider the effect of some major risk factors for diabetes, such as initial FG levels and the concomitant medications, and therefore, could not provide an accurate measurement of the association between ALP and incident diabetes. In addition, a recent mendelian randomization study demonstrated that there was a modest negative effect of genetically predicted ALP on type 2 diabetes (OR, 0.91; 95% CI: 0.86, 0.97). [18]

Type 2 diabetes is an inflammatory atherothrombotic condition associated with a high prevalence of cardiovascular disease. In patients with type 2 diabetes, low grade inflammation is reflected by increased plasma levels of several biomarkers of inflammation such as C-reactive protein (CRP). Small increases in CRP predict the likelihood of developing cardiovascular events both in diabetic and nondiabetic populations. In addition, in apparently healthy subjects, increased levels of CRP predict the risk of developing type 2 diabetes. There is some evidence that CRP, besides its predictive role in determining cardiovascular risk, may represent an active participant in atherogenesis. CRP is expressed in human atherosclerotic plaques and both vascular cells and monocytes/macrophages appear to represent a significant source of CRP in the inflammatory vessel wall. [19]

By activating the main cell types present in the atherosclerotic lesions, CRP generated within the coronary plaques may contribute to the development and progression of atherosclerosis. Data on vascular CRP regulation are scarce. Current evidence suggests that inflammatory and metabolic factors associated with diabetes, such as high glucose, adipokines, modified lipoproteins and free fatty acids may trigger CRP production by endothelial cells, smooth muscle cells and monocytes/macrophages. [20] These data suggest that local CRP concentration in diabetic atherosclerotic plaques could be higher than in nondiabetic ones. Given the possible correlation between local CRP production and the degree of severity of coronary artery disease or the nature of the lesion, such alteration may contribute to the accelerated development of vascular disease in patients with type 2 diabetes. [21]

CONCLUSION

The present study suggests that serum ALP and hsCRP concentration is significantly increased in type 2 diabetes mellitus. Both are further increased in diabetic patients with complications and poor glycemic control. There is a significant positive correlation between serum ALP activity and hsCRP. Serum ALP level and hsCRP concentration was independently and positively

correlated with FBS, PP2BS and HbA1c (markers of glycemic control). All these finding suggesting a link between CVD, inflammation and glycemic control in patient with type 2 diabetes mellitus.

CONFLICT OF INTEREST: All authors declare that there is no conflict of interest existing.

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