Research Article

Serum alkaline phosphatase and high sensitivity C-reactive protein in type II diabetes mellitus: a risk of cardio vascular disease in South Indian population

G. Deepika*, N. Veeraiah, Syed Naveed, M. V. Ramana

Department of Biochemistry, Mahavir Institute of Medical Sciences, Vikarabad, Telangana, India

Received: 13 February 2016
Revised: 16 February 2016
Accepted: 08 March 2016

*Correspondence:
Dr. G. Deepika,
E-mail: dr.deepikapavan@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: 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. The aim of the study was to examine the relationship between alkaline phosphatase (ALP) and high sensitive C reactive protein (hsCRP), in type 2 diabetic patients. We assessed the association of ALP and hsCRP levels with CVD complication and determined its utility for CVD risk prediction in type 2 DM subjects with good and poor glycemic control. Further, we investigated correlation between serum ALP and hsCRP level with glycemic control (FBS, PP2BS, HbA1c) in subjects.

Methods: A cross sectional study consists of 390 patients out of which 100 normal healthy control (Group I), 120 patients having type 2 DM with good control (Group II), 170 patients with type 2 DM with poor control (Group III) were selected. Serum ALP, serum hsCRP, FBS, PP2BS, HbA1c, and other biochemical investigations including serum liver enzymes and lipid profile were measured.

Results: In Study I Mean serum ALP(145.17±23.91) and hsCRP (2.53±0.76) concentration in group II patients when compared to group I serum ALP(142.17±16.48) and Hscrp (1.51±0.15) shows a significance of ALP (p<0.05) and Hscrp (p<0.001).Study II Mean serum ALP(145.17±23.91) and hsCRP (2.53±0.76) concentration in group II patients when compared to group III serum ALP (147.79±28.95) and Hscrp (3.84±0.47) group shows a significance of ALP (p<0.001) and Hscrp (p<0.05). Study III Mean serum ALP (147.79±28.95) and hsCRP (3.84±0.47) concentration in group III patients when compared to group I serum ALP (142.17±16.48) and Hscrp (1.51±0.15) shows a high significance of both ALP and Hscrp (p<0.001). Further significant positive correlation was observed between ALP and hsCRP concentration as well as with HbA1c, FBS, and PP2BS.

Conclusions: Inflammation along with the poor glycemic control in diabetes play a role in diabetic macrovascular complication like CVD. All these finding are showing a link between CVD, inflammation and glycemic control in patient with type 2 diabetes mellitus.

Keywords: Inflammation, Poor glycemic control, Diabetes, CVD

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
Diabetes mellitus (DM) comprises a group of common metabolic disorders that share common phenotype of hyperglycemia. 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. Alkaline phosphatases (ALP) is a hydrolase enzyme, which is widely expressed in human tissues, but is highly concentrated in the liver, bone, and kidney. 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 macrovascular 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). 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. 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. 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. 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.

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.

The purpose of study was to investigate a possible correlation between raised ALP levels in type 2 diabetics and non-diabetics. 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. Further, we investigated correlation between serum ALP and hsCRP with glycemic control in subjects. Therefore, to put the interdependence between ALP and hsCRP levels into clinical perspective, we also aimed to determine whether the ALP-CVD relationship is confounded or modified by hsCRP. Finally, we aimed to investigate for the extent to which ALP measurements could improve the prediction of first-onset CVD outcomes in diabetes.

**METHODS**

This study was a hospital based cross sectional study conducted at Mahavir Institute of Medical sciences, Vikarabad, Telangana (India) between May 2015 to Jan 2016. A cross sectional study consists of 390 subjects out of them 120 patients having type 2 DM with good glycemic control (Group II), 170 patients with type 2 DM with poor glycemic control (Group III) and 100 normal healthy control (Group I) were selected. Subjects were recruited according to simple random sampling method meeting the selection criteria.

**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 – Type 2 DM patients with good glycemic control (n=120) this group consisted of patients with type 2 DM with duration less than 8 years, HbA1c level <7%. They were on life style modifications and oral
hypoglycemic drugs and free from clinical evidence of any complication of diabetes mellitus.

Group III – Type 2 DM patients with poor glycemic control (n=170) this group consisted of patients with type 2 DM with duration more than 8 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.

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 (Kit: Quantitative determination blood sugar by glucose oxidase peroxidase method mfg by Spinreact). Glycated hemoglobin (HbA1c) concentration was measured by High Performance Liquid Chromatography (HPLC) method (Kit: Quantitative determination of Bio-rad D10 (HbA1c) in human blood by mfg . Serum ALP activity was determined by carboxy substrate kinetic method. (Kit:Quantitative determination of Alp by carboxy substrate method mfg by Coral Crest biosystems). Serum hsCRP level is measured by immunoturbidimetric method (Kit: Quantitative determination of hsCRP in human blood by latex turbidimetry assay mfg by Spinreact). All other biochemical investigation includes serum liver enzymes, lipids, and other biochemical blood measurements were determined using standard laboratory procedures on semi autoanlyser 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 <0.05 was considered statistically significant. Pearson linear correlation was used to Study correlation between parameters.

RESULTS

Our study shows Study I Mean serum ALP (145.17±23.91) and hsCRP (2.53±0.76) concentration in group II patients when compared to group I serum ALP(142.17±16.48) and Hscrp (1.51±0.15) shows a significance of ALP as (p<0.05) and Hscrp as (p<0.001). Study II Mean serum ALP (145.17±23.91) and hsCRP (2.53±0.76) concentration in group II patients when compared to group III serum ALP (147.79±28.95) and Hscrp (3.848±0.47) group shows a significance of ALP as (p<0.001) and Hscrp as (p<0.05). Study III Mean serum ALP (147.79±28.95) and hsCRP (3.848±0.47) concentration in group III patients when compared to group I serum ALP (142.17±16.48) and Hscrp (1.51±0.15) shows a high significance of both ALP and Hscrp (p<0.001). We have found a statistically significant positive between serum ALP and serum hsCRP concentration in concordance with glycemic control (HbA1c, FBS, and PP2BS) (Table 2, Figures 1-3).
Characteristics like age, sex, were not differing between groups. We found increased serum alanine transaminase (ALT) and aspartate transaminase (AST) concentration in group III compared to group I and group II. 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.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>100</td>
<td>120</td>
<td>170</td>
</tr>
<tr>
<td>Sex (M/F)%</td>
<td>63/37</td>
<td>70/30</td>
<td>108/62</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>53</td>
<td>54</td>
<td>53</td>
</tr>
<tr>
<td>Duration of diabetes (In years)</td>
<td>-</td>
<td>5.11</td>
<td>12.28</td>
</tr>
<tr>
<td>Serum ALT concentration (IU/L)</td>
<td>142.17±16.48</td>
<td>145.17±23.91</td>
<td>147.79±28.95</td>
</tr>
<tr>
<td>Serum hsCRP concentration (mg/L)</td>
<td>1.51±0.15</td>
<td>2.50±0.76</td>
<td>3.848±0.47</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.54±0.31</td>
<td>6.45±0.29</td>
<td>8.04±0.643</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>90.68±13.20</td>
<td>127.70±30.0</td>
<td>190.59±37.48</td>
</tr>
<tr>
<td>PP2BS (mg/dl)</td>
<td>112.18±8.98</td>
<td>153.47±16.29</td>
<td>293.04±30.1</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>150.99±16.7</td>
<td>193.32±30.87</td>
<td>198.08±40.69</td>
</tr>
<tr>
<td>Triglycerides total (mg/dl)</td>
<td>110.41±13.55</td>
<td>132.83±20.2</td>
<td>139.22±44.43</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>18.68±3.95</td>
<td>20.46±4.72</td>
<td>25.82±2.43</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>19.03±3.75</td>
<td>22.78±10.83</td>
<td>25.8±2.43</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Study groups</th>
<th>ALP (IU/L)</th>
<th>HsCRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>142.17±16.48</td>
<td>1.52±0.15</td>
</tr>
<tr>
<td>Group II</td>
<td>145.17±23.91</td>
<td>2.53±0.76</td>
</tr>
<tr>
<td>t value</td>
<td>1.45</td>
<td>13.43</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.05</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 3: Values of serum ALP and HsCRP concentration between study group II and group III.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>ALP (IU/L)</th>
<th>HsCRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>145.17±23.91</td>
<td>2.53±0.76</td>
</tr>
<tr>
<td>Group III</td>
<td>147.79±28.95</td>
<td>3.88±0.61</td>
</tr>
<tr>
<td>t value</td>
<td>3.66</td>
<td>16.50</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.0001</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

DISCUSSION

Our study shows statistical significantly increased concentration of ALP and hsCRP in serum in patient with type 2 DM with poor glycemic control compared to healthy persons as well as subjects having type 2 DM with good glycemic control. Also we found a significant positive linear relationship between ALP and hsCRP concentration as well as both with HbA1C, FBS, and PP2BS. These findings suggest a link between Table 1 Values of Serum ALP and hsCRP concentration between Study Groups I, II, III.

Activity indicated by increased serum ALP concentration, inflammation (raised hsCRP concentration) and glycemic control in patients with type 2 DM and related complications. Also at levels of ALP and hsCRP considered well within the normal range, there was a substantial and significant increased concentration in patients with type 2 DM with good glycemic control compared to healthy subjects. This suggests a role of oxidative stress and chronic low grade inflammation in pathogenesis of type 2 diabetes patients. Several possible mechanisms which explain increased
Table 5: Pearson’s correlation analysis between serum ALP and hsCRP and glycemic control.

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient r value</th>
<th>Two tailed p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALP with hsCRP</td>
<td>0.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum ALP with HbA1c</td>
<td>0.84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum ALP with FBS</td>
<td>0.38</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum hsCRP with ALP</td>
<td>0.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum hsCRP with HbA1c</td>
<td>0.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum hsCRP with FBS</td>
<td>0.37</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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 insulinaemia. Thus, ALP could serve as a marker of the insulin resistance syndrome in the pathogenesis of diabetes. There is now growing evidence to suggest that ALP is not only a marker of fatty liver but also a marker of CVD. Experimental studies have reported that ALP has a central role in the maintenance of intracellular antioxidant defences through its mediation of extracellular glutathione transport into most types of cells. It is an ectoenzyme normally present at the outer side of the cell membrane that has the primary function of maintaining intracellular concentrations of glutathione (GSH), a critical antioxidant defence for the cell. 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.

Inflammatory tissue is currently suggested as mechanism underlying diabetes and diabetic complications. In recent years, much attention has been focused on the role of CVD, and it has been reported that inflammatory may constitute the key and common event in the pathogenesis of secondary diabetic complications. 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.

Our finding of relationship between ALP and CVD risk is compatible with some previous reports. In the 20th year follow-up examination and analyses of the British Regional Heart Study (BRHS), the results were suggestive of a non-linear association between ALP and stroke/CVD events in older men aged 60 to 79 years. It is therefore suggested that measurements of other inflammatory markers including C-reactive protein by a validated high-sensitivity assay be added in an attempt to substantiate this hypothesis.

Figure 4: Correlation between serum HSCRP and HBA1C concentration in patients.

Figure 5: Showing correlation between serum HSCPR and FBS concentration in patients.

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. Ahmed Khan D, et al studied diabetic patients had significantly elevated median of HbA1c, hsCRP, total cholesterol, nitrate and GGT as compared to controls. HbA1c showed a positive correlation with hsCRP, total cholesterol, nitrate and ALP inflammatory markers should be used in addition to HbA1c for assessment of increased cardiac risk in uncontrolled diabetic patients because of accelerated atherosclerosis due to free radical injury. Sarinannaporn V, et al found that hsCRP levels correlated with HbA1c levels. Mean HbA1c levels were significantly higher in patients who had hsCRP levels of mg/L or more. Other factors such as age, LDL cholesterol, Screenings correlated with hsCRP level. Also Bahceci M, et al compare serum hsCRP levels in type 2 diabetic men without coronary heart diseases (CHD), non-diabetic CHD patients and type 2 DM patients with CHD and shows type 2 DM men without CHD had similar CRP levels with non-diabetic CHD patients, whereas CRP levels of type 2 DM men with CHD were higher than non-diabetic men with CHD.
Because of a positive correlation between serum hsCRP and HbA1c, fasting insulin and HOMA-IR, inflammation, insulin resistance and hyperglycemia jointly contribute to the cardiovascular risk in type 2 DM men. Other lines of evidence support a relationship between elevated serum GGT and poor glycemic control and metabolic syndrome are also found. Higher ALP levels are accompanied by more insulin resistance and greater risk for developing type 2 DM and poor glycemic control. The strong association of serum ALP activity with some diabetes related metabolic disorders, such as atherogenic dyslipidemia and poor glycemic control, may be explained by underlying, not mutually exclusive, biological mechanisms such as fatty liver, insulin resistance, and enhanced oxidative stress.

Possible that the occurrence of ALP reactions plays a direct role in the pathogenesis of atherogenic dyslipidemia and poor glycemic control, independently of the presence of fatty liver, possibly through the induction of chronic inflammation and insulin resistance. Supporting a role of serum ALP in the inflammation levels of inflammation markers, such as fibrinogen, uric acid, CRP, and F2-isoprostanes, in a dose response manner. Several studies demonstrate that hsCRP remained a significant predictor of diabetes risk even after adjusting with body mass index, family history of diabetes mellitus, smoking and other factors. In general, serum ALP concentration is closely related with other enzymes more specific to the liver, serum ALT or AST concentration, so we did parallel analyses with ALT and AST to further explore the possible role of liver damage in the association of ALP with diabetes. Within their normal ranges, ALT and AST showed significant increase in type 2 diabetes patients with poor control (p value <0.001).

Moreover, markers of hepatic fat content, such as serum GGT activity and other liver enzymes, have been shown in large prospective studies to predict the incidence of type 2 diabetes, insulin resistance, and cardiovascular disease independently of obesity. Our results also suggest that liver enzymes are closely associated with the risk of metabolic syndrome and type 2 diabetes and that among these enzymes serum ALP is the most powerful risk indicator for developing the metabolic syndrome and type 2 diabetes. Another possible path physiological mechanism is that elevated liver enzymes may reflect inflammation, which impairs insulin signalling both in the liver and systemically.

ALP showed a significant rise in both diabetic and non-diabetic patients as compared to control. Comparing diabetics (Group II and III) and non diabetics (Group I) 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 ALP in diabetics (Group II and III) and non-diabetics (Group I) and hsCRP during follow-up was not included in the analysis. Further, we could not include several confounding variables in this study, such as fasting insulin concentration. Therefore, fasting insulin concentration should be included in future studies.

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 and poor glycemic control. Also there is a significant positive correlation between serum ALP activity and hsCRP. Both are also independently positively correlated with HbA1c, FBS and PP2BS (short and long term glycemic control). So far, the underlying pathophysiological mechanisms are not entirely clear. It seems that insulin resistance, oxidative stress and chronic low grade systemic inflammation may be involved. All these finding suggesting a link between oxidative stress, inflammation and glycemic control in patient with type 2 diabetes mellitus. Further studies are needed to investigate the biological mechanisms underlying this association.

CONCLUSION

In 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.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

35. Hsueh WA, Quinones MJ: Role of endothelial dysfunction in insulin resistance. Am J Cardiol. 2003;92:10J-17J.