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Original research

Evaluation of serum alkaline phosphatase and calcium in type 2 diabetes mellitus patients at Lucknow, India

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Abstract

There is an increasing demand and requirement to develop new biomarkers for the early diagnosis and detection of diabetes mellitus (DM). The present study is designed to estimate serum alkaline phosphatase (ALP) and serum calcium levels in type 2 diabetes mellitus (T2DM) patients. The serum alkaline phosphatase and calcium levels were estimated using commercially available kits on a semiautoanalyzer. The fasting blood sugar (FBS) of the subjects was measured by the glucose oxidase-peroxidase (GOD-POD) method using a commercially available kit. The level of ALP was found to be statistically significant in T2DM patients when compared with matched healthy individuals ($p < 0.001$), whereas the association of serum calcium level was found to be non-significant ($p = 0.07$). Although there is a decrease in serum calcium level in T2DM patients when compared with healthy controls. It is concluded that the management of T2DM and its associated complications may require regular estimation of serum ALP and calcium levels. The estimations may also be significant in the management of osteoporosis in T2DM patients. High utilization of calcium and vitamin D especially from supplements may lower the risk of DM.

Keywords: Alkaline phosphatase, Diabetes mellitus, Osteoporosis, Serum calcium, T2DM

1. Introduction

Diabetes is considered to be an important health issue with a significant burden worldwide [1]. The number of people with type 2 diabetes mellitus (T2DM) worldwide is predicted to double between 2000 and 2030 [2], and India will be one of the countries with the maximum number of people having T2DM including China and United States [3]. The incidence and prevalence of DM is increasing because of the aging population and the prevalence of obesity and sedentary lifestyle. About 195 million world population in different age groups are affected by DM and it is one of the major causes of impairment and death. T2DM patients, having progressive insulin deficiency, longer duration of diabetes, and glycaemic control increase the risk of hyperglycaemia in patients [2]. In T2DM, the variations in blood sugar were positively correlated with the development of coronary artery disease (CAD) and blood vessel endothelium is damaged by chronic persistent hyperglycaemia.

Increase in ALP concentration may occur due to increased osteoblastic activity which occurs due to depletion of calcium bone mineral content leading to alveolar bone loss or fractures. Patients with T2DM may be in the condition of oxidative stress, during which the ALP is released in order to facilitate the increased transport of glutathione (GSH) into the cell. Hence, increased ALP level plays a vital role in the maintenance of intracellular antioxidant defence mechanism. Calcium is an important element found in

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teeth and bones, providing strength and has a structural function [4]. It is important element for maintaining and regulating metabolic activities of cells, transmission of nerve impulse and contraction of muscles [5]. More than of the calcium in the body is stored in bone as hydroxyapatite which provides skeletal strength as well as a reservoir for calcium to be released into the serum. In serum, calcium exists in 3 forms namely, protein-bound, ionized (free), and complexed (chelated). The protein-bound (albumin and calmodulin) calcium accounts for 40% of the serum calcium and cannot be used by tissues. The serum calcium is often chelated into the ionic complexes of calcium phosphate, calcium carbonate, and calcium oxalate and allows calcium to be absorbed by various tissues of the body. However, the free calcium, which accounts to about 50% of the serum calcium, is utilized by the body to maintain physiologic functions [5]. Alteration in serum calcium levels in plasma of T2DM patients results in peripheral insulin resistance due to impairment in signal transmission of insulin due to which the activity of glucose transporter protein 4 (GLUT4) is decreased [6]. In hyperglycemia, calcium is mostly excreted from the bone in urine in T2DM patients [7]. Decrease level of serum calcium in T2DM patients may be due to hyperglycemia which results in excessive urinary loss of calcium. Hence, the study was designed to evaluate the activity of serum alkaline phosphatase and calcium levels in T2DM patients and control subjects. We also wanted to determine the co-relation (if any) in the studied parameters in T2DM patients.

2. Materials and Methods

2.1 Subject selection

This case-control study was approved by Institutional Ethical Committee of the IIMS&R, Integral University, Lucknow (IEC/IIMS&R/2019/35). A total of 92 subjects aged from 30-65 years were included in the study, 46 were T2DM patients and 46 healthy controls. Subjects were enrolled after taking proper medical and family history and informed consent from each subjects enrolled was taken and recorded. T2DM was diagnosed if the fasting plasma glucose was estimated ≥ 126 mg/dl (≥ 7 mmol/l) or 2 hour post glucose was ≥ 200 mg/dl (≥ 11.1 mmol/l) [8, 9].

2.2 Biochemical estimations

Estimation of blood sugar: the fasting blood sugar (FBS) was estimated by the glucose oxidase/peroxidises method [10]. Glucose is oxidized by glucose oxidase (GOD) into gluconic acid and hydrogen peroxide. Hydrogen peroxide is the presence of peroxidase (POD) oxidized the chromogen 4-Amino antipyrine/phenolic compound to a red coloured compound. The intensity of the red coloured is proportion to the glucose concentration and is measured at 505 nm (489-530 nm). The optical dentisty (OD) of the samples was measured at 505 mm and the concentration of glucose in the sample was estimated using the following equation:

Glucose (mg/dl) = A sample/A standard × concentration of standard (100 mg/dl)

Estimation of the serum alkaline phosphatase: Serum ALP was estimated by using commercially available kits (Coral, Tulip Diagnostics Pvt. Ltd, India) using a semi-autoanalyzer [7]. The activity of ALP was analyzed by the kinetic method using the p-nitrophenyl phosphate (p-NPP) [11-13].

Estimation of serum calcium by OCPC method: serum calcium levels was measured using commercially available kit (Coral, Tulip Diagnostics Pvt. Ltd., India) using a semi-autoanalyzer [4]. The serum calcium was estimated by the o-cresolphthalein complexone (OCPC) method [14, 15]. Calcium in alkaline medium combines with o-cresolphthalein complex to form a purple colored complex. Intensity of the color formed is directly proportional of the amount of calcium present in the sample and was measured at 570 nm.

2.3 Statistical analysis

Statistical analysis was done using SPSS software version 20.0. The data are represented as Mean±SD (Standard Deviation), $p<0.05$ value was considered statistically significant. The correlation was determined by using Karl's Pearson's correlation coefficient.

3. Results

After taking clinical history which included occupation, age, sex, and integrated risk factors promoting the illness, the patients we selected for the study. The patients with serious medical conditions and with mineral supplementation or any drugs that affected mineral metabolism were excluded from the

study. A total of 46 diagnosed T2DM patients aged between 30-65 years and a similar number of apparently healthy individual of age 30-65 years as controls were taken for the study.

It was found that ALP was significantly increased in patients with mean and SD (264.79±44.85) when compared to healthy controls with mean and SD (143.17±29.96) as shown in Table 1. It was found that the ALP is raised in T2DM patients when compared to the healthy individual which is statistically significant i.e. p <0.001 (Table 1).

However, it was found that calcium levels in T2DM patients was not significantly different (9.05±0.86) when compared to healthy controls (9.46±0.92) as shown in Table 2. It was found that the estimated calcium level was almost same in both the groups i.e. cases and controls, hence the association was not statistically significant i.e. (p = 0.07) (Table 2).

It was observed that the clinical parameters such as FBS, ALP have significant positive co-relation in T2DM patients (p <0.001) but calcium level was statistically not significant (p= 0.07) shown in Table 3.

Table 1. Estimation of ALP in T2DM patients and controls

Group	Mean	SD	p-value
Control (n=46)	143.17	29.96	-
T2DM (n=46)	264.79	44.85	<0.001

Table. 2 Estimation of serum calcium in T2DM patients and controls

Group	Mean	SD	p-value
Control (n=46)	9.46	0.92	-
T2DM (n=46)	9.05	0.86	0.07

4. Discussion

ALP physiologically increases during bone growth, while pathological increases are mostly associated with hepato-biliary disorders. Alkaline phosphatase enzyme may be raised in different conditions like pregnancy, bone disorders, hyper-parathyroidism, and chronic alcoholism [16]. Due to extra deposition of fat in liver, serum ALP level may raise and, termed as non – alcoholic fatty liver disease. Fatty liver may result in hepatic insulin resistance and may develop systemic insulin resistance and insulinemia. Hence ALP is said to be the marker of insulin resistance group of symptoms which includes in the pathogenesis of diabetes. Research studies reported that ALP participates in the maintenance of intracellular antioxidant defenses mechanism via extracellular glutathione transport into various cells. ALP is normally present outside of the cell membrane having primary function of maintaining level of glutathione (GSH), an important antioxidant. Increases in ALP activity can be due to oxidative stress, resulting increased transport of GSH precursors into cells [4, 16].

Table 3. Correlation of clinical parameters in T2DM patients

Variables		FBS	ALP	Calcium
FBS	Pearson Correlation	1	.572**	-.323
	Sig. (2-tailed)	-	.001	.071
	N	46	46	46
ALP	Pearson Correlation	.572**	1	-.410*
	Sig. (2-tailed)	.001	-	.020
	N	46	46	46
Calcium	Pearson Correlation	-.323	-.410*	1
	Sig. (2-tailed)	.071	.020	-
	N	46	46	46

Calcium is present in the bone and teeth, establishing a structural function [17]. It act as intracellular second messengers that affects activity of the enzymes and secretion of hormones such as insulin, aldosterone, and anti-diuretic hormone. It is important for stabilizing cell metabolism, nerve impulse transmission, and muscular contraction [18]. It plays various important functions in formation, release, and receptor response to neurotransmitter [19]. Bone formation rate is decreased in hyperglycemic condition and calcium loss via urine in Diabetes is originated from the bone [20]. Insulin is also necessary for normal bone mineralization. Osteoblast controls mineralization by regulating the passage of calcium and phosphate ion across their cell membrane, these cells contain phosphatase that is used to generate phosphate ion from inorganic phosphates [21]. Serum calcium level is decreased in T2DM patients due to hyperglycemia which results in increase urinary loss of calcium in proportion to the

degree of glucosuria [22]. Patient having DM are at the risk of developing complications like diabetic foot syndrome, Charcot's arthropathy and osteoporosis [23].

The present case-control study was designed to estimate the serum ALP and calcium levels in diagnosed T2DM patients and healthy controls. It was observed that the concentration of ALP was significantly increased when compared with healthy control subjects. Botolin and McCabe have shown that chronic hyperglycemia increases ALP activity [24]. The increase in ALP concentration could be due to osteoblastic activity as a result of depletion of calcium mineral content which may lead to alveolar bone loss or fractures. It was analyzed that the calcium level was decreased in T2DM subjects when compared with healthy individuals but it was not statistically significant. A Canadian study by Sun et al., found that calcium is altered significantly with abnormalities in FBS [25]. The decreased level of serum calcium in T2DM patients may be due to hyperglycemia which results in excessive urinary loss of calcium. However, due to small sample size, further studies with more patients are required to substantiate the findings of the present study.

We conclude that ALP levels in T2DM were significantly higher when compared with healthy individuals. The calcium levels was not significantly different, between T2DM patients and healthy controls. The FBS and ALP was significantly correlated. Hence, the analysis of serum ALP and calcium levels in T2DM patients is an important tool for the management of osteoporosis and related bone disorders associated with DM.

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Authors' contributions

MDK: data collection and analysis of results, drafted the manuscript. RA: conception or design of work, analysis and interpretation of results. HA: analysis and interpretation of results, drafted the manuscript, critically revised the manuscript. MMK: analysis and interpretation of results, statistical analysis of data. SK: conception or design of work, analysis and interpretation of results. All authors approved the final version to be published.

Conflict of interests

There are no potential conflicts of interest to declare.

Ethical declarations

Ethics approval and consent to participate: The study was conducted through appropriate consent and approval of Institutional Ethical Committee, IIMS&R, Lucknow (IEC/IIMS&R/2019/35).

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