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Original Research Article

Study of Association of C - reactive protein and Alkaline Phosphatase in Type 2 Diabetes Mellitus Patients

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Abstract

Background: The prevalence and incidence of type 2 diabetes are rising rapidly worldwide, especially in Asia. Diabetes has been linked to a shorter life expectancy mainly because of its complications, including heart disease, strokes, eye disease, and kidney failure and bone disease. The aim of the study was to examine the relationship between high sensitive C reactive protein (hsCRP) and alkaline phosphatase (ALP) in type 2 diabetes patients. Furthermore, we investigated correlation between serum hsCRP and ALP level with glycaemic triad (FBS, PPBS, HbA1c) in case and control group. ***Methods:*** A cross sectional study consists of 200 patients out of which 100 normal healthy controls (Group I), case - 100 patients having type 2 DM (Group II). FBS, PPBS, HbA1c, hsCRP and ALP were measured. ***Results:*** Mean serum hsCRP and ALP level were statistically significantly higher in case group compared to control group. Moreover, significant positive correlation was observed between hsCRP and ALP level as well as both with FBS, PPBS and HbA1c. ***Conclusions:*** Oxidative stress and inflammation appears to be a key component and also associated with poor glycaemic control and further pathogenesis of diabetes and its complications. All our finding suggesting a link between oxidative stress, inflammation and glycaemic control in patient with type 2 diabetes mellitus.

Keywords: C - reactive protein Alkaline Phosphatase Mellitus Patients Diabetes.

INTRODUCTION

Diabetes is one of the most challenging health problems in 21st century [1]. Diabetes mellitus (DM) is a clinical syndrome characterized by abnormal metabolism of carbohydrate, protein and fat resulting in hyperglycemia due to absolute or relative deficiency of insulin ending up in vascular complications leading to retinopathy, neuropathy and nephropathy. It can be divided into two main categories. Insulin Dependent Diabetes Mellitus (IDDM) now labeled as type-1 Diabetes Mellitus and Non-Insulin Dependent Diabetes Mellitus (NIDDM) known as type-2 Diabetes Mellitus (type 2 DM). Diabetes mellitus (DM) comprises a group of common metabolic disorders that share common phenotype of hyperglycemia [2] Hyperglycemia not only defines the disease but is the cause of its most characteristic symptoms and long-term complications. Understanding the pathogenesis and preventing long-term complications have been major goals of research in diabetes mellitus. Research in the past few years has linked inflammation to β-cell dysfunction resulting from chronic exposure to hyperglycemia. A growing body of data reinforces the concept that inflammation plays an important role in the pathogenesis of type 2 DM and links DM with concomitant conditions with inflammatory components [3].

Alkaline phosphates (ALP) is a hydrolase enzyme, which is widely expressed in human tissues, but is highly concentrated in the liver, bone, and kidney [4, 5]. Physiological increases are found during bone growth, while pathological increases are largely associated with hepatobiliary and bone diseases. Type-2 Diabetes Mellitus constitutes 85-90% of diabetic patients. Uncontrolled diabetes (chronic hyperglycemia) is associated with several long-term complications, related with micro-vascular diseases including Retinopathy, nephropathy, neuropathy and macro vascular diseases such as cardiovascular and cerebro vascular, increased susceptibility to infection; and poor wound healing. It has been reported that many diabetics may also exhibit elevated serum alkaline phosphates level. Alkaline phosphatase is an inflammatory mediator like C-reactive protein (CRP) (a novel risk marker for cardiovascular disease [6]. Both ALP and

CRP have consistently been shown to be directly and significantly associated with each other, with suggestions that they share common biological pathways [7]. Over the past decade, serum ALP has sparked interest as an emerging marker for cardiovascular risk in the general population, but uncertainty exists because important questions pertaining to its association with CVD remain unresolved.

High sensitivity C-reactive protein (hsCRP) is a C-reactive protein measured by a highly sensitive assay. CRP represents the classical acute-phase protein produced in the liver in response to inflammatory stimuli, and plasma levels of hsCRP provide a sensitive marker of increased inflammatory activity in the arterial wall [8, 9]. Chronic, systemic subclinical inflammation has also been identified as a driving force for insulin resistance, metabolic syndrome, and type 2 DM. Some related metabolic disorders include abdominal adiposity, hypertension, High sensitivity C-reactive protein (hsCRP) is a C-reactive protein measured by a highly sensitive assay. CRP represents the classical acute-phase protein produced in the liver in response to inflammatory stimuli, and plasma levels of hsCRP provide a sensitive marker of increased inflammatory activity in the arterial wall [8, 9]. Chronic, systemic subclinical inflammation has also been identified as a driving force for insulin resistance, metabolic syndrome, and type 2 DM. Some related metabolic disorders include abdominal adiposity, hypertension, endothelial dysfunction, and glucose intolerance, which often occur in a cluster. Insulin resistance correlates closely with the risk of cardiovascular diseases (CVD), explaining some of the excess morbidity and mortality in type 2 DM patients [10]. Because the development of complications is linked to the accumulation of glycation adducts in tissue proteins. The core of the issue is glycemic control. Optimal monitoring of glycemic control involves plasma glucose measurements (fasting and postprandial blood sugar) and measurement of glycated hemoglobin (HbA1c). These measurements are complementary: the patient's glucose measurements provide a picture of short-term glycemic control, whereas HbA1c reflects average glycemic control over the previous 3 months [11].

In a recently published literature-based Meta-analysis of studies assessing the associations of liver enzymes and CVD risk in participants recruited from approximately general populations, the results suggested a modest positive linear association between ALP activity and CVD risk [12]. Since the inflammation appears to be a key component of many reactions associated with poor glycemic control and further pathogenesis of diabetes and its complications; we found it interesting to study serum ALP activity (marker of CVD) and hsCRP level (an inflammatory marker) in diabetic subjects.

AIM AND OBJECTIVE

- The purpose of study to investigate a possible correlation between raised ALP levels in type 2 diabetics and non-diabetics.
- We would investigate correlation between serum ALP and hsCRP with glycemic control in subjects
- We also aim to determine whether the ALP-CVD relationship is confounded or modified by hsCRP.
- We would aim to investigate for the extent to which ALP measurements could improve the prediction of first-onset CVD outcomes in diabetes

MATERIAL AND METHOD

Ethical approval

The study will be conducted after approval from medical ethical committee, Govt medical college and hospital

Study type

This study was a hospital based cross sectional study of the relationship between alkaline phosphatase (ALP) and high sensitive C -reactive protein (hsCRP), in type 2 diabetic patients.

Sampling method: Random
Sample size: 200
Study period: 6 months

Inclusion Criteria

The subjects selected for study were grouped as follows:

Group I – Control group (n=100): This group consisted of age and sex matched healthy subjects. They were taken from general population who came for routine checkup.

Group II – Case group - Type 2 DM patients (n=100) as ADA diagnostic criteria

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 anemia, hemoglobin variants were excluded from this study.

Sample Collection and Analysis

A 5 ml of venous blood was drawn from each volunteer using a disposable vacationer system in fasting condition (Plain, EDTA and Fluoride).

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 & PPBS) estimated by glucose

oxidase-peroxidase (GOD-POD) enzymatic end point method (Kit: Quantitative determination blood sugar by glucose oxidase peroxidase method cobas integra). Glycated hemoglobin (HbA1c) Concentration was measured by immuno turbidimetric method. Serum ALP activity was determined by carboxy substrate kinetic method. (Kit: Quantitative determination of ALP by carboxyl substrate method cobas integra). Serum hsCRP level is measured by immuno turbidimetric method (Kit: Quantitative determination of hsCRP in human blood by latex turbidity assay cobas integra).

Definition

The American Diabetes Association (ADA) criteria for the diagnosis of diabetes are any of the following [13].

- A haemoglobin A1c (HbA1c) level of 6.5% or higher; the test should be performed in a laboratory using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay, or
- A fasting plasma glucose (FPG) level of 126 mg/dL (7 mmol/L) or higher; fasting is defined as no caloric intake for at least 8 hours, or
- A 2-hour plasma glucose level of 200 mg/dL (11.1 mmol/L) or higher during a 75-g oral glucose tolerance test (OGTT), or
- A random plasma glucose of 200 mg/dL (11.1 mmol/L) or higher in a patient with classic symptoms of hyperglycemia (i.e., polyuria, polydipsia, polyphagia, weight loss) or hyperglycemic crisis

DATA COLLECTION

Demographic data, laboratory result and clinical details of all potential candidates were recorded in an especially designed questionnaire

STATISTICAL ANALYSIS

Un-paired t-test was applied to analysed the study data

RESULTS

In our study characteristics of the study population shows the demographic and clinical characteristics of the case and control groups.

Table-1: Comparison of baseline characteristics between case and control groups

	Case (n=100) Mean ± SD	Control (n=100) Mean ± SD	p - value
Age	53.33 ± 8.23	49.21± 7.01	0.78
Sex (Male/female)	67/33	61/39	
BMI (kg/cm^2)	26.9	23.1	< 0.001
WC (cm)	89.21 ± 8.21	82.92 ± 6.93	< 0.001
HIP (cm)	94.94 ± 7.23	92.14 ± 6.42	< 0.001
WHR	0.94	0.85	< 0.001
SBP (mmHg)	137 ± 14	123 ± 13	< 0.001
DBP (mmHg)	89 ± 11	81 ± 9	< 0.001

Baseline characteristics of case and control group as well other biochemical parameter are given in Table 1. Baseline characteristics like age and gender in case and control group were not significant difference. Whereas, there were significant differences in BMI, waist circumference (WC), hip circumference (HIP), waist-to-hip ratio (WHR), Systolic blood pressure (SBP) and diastolic blood pressure (DBP) between case and Control groups (p values < 0.001).

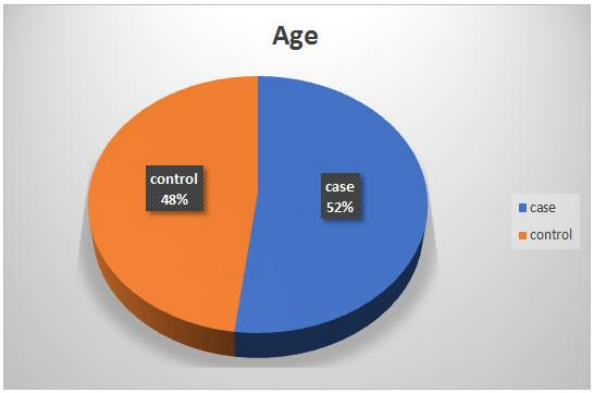

Fig-1: Comparison of age between case and control group

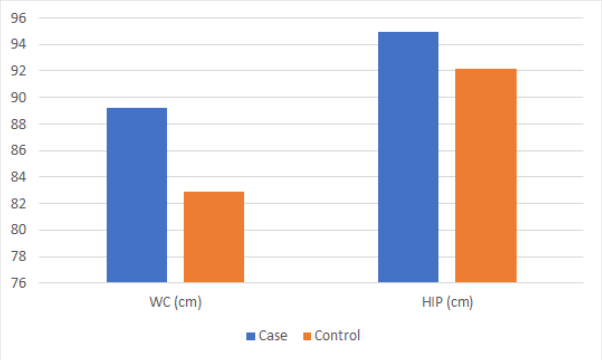

Fig-2: Comparison of age between case and control group

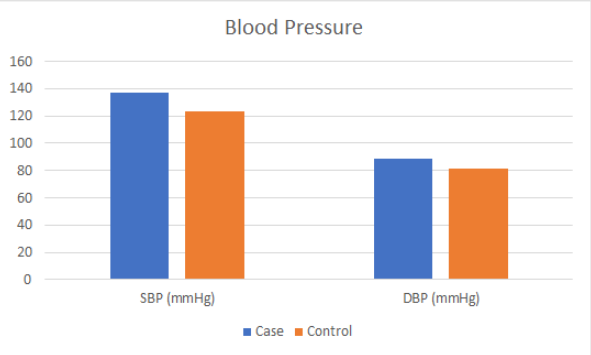

Fig-3: Comparison of blood pressure between case and control group

Table-2: Comparison between case and control group

	Case	Control	p - value
FBS (mg/dl)	168.03 ± 22.45	96.21 ± 14.45	< 0.001
PPBS (mg/dl)	194.78 ± 29.53	121.67 ± 18.34	< 0.001
HbA1c (%)	7.21± 0.83	5.89 ± 0.41	< 0.001
ALP (IU/L)	151 ± 19.54	139 ± 16.23	0.71
hsCRP (mg/dl)	3.17 ± 0.61	1.21± 0.23	< 0.001

Our study shows that mean serum FBS level in case group was significantly higher compared to control (p values < 0.001). Also, PPBS level in case was significantly higher compared to control group (p values < 0.001). Furthermore, mean HbA1c % in case were significantly higher compared to control (p values < 0.001). But serum alkaline phosphatase (ALP) concentration is not increased between case and control groups (p value is 0.71 which considered not significant). In addition, mean hsCRP levels in case were significantly higher compared to control group (p values < 0.001) (Table 2).

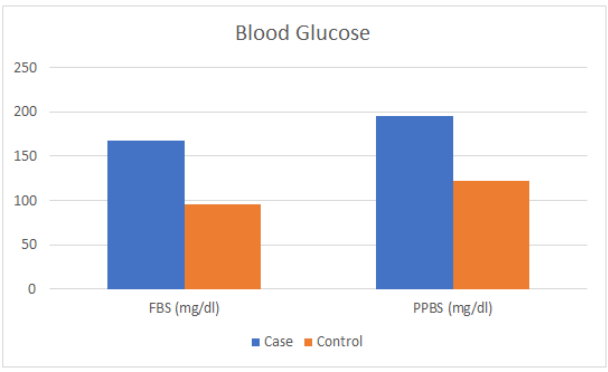

Fig-4: Comparison of blood glucose level between case and control group

Table-3: Values of serum hsCRP and ALP concentration between case and control group

	hsCRP (mg/dl)	ALP (IU/L)
Case	3.17 ± 0.61	151 ± 19.54
Control	1.21± 0.23	139 ± 16.23
t- value	31.23	21.43
P value	<0.0001	<0.0001

There were significant differences in serum hs-CRP and ALP levels between case and control groups (both p < 0.0001). Compared to controls group, there were significantly higher hs-CRP levels and serum ALP in the case group (both p < 0.001) ((Table 3/Figure).

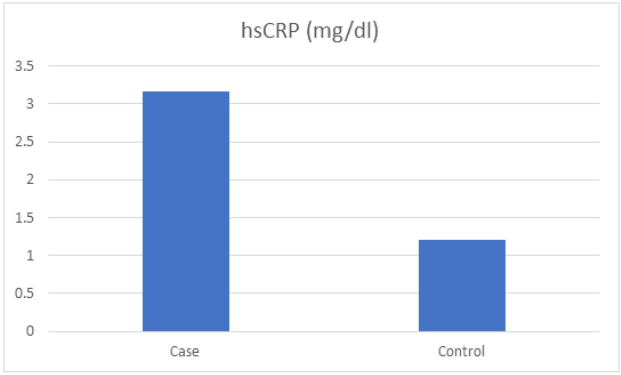

Fig-5: Comparison of hsCRP concentration between case and control group

Table-4: Pearson's correlation analysis between serum hsCRP, ALP and glycaemic control

	Correlation coefficient r value	Two tailed p value
Serum hsCRP with ALP	0.32	<0.0001
Serum hsCRP with FBS	0.37	<0.0001
Serum hsCRP with PPBS	0.36	<0.0001
Serum hsCRP with HbA1c	0.43	<0.0001
Serum ALP with FBS	0.33	<0.0001
Serum ALP with PLBS	0.41	<0.0001
Serum ALP with HbA1c	0.72	<0.0001

Correlations of hs-CRP levels with clinical and laboratory parameters between case-control groups

Univariate correlation analysis demonstrated that statistically significant correlations of hs-CRP with other clinical and quantitative laboratory parameters were found (all $p < 0.0001$). In addition, in normal controls, there was a statistically significant positive correlation of serum ALP levels with hs-CRP and glycaemic triad (FBS, PPBS and HbA1c) in the case group (all $p < 0.0001$) (Table 4).

DISCUSSION

The aim of our study is to correlate serum hs-CRP, serum ALP and glycaemic triad (FBS, PPBS and HbA1c) in case and control group. Our study shows that statistical significantly increased concentration of hs-CRP and ALP in patients with case group (type 2 DM) compared with control group (healthy persons). Also, we found a significant positive linear relationship between hs-CRP and ALP concentration as well as both with FBS, PPBS and HbA1c. These findings suggest a link between inflammations (raised hsCRP concentration), oxidative stress (indicated by increased serum ALP concentration) and glycaemic control in patients with type 2 DM and related complications. Moreover, concentration of hs-CRP and ALP significant increased levle in patients with type 2 DM compared to healthy subjects.

Glycaemic triad such as FBS, PPBS and HbA1c showed a significant rise in type 2 diabetes then control group. Comparing diabetics and non-diabetics have, raised blood glucose level in diabetic patients and change in medium make the individual susceptible to infection due to depressed immunity. It can be seen that significant increase of HbA1c in diabetics then non-diabetics.

Several possible mechanisms which explain increased FBS, PPBS and HbA1c concentration in patients with type 2 DM and its correlation with glycaemic control. Sarinnapakorn V, *et al.* found that hsCRP levels correlated with HbA1c levels [14]. Mean HbA1c levels were significantly higher in patients who had more hsCRP levels. Other factors such as age, BMI, blood pressure, screenings correlated with hsCRP level. Because of a positive correlation between serum hsCRP and FBS, PPBS, HbA1c, and inflammation, insulin resistance and hyperglycaemia jointly contribute to the cardiovascular risk in type 2 DM. This suggests a role of oxidative stress and chronic low-grade inflammation in pathogenesis of type 2 diabetes patients [15].

There are various studies which support our results. R Sharma *et al*. shows rise in levels of hsCRP and ALP in diabetic subjects and their significant association which might be a result of inflammation in diabetes mellitus[16] Ahmed Khan D, *et al*. studied diabetic patients had significantly elevated median of HbA1c, hsCRP and GGT as compared to controls [17] HbA1c showed a positive correlation with hsCRP, ALP and inflammatory markers used in addition to HbA1c for assessment of increased risk in diabetic patients because of accelerated atherosclerosis due to free radical injury[18].

In the present study, our results showed a significantly higher hs-CRP level in the type 2 diabetes patients than in normal controls. Serum hs-CRP is positively associated with the metabolic syndrome and has been acknowledged to be an independent risk factor for development of diabetic neuropathy, diabetic foot ulcers and CV complications [19]. Recently, Aryan *et al*. performed a larger sample population-based study to evaluate the predicted value of hs-CRP for complications of T2DM [20]. Hs-CRP has been applied in clinical settings to monitor chronic and acute inflammatory conditions [21]. In addition, it has also been reported that hs-CRP has an association with insulin resistance, of which CRP may contribute to vascular inflammation and cause injury of vascular cells and further contribute to the development of insulin resistance [22].

Our results also suggest that liver enzymes are closely associated with the risk of metabolic syndrome and type 2 diabetes and that among this serum ALP is the most powerful risk indicator for developing the metabolic syndrome and type 2 diabetes. Similar study conducted Vazarova et stated that serum ALP levels mild increase in Type 2 diabetes patients [23]. Possible pathophysiological mechanism is that elevated liver enzymes may reflect due to obesity, fatty liver, hepatosteatosis, inflammation, which impairs insulin signalling both in the liver and systemically [24]. Elevation of serum ALP could be the expression of an excess deposition of fat in the liver, termed non-alcoholic fatty liver disease. Fatty liver is thought to cause hepatic insulin resistance and to contribute to the development of systemic insulin resistance and hyper insulinemia[25]. Elevated Serum ALP level may be a simple and reliable marker of visceral and hepatic fat and, by inference, of hepatic insulin resistance. In addition, implication of inflammatory in the pathogenesis of diabetes is suggested, not only by oxygen free-radical generation, but also due to non-enzymatic protein glycosylation, autoxidation of glucose, impaired glutathione metabolism [26]. Increases in ALP activity can be a response to oxidative stress, facilitating increased transport of GSH precursors into cells. In addition, ALP is leaked into the serum possibly as a result of normal cell turnover and cellular stresses. These findings suggest that a raised serum ALP level is an independent risk factor for type 2 diabetes [27].

Despite these potential limitations, our findings, which were obtained from a cross sectional study shows that serum ALP activity and hsCRP level

is significantly increased in patients with type 2 diabetes mellitus compared to healthy control. Both are further increased in diabetic patients with complications. Also, there is a significant positive correlation between serum ALP activity and hsCRP. Both are also independently positively correlated with HbA1c, FBS and PPBS (short and long term glycaemic control). So far, the underlying pathophysiological mechanisms due to insulin resistance, oxidative stress and chronic low grade systemic inflammation may be involved. All these finding suggesting a link between oxidative stress, inflammation and glycaemic control in patient with type 2 diabetes mellitus. Further studies are needed to investigate the biological mechanisms underlying this association.

CONCLUSION

Although FBS, PPBS, HbA1c, hs-CRP and ALP have been studied in many metabolic diseases, there are very few studies regarding the expression of FBS, PPBS, HbA1c, hs-CRP and ALP and their association with Type 2 DM. In the present study, we investigated the serum FBS, PPBS, HbA1c, hs-CRP and ALP concentrations in Type 2DM and their relations with clinical and laboratory features. The current study demonstrated that, in comparison to healthy controls, there were significantly increased FBS, PPBS, HbA1c, hs-CRP and ALP levels in Type 2DM. Furthermore, hs-CRP level was correlated with BMI, ALP in the Type 2DM group, and was associated with FBS, PPBS and HbA1c. 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, PPBS and HbA1c (markers of glycaemic control). All these finding suggesting a link between oxidative stress, inflammation and glycaemic control in patient with type 2 diabetes mellitus.

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