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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 Mellitus is a metabolic disorder and associated with number of disorders including bone, kidney, liver, nerves, had and blood vessels dysfunction. C- reactive protein (CRP) is a sensitive physiological biomarker of subclinical inflammation associated with hyperglycemia. The objective of the present study was to establish the relationship between CRP with blood sugar and Alkaline Phosphatase (ALP) in serum of Diabetes Mellitus (DM2).

Materials and methods: This study was a hospital based cross sectional study conducted in the Department of Biochemistry at Tertiary Care Teaching Hospital over a period of 1 year. A cross sectional study consists of 180 subjects out of them 90 normal healthy subjects control Group (Group I) and 90 patients with type 2 DM with poor glycemic control were selected. Subjects were recruited according to simple random sampling method meeting the selection criteria. Group I – Control group (n=90): 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=90) this group consisted of patients with type 2 DM with duration more than 5 years, HbA1c Level >7%.

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Results: Characteristics like age, sex, were not differing between groups. We found increased 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 <0.001 is considered significant). In our study shows that in group I Mean serum ALP (144.13±18.52) and hsCRP (1.35±0.20) when compared to group II serum ALP (147.23±25.95) and hsCRP (4.55±0.79).

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.

Keywords: Alkaline phosphatase, C-reactive protein, Diabetes Mellitus.

INTRODUCTION

Diabetes is a metabolic — disorder with inappropriate hyperglycemia either due to an absolute or relative deficiency of insulin secretion or reduction in the biologic effectiveness of insulin or both. It is also associated with disturbance concerned with protein, carbohydrate and lipid metabolism. (1) The decreased uptake of glucose into muscle and adipose tissue leads to chronic extra cellular hyperglycemia which results in tissue damage and chronic vascular complications (2).

The international diabetic federation (IDF) estimated in 2011 that 366 million adults aged 20-79 years, of the world's 7 billion population have diabetes mellitus. This gives a comparative prevalence of 8.5%. (3) Since more than 90% of the global cases of Diabetes mellitus are type 2, it is evident that the epidemic was mainly due to the escalation of the causes of Diabetes mellitus type 2. (4)

Among several markers of inflammation, CRP is found to be significant in people with diabetes. CRP, a pentameric protein produced by the liver has emerged as the "golden marker for inflammation". It is a non-immunoglobin protein having five identical subunits. It is a member of pentraxin family protein. (5) The C-reactive protein (CRP) derives from the fact that it reacts with capsule polysaccharide of streptococcus pneumonia. It is an acute phase response protein markedly increased in both inflammatory and infection diseases. It plays an important role in innate immunity. (6) Experimental evidence demonstrated that CRP as a sensitive physiological marker of sub clinical systemic inflammation is associated with hyperglycemia, insulin resistance and overt DM2 (7).

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Insulin, together with insulin-like growth factor; stimulates bone matrix synthesis. The stimulatory effect of insulin on bone matrix results from its action on the differentiating function of osteoblasts. Insulin is also necessary for normal bone mineralization. Osteoblasts control mineralization by regulating the passage of calcium (Ca+2) and phosphate ion (P04-2) across their cell membrane; these cells contain alkaline phosphatase (ALP) that is used to generate P04-2 from inorganic phosphates (8).

It has been reported that DM and connected with the disease metabolic disturbances lead to important alterations in bone metabolism. It has been reported that poorly controlled DM2 may be consequence of increased osteolysis and can lead to increased susceptibility to bone loss and development of bone changes as osteopenia. (9) In the majority of recent studies it has been reported that bone turnover in patients with DM2 is decreased due to the decrease in the number of osteoclasts and delay in formation and mineralization of the osteoid. (10) 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 a hospital based cross sectional study conducted in the Department of Biochemistry at Tertiary Care Teaching Hospital over a period of 1 year. A cross sectional study consists of 180 subjects out of them 90 normal healthy subjects control Group (Group I) and 90 patients with type 2 DM with poor glycemic control were selected. Subjects were recruited according to simple random sampling method meeting the selection criteria.

Inclusion criteria

Group I – Control group (n=90): 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=90) 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.

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The objectives of study were explained to all eligible subjects for this study. Informed written consent of all subjects included in the study was obtained for Involvement in study groups and for venipunctures. Emphasis was given that participation in this study was voluntary.

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.

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

RESULTS

In table 1, Characteristics like age, sex, were not differing between groups. We found increased 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 <0.001 is considered significant).

Table 1: Comparison of baseline characteristics and other biochemical parameters between study groups.

	Group I	**Group II**
Number of subjects	90	90
Sex (M/F)%	60/30	63/27
Age (in years)	39	43
Duration of diabetes (In years)	-	6.15
Serum ALP concentration (IU/L)	144.13±18.52	147.23±25.95
Serum hsCRP concentration (mg/L)	1.35±0.20	4.55±0.79
HbA1c (%)	4.59±0.39	7.49±0.34

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FBS (mg/dl)	72.73±15.25	129.75±32.0
PPBS (mg/dl)	114.24±10.99	155.53±18.35
Total cholesterol (mg/dl)	152.99±18.9	195.35±32.91
Triglycerides total (mg/dl)	112.45±15.59	134.88±22.5
ALT (U/L)	20.73±5.99	22.49±6.78
AST (U/L)	21.05±5.79	24.83±12.88

Table 2: Values of serum ALP and hsCRP concentration between study groups I and group II.

Study groups	ALP (IU/L)	Hs CRP (mg/l)
Group I	144.13±18.52	1.35±0.20
Group II	147.23±25.95	4.55±0.79
t value	3.49	15.48
p value	<0.05	<0.0001

In table 2, our study shows that in group I Mean serum ALP (144.13±18.52) and hsCRP (1.35±0.20) when compared to group II serum ALP (147.23±25.95) and hsCRP (4.55±0.79).

Table 3: Pearson's correlation analysis between serum ALP and hsCRP and glycemic control.

	Correlation coefficient r value	Two tailed p value
Serum ALP with hsCRP	0.35	<0.0001
Serum ALP with HbA1c	0.89	<0.0001
Serum ALP with FBS	0.43	<0.0001
Serum hsCRP with ALP	0.35	<0.0001
Serum hsCRP with HbA1c	0.39	<0.0001
Serum hsCRP with FBS	0.42	<0.0001

DISCUSSION

Our study demonstrated that there was a positive association between baseline serum ALP levels and diabetes, independent of other liver aminotransferases, treated BP and other important confounders, among hypertensive patients. Moreover, our study expanded the results of previous studies by demonstrating that the positive association between baseline serum ALP levels and diabetes was more pronounced in participants with lower or higher FG levels.

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. [11] 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

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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). [12]

At the same time, another mendelian randomization study suggested that ALP was not associated with the risk of diabetes. [13] It must be pointed out that both studies only included European origin participants whose genetic background may be different with other population. Overall, to data, the association between ALP and incident diabetes remains uncertain. The explanations for these discrepant results might be due to differences in study population characteristics and/or sample sizes. More importantly, no previous study has comprehensively investigated the modifiers on the relation of ALP with diabetes. [14]

Our study provided a rare opportunity to evaluate the temporal and dose–response relation of serum ALP with diabetes in adults, with a comprehensive adjustment and stratified analysis for almost all the pertinent clinical information and laboratory measurements. Our study has made some new contributions to the Feld. First, we demonstrated that higher serum ALP associated with increased diabetes in patients, independent of other liver enzymes, treated BP and traditional or suspected risk factors. Our study findings are biological plausible based on available literature, although the potential mechanisms by which serum ALP increases diabetes risk remains to be delineated. ALP was reported to contribute to vascular calcification, which linked to insulin resistance, subsequently leading to the development of diabetes. [15]

Animal experiments showed that ALP upregulation was demonstrated in the vascular wall of diabetic rat and mouse models of vascular calcification. [16] Higher serum ALP was associated with increased risk of endothelial dysfunction, a process related to insulin resistance, an initial process to diabetes. This was explained that ALP could reduce nitric oxide (NO) bioavailability by inhibiting tyrosine kinase [17] activity into endothelial cells, leading to the consequent impairment of endothelial NO synthase function. [18] Higher serum ALP levels had been reported to be associated with increased inflammation status in CKD patients or general population. [19] Notably, both endothelial dysfunction and chronic inflammation has been considered as the early events in the development of the diabetes. Taken together, the aforementioned biological functions of ALP may be in part underlying our observed positive association between ALP and incident diabetes. However, more mechanistic studies are still needed. [20]

Second, our results showed that CRP and FBG levels significantly modified the association between serum ALP and the risk of diabetes. The higher FG levels may partially represent the abnormal glucose metabolic state, due to the impairment of pancreatic alpha and beta cell function and the induced impaired insulin secretion. [20] This population usually had a

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significantly increased risk of diabetes. [21] Since higher ALP was mainly associated with insulin resistance, our results suggested that increased ALP and higher FG levels may synergistically increase the risk of incident diabetes. On the other hand, it had been reported that elevated CRP could also promote the inflammation and oxidative stress. [22] It seemed that elevated CRP and ALP levels may share some common pathway in the development of diabetes. As such, the detrimental effects of higher CRP levels may attenuate the positive relation of serum ALP levels with the risk of diabetes. Our studies suggested that the combination of optimal ALP, CRP and FG levels may be a better strategy for the primary prevention of diabetes in adults.

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.

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