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Original Article

Correlation between Serum Alkaline Phosphatase And High Sensitivity C-reactive Protein in Type 2 Diabetes Mellitus Patients

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Abstract

Background: Diabetes mellitus is a lifelong chronic disease and is one of the most challenging health problems in the 21st century, rapidly rising all over the globe at an alarming rate. Alkaline phosphatase (ALP) is a widely used marker for skeletal and hepatobiliary disorders, but its activity is also increased in atherosclerosis and peripheral vascular disease. High-sensitivity C-reactive protein (hsCRP) is an acute-phase reactant and a sensitive marker of inflammation.

Aims: The aim of the study was to examine the relationship between serum ALP and hsCRP levels in type 2 diabetes mellitus (T2DM) patients, along with parameters of glycemic control (fasting blood glucose and glycosylated hemoglobin).

Materials and Methods: This was a case–control-study in which the study group included 50 patients above the age of 30 with clinically diagnosed T2DM who were either visiting outpatient departments or admitted in the Department of Medicine, Guru Nanak Dev Hospital attached to Government Medical College, Amritsar. Fifty-year-old and sex-matched individuals were taken to serve as controls. All the individuals were investigated for fasting blood glucose, glycosylated hemoglobin, serum ALP, high-sensitivity CRP, and serum transaminases (alanine aminotransferase and aspartate aminotransferase).

Result: The mean serum ALP and high-sensitivity CRP levels were statistically significantly higher in T2DM patients compared to controls. Furthermore, a significant positive association was observed between serum ALP and high-sensitivity CRP levels, as well as both with fasting blood glucose and glycosylated hemoglobin.

Conclusion: Oxidative stress and inflammation appear to be a key component in the pathogenesis of DM and its complications. All these findings suggest a link between oxidative stress, inflammation, and glycemic control in T2DM patients.

Key words: Serum alkaline phosphatase, Glycosylated hemoglobin, High sensitivity C-reactive protein, Serum transaminases (alanine aminotransferase and aspartate aminotransferase)

INTRODUCTION

Diabetes mellitus is not one disease but rather is a heterogeneous group of multifactorial, primarily polygenic syndromes that are characterized by abnormal metabolism of carbohydrate, protein, and fat, resulting in hyperglycemia due to an absolute or relative deficiency of insulin, ending up in vascular complications leading to retinopathy, neuropathy, and nephropathy.[1] It is constantly increasing worldwide due to the aging population, urbanization, and obesity. Over the last few decades, the number of patients with diabetes, especially type 2 diabetes mellitus (T2DM), has risen to 350 million worldwide, and it is estimated that this figure will further increase to 592 million worldwide by the year 2035.[2] T2DM covers about 90% of all cases and results from the interaction between genetic, environmental, and behavioral risk factors with the features of hyperglycemia, insulin resistance, and relative insulin deficiency.[3] Hyperglycemia not only defines the disease but is the major cause of its most characteristic symptoms and long-term complications.[1,3] Insulin is the

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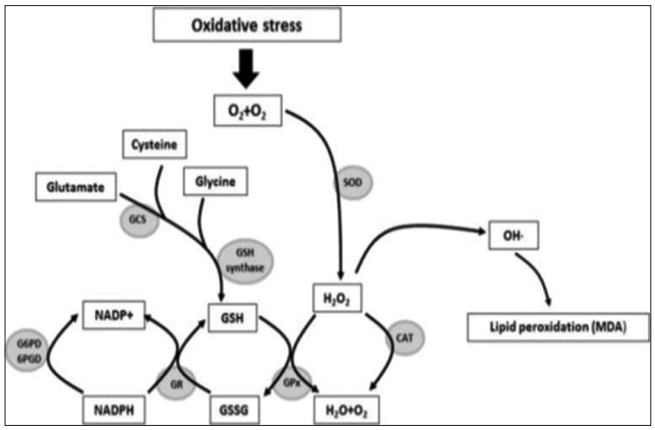

Figure 1: The role of oxidative stress in tissue injury[6]

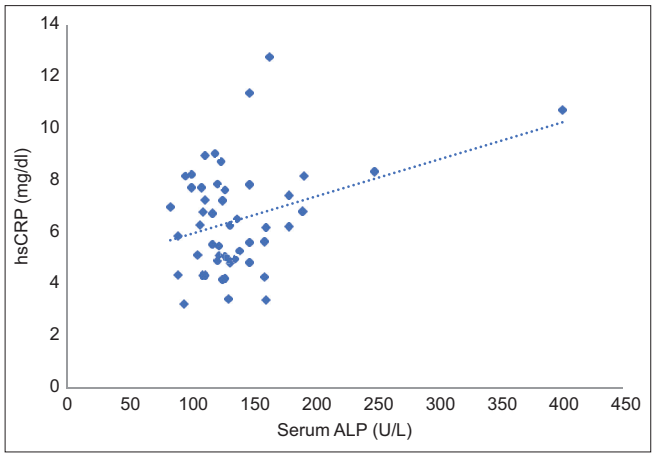

Figure 2: Correlation between serum alkaline phosphatase and high-sensitivity C-reactive protein in Type 2 diabetes mellitus patients with an r-value of +0.34

principle hormone that regulates the uptake of glucose from the blood into cells, including skeletal muscle cells and adipocytes.[3]

Understanding the pathogenesis and preventing long-term complications have been the 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 T2DM and links it with concomitant conditions with inflammatory conditions.[4]

Alkaline phosphatase (ALP) belongs to a group of ubiquitous metalloenzymes that are widely expressed in human tissues, but it is highly concentrated in human tissues, especially in the liver, bone, and kidney. ALP is a generally accepted clinical marker of hepatic or bone disease. It has been reported that many diabetics also exhibit elevated ALP levels.[4,5]

The liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are routinely used in the evaluation of liver function. AST and ALT are considered markers of hepatocellular death. ALT is the most specific marker of liver pathology, whereas AST is also found in other tissues and is a less specific marker of liver function.[4] Mild elevations of liver enzymes are associated with higher plasma C-reactive protein (CRP) concentrations. Hepatic inflammation secondary to non-alcoholic fatty liver disease is a potential contributor to the chronic low-grade inflammation associated with metabolic risk factors and the metabolic syndrome.[5,6]

There has been an increasing interest in the involvement of low-grade inflammation in the pathogenesis of T2DM. The mechanisms by which chronic inflammation can evoke T2DM are not clear. However, it is known that adipose tissue can synthesize and release the main pro-inflammatory cytokines, tumor necrosis factor-alpha, interleukin-1 (IL-1), and IL-6, and inflammatory markers that are associated with body fat mass.

Pro-inflammatory cytokines and acute-phase reactants are involved in multiple metabolic pathways relevant to insulin resistance.[6] CRP, measured as high-sensitivity CRP, an acute-phase reactant produced by the liver, is an extremely sensitive marker of systemic inflammation.

In addition, high-sensitivity C-reactive protein (hsCRP) has also emerged as a powerful predictor of cardiovascular disease. However, hsCRP levels are known to vary among populations, influenced by gender, age, and obesity.[7]

ALP is an inflammatory mediator like hsCRP (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 a common biological pathway. Chronic, systemic subclinical inflammation has also been identified as a driving force for insulin resistance, metabolic syndrome, and T2DM.[5,7] 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 measurement and the measurement of glycosylated hemoglobin A1c (HbA1c). These measurements are complementary, as the patient's glucose measurements provide a picture of short-term glycemic control, whereas HbA1c reflects the average glycemic control over the previous 3 months.[8]

Since inflammation appears to be a key component of many reactions that are associated with poor glycemic control and the further pathogenesis of diabetes and its complications, the purpose of the study was to find an

Table 1: Comparison of baseline characteristics between T2DM patients and controls

Baseline parameter	T2DM patients Mean±SD	Controls Mean±SD	*P*–value
Age	56.26±7.41	42.68±12.16	0.02
Height (cm)	165.6±8.47	171.2±9.55	>0.05
Weight (Kg)	71.44±8.80	67.92±7.71	>0.05
BMI (Kg/m^2)	25.99±2.12	23.77±2.38	0.01
WC (inches)	20.43±3.88	18.83±3.99	0.01

T2DM: Type 2 diabetes mellitus, BMI: Body mass index, WC: Waist circumference, SD: Standard deviation

Table 2: Variations in fasting blood glucose and glycosylated hemoglobin levels in T2DM patients and controls

Groups	FBG (mg/dL)	HbA1c (%)
T2DM patients	166.9±3.88	7.68±1.32
Controls	83.72±7.49	4.90±0.40
P-value	0.001	0.001

T2DM: Type 2 diabetes mellitus, FBG: Fasting blood glucose, HbA1c: Hemoglobin A1C

Table 3: Variations in serum ALP and high-sensitivity C-reactive protein levels in T2DM patients and controls

Groups	Serum ALP (U/L)	hsCRP (mg/dL)
T2DM patients	137.48±48.88	6.45±2.04
Controls	78.2±16.24	0.32±0.26
P-value	0.001	0.001

ALP: Alkaline phosphatase, hsCRP: High sensitivity C-reactive protein, T2DM: Type 2 diabetes mellitus, HbA1c: Hemoglobin A1C

Table 4: Variations in serum transaminases levels in T2DM patients and controls

Groups	Serum ALT (U/L)	Serum AST (U/L)
T2DM patients	57.6±18.46	59.96±17.44
Controls	27.8±7.54	26.44±5.59
P-value	0.001	0.001

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, T2DM: Type 2 diabetes mellitus

Table 5: Variations in serum fasting blood glucose, glycosylated hemoglobin, serum ALP, high-sensitivity CRP, and serum transaminase levels in T2DM patients residing in urban and rural area

T2DM patients	Urban area	Rural area	*P*-value
Serum FBG	167.2±28.97	167.95±24.79	>0.05
HbA1c	7.69±1.17	7.68±1.51	>0.05
Serum ALP	141.0±61.17	134.9±33.62	0.67
hsCRP	6.29±2.01	6.57±2.13	0.64
Serum ALT	53.96±17.59	61.08±19.33	>0.05
Serum AST	56.44±14.33	59.12±19.04	>0.05

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CRP: C-reactive protein, ALP: Alkaline phosphatase, hsCRP: High sensitivity C-reactive protein, FBG: Fasting blood glucose, T2DM: Type 2 diabetes mellitus, HbA1c: Hemoglobin A1C

association between serum ALP and hsCRP in T2DM along with the parameters of glycemic control.

MATERIALS AND METHODS

This was a case–control study comprising of 100 individuals. The study group included 50 patients above the age of 30 with clinically diagnosed T2DM who were either visiting outpatient departments or admitted in the Department of Medicine, Guru Nanak Dev Hospital attached to Government Medical College, Amritsar. Fifty normal, healthy volunteers from the general population served as controls. The following subjects were excluded from the study:

- Patients with Type 1 diabetes mellitus
- High (>120 g/L) alcohol consumption
- Chronic liver diseases
- Chronic renal diseases
- Any chronic infections like tuberculosis
- Smokers
- Thyroid disorders.

All the subjects included in the study were investigated for:

Fasting blood glucose levels were estimated by the GOD-POD Method[9], glycated (HbA1c) levels were measured by the Ion-Exchange Resin Method[10], liver enzymes (ALT, AST, and ALP)[11-13] were estimated using the IFCC Method, and hsCRP was measured by a turbidimetric immunoassay.[14]

Statistical Analysis

Mean ± SD was calculated for all the parameters analyzed and were compared by the Student's t test and analysis of variance (SPSS version 17) for correlation among different parameters. *P*-value considered:

$P < 0.05$ – Significant

$P < 0.001$ – Highly Significant

RESULTS

It was observed that levels of fasting blood glucose (FBG) and HbA1c were increased significantly ($P \leq 0.001$) in T2DM patients as compared to healthy individuals.

The mean serum levels of ALP and hsCRP were high in T2DM patients as compared to controls ($P \leq 0.001$).

It was observed that levels of serum ALT and serum AST were significantly raised in T2DM patients as compared to controls ($P \leq 0.001$).

It was found that in T2DM patients of urban regions, the level of fasting blood glucose was 167.2 ± 28.97 mg/dL and in rural regions, it was 167.95 ± 24.79 mg/dL, whereas the HbA1c levels in urban regions were 7.69 ± 1.17% and in rural regions, they were 7.68 ± 1.51%.

Levels of serum ALP were higher in T2DM patients residing in urban areas and lower in patients of T2DM in rural area, whereas serum hsCRP was higher in T2DM patients residing in rural areas and lower in urban areas.

It was also observed that T2DM patients residing in rural areas show a higher level of serum transaminases (ALT and AST) as compared to T2DM patients residing in urban regions [Figures 1 and 2 and Tables 1-5].

DISCUSSION

A statistically significant increase was observed in the levels of serum ALP and hsCRP in T2DM patients as compared with controls. Furthermore, a significant positive linear relationship was observed between serum ALP and hsCRP concentrations, along with FBG and HbA1c. These findings suggest a link between oxidative stress (indicated by increased serum ALP concentration), inflammation (indicated by raised hsCRP concentration), and glycemic control (raised levels of FBG and HbA1c) in T2DM patients as compared to controls.

Glycemic markers such as FBS and HbA1c showed a significant rise in T2DM patients than controls. Sarinnapakorn and Wanicagool,[15] found that hsCRP levels correlated with HbA1c levels. Because of a positive correlation between serum hsCRP and FBS, PPBS, HbA1c, and inflammation, insulin resistance and hyperglycemia jointly contribute to the cardiovascular risk in T2DM.[16] This suggests the role of oxidative stress and chronic low-grade inflammation in the pathogenesis of T2DM.

The pathophysiology of T2DM revealed that oxidative stress is one of the factors that play a role in the pathogenesis of insulin resistance, impaired insulin secretion, glucose utilization, and impaired hepatic glucose metabolism, coupled with the activation of pro-inflammatory cytokines.[17] Oxidative stress in pancreatic beta cells is known to be induced by high glucose levels, hyperlipidemia, and inflammatory responses.

Elevation in serum ALP levels reflects an excess deposition of fat in the liver, termed non-alcoholic fatty liver disease. Fatty livers are thought to cause hepatic insulin resistance and also contribute to the development of systemic insulin resistance and hyperinsulinemia.[4] Thus, ALP could serve as a marker of insulin resistance in the pathogenesis of diabetes. Experimental studies have reported that ALP has a central role in the maintenance of intracellular antioxidant defenses through its mediation of extracellular glutathione (GSH) transport into most of the cells. Its primary function is to maintain the intracellular concentrations of GSH, a critical antioxidant defense for all cells. An increase in ALP activity can be a response to oxidative stress, facilitating increased transport of GSH precursors into the cells. In addition, ALP is leaked into the serum, possibly as a result of normal cell turnover and cellular stresses.[18] These findings suggest that a raised serum ALP level is an independent risk factor for T2DM.

Our results suggest that liver enzymes are closely associated with the risk of metabolic syndrome and T2DM, and among this serum ALP is the most powerful risk indicator for developing metabolic syndrome and T2DM. According to Vozarova *et al.*,[19] it was estimated that the liver enzymes AST, ALT, and ALP were significantly higher in diabetic patients as compared to non-diabetic controls.

Our study showed significantly higher hsCRP levels in T2DM patients as compared to controls. hsCRP is positively associated with the metabolic syndrome and has been acknowledged to be an independent risk factor for the development of diabetic neuropathy, diabetic foot ulcers, and CV complications.[9] In addition, it has also been reported that hsCRP has an association with insulin resistance, which may contribute to vascular inflammation, cause injury of vascular cells, and further contribute to the development of insulin resistance.[5]

In the present study, when the FBG levels and HbA1c levels were compared among T2DM patients residing in urban and rural areas, it was found that in T2DM patients, the levels of fasting blood glucose and HbA1c do not show any significant difference ($P > 0.05$). It was found that in T2DM patients of urban regions, the level of fasting blood glucose was 167.2 ± 28.97 mg/dL and in rural regions, it was 167.95 ± 24.79 mg/dL, whereas the HbA1c levels in urban regions were 7.69 ± 1.17% and in rural regions, they were 7.68 ± 1.51%. It was observed that the levels of serum ALP were higher in T2DM patients in urban regions with a mean ± SD of 141.04 ± 61.17 U/L and lower in patients of T2DM in rural regions with a mean ± SD of 134.95 ± 33.62 U/L. The level of hsCRP was higher in rural regions with a mean ± SD of 6.57 ± 2.13 mg/dl, and in urban regions, the level of hsCRP was lower with a mean ± SD of 6.29 ± 2.01 mg/dl. In adults, the prevalence of diabetes is 2–3 folds greater in urban than in rural population. National surveys showed that there has been a marked decrease in undernutrition and a significant

increase in the prevalence of overweight and obesity, more specifically among the urban populations of India.

CONCLUSION

The present study demonstrated that, in comparison to healthy controls, there were significantly increased serum FBG, HbA1c, serum ALP, and hsCRP levels in T2DM patients. There is a significant positive correlation between serum ALP and hsCRP levels. Serum ALP and hsCRP concentrations were independently and positively associated with serum FBG and HbA1c (markers of glycemic index). All these findings suggest a link between oxidative stress, inflammation, and glycemic control in T2DM patients. Furthermore, the raised levels of hsCRP in T2DM patients may contribute to atherosclerotic processes leading to the development of coronary heart disease in these patients, and this can be used as a marker of the development of atherosclerosis in T2DM patients. Furthermore, this study suggested that liver transaminases (ALT and AST) have shown higher activity in T2DM patients than in healthy individuals. The reasons for elevated transaminases in an insulin-resistant state include oxidative stress from reactive lipid peroxidation, peroxisomal β-oxidation, and recruited inflammatory cells. Elevation of these enzymes could also be due to the direct hepatotoxic effect of fatty acid on the liver when it is produced in excess.

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