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*Full Length Research Paper*

# Cadmium, lead, arsenic and selenium levels in patients with type 2 diabetes mellitus

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**There is accumulating evidence that the metabolism of several trace metals are altered in diabetes mellitus and these micronutrients might have specific roles in the pathogenesis and progression of the disease. The aim of this study was to investigate the level of toxic elements: lead (Pb), cadmium (Cd) and arsenic (As) levels in whole blood and selenium (Se) (an antioxidant element) in serum of patients with type 2 diabetes mellitus. Fifty diabetic patients and 40 apparently healthy non-diabetic individuals were recruited into this study. After an overnight fasting, blood was collected from each subject and blood/serum concentrations of these elements were measured by atomic absorption spectrophotometer after acid digestion. The mean value of Pb and Cd were significantly higher in the serum of diabetic patients when compared with the control ($p < 0.01$) but there was no significant difference in the concentration of As ($p > 0.05$). The serum concentration of Se was significantly lower in diabetic patients than in healthy control group ($P < 0.01$). Also, the concentration of the toxic elements showed positive correlation with fasting plasma glucose (Cd $r = 0.378$, Pb $r = 0.425$, $p < 0.01$) and inverse correlation with serum selenium ($r = -0.599$, $p < 0.01$). This study showed that, increased toxic metals are associated with diabetes mellitus. Thus, these elements may play a role in the development and pathogenesis of diabetes mellitus. In addition, depression in antioxidant concentration (especially, Se) may further aggravate this effect.**

**Key words:** Toxic elements, antioxidant, diabetes mellitus, adult Nigerians.

## INTRODUCTION

Diabetes mellitus is a syndrome characterized by disorder in metabolism and abnormally high blood sugar (hyperglycaemia) resulting from low levels of insulin with or without abnormal resistance to the action of insulin (Tierney et al., 2002; Tierney et al., 2004). The characteristic symptoms are excessive urine productions (polyuria), excessive thirst and increased fluid intake (polydipsia), blurred vision, unexplained weight loss and lethargy. These symptoms are likely to be absent if the blood sugar is only mildly elevated (Lionel, 2007).

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Increasing rates of type 2 diabetes in the developed and developing countries suggest that environmental factors may be involved in the aetiology of type 2 diabetes mellitus (Jones et al., 2005). Acute arsenite toxicity, including its effects on glucose metabolism, is generally attributed to its reactivity toward thiol (SH) groups (Aposhian, 1989; Ratnaike, 2003). During acute poisoning, arsenite inhibits pyruvate and α-ketoglutarate dehydrogenases (Aposhian, 1989), essential enzymes for gluconeogenesis and glycolysis. Arsenate, on the other hand, can replace phosphate in energy transfer pathways of phosphorylation and also uncouples oxidative phosphorylation (Kennedy and Lehninger, 1949).

In epidemiologic studies, the association between arsenic exposure and diabetes across different populations and different sources of exposure was inconsistent. For

example, epidemiologic studies have implicated arsenic as a possible cause of type 2 diabetes and a role for other environmental toxins is strongly suspected (Chong et al., 2006). In populations exposed to high arsenic levels via drinking water in Taiwan and Bangladesh, diabetes risk was consistently increased. In occupational settings, diabetes mortality was increased in some studies and decreased in others (Tseng et al., 2000). Finally, no association with diabetes was observed in four studies of general populations outside of Taiwan or Bangladesh (Tseng et al., 2000).

There are reports linking human exposure to cadmium with pancreatic cancer (Schwartz and Reis, 2000) and because of the association between pancreatic cancer and type 2 diabetes (Eberhart et al., 2004), it is possible that type 2 diabetes is also associated with cadmium exposure.

The effects of lead poisoning in diabetes subjects have been recognized (Wedeen et al., 1975; Lin et al., 2003; Tsaih et al., 2004). Epidemiologic studies, mortality studies and experimental studies in animals have reported lead toxic effects at high levels of exposure and it is possible that high blood concentration of lead may contribute progression of diabetic complications in diabetic patients (Lin et al., 2003).

It has been reported that oxidative stress reduces insulin secretion and increases insulin resistance in some experimental models and may thus play a causal role in the pathogenesis of diabetes (West, 2000; Stumvoll et al., 2005; Evans et al., 2005). Selenium, an essential trace element, is involved in the complex system of defence against oxidative stress through selenium-dependent glutathione peroxidases and other selenoproteins (Burt, 2007). Because of its antioxidant properties, selenium might thus prevent the development of complications in diabetic patients. In addition, selenate, an inorganic form of selenium, mimics insulin activity in experimental models (Mueller, 2006; Mueller et al., 2008; Pallauf et al., 2008).

The role of selenium in the regulation of free radical production has been documented; its role in preventing glucose intolerance and the complications of diabetes mellitus has also been postulated. For instance, insulin reserves are decreased with selenium deficiency causing glucose intolerance. We therefore, hypothesised that elevation of heavy metals such as cadmium, lead, arsenic and deficiency of antioxidant trace element such as selenium may contribute to the pathogenesis of diabetes mellitus.

## Aims and objectives of the study

The study was designed to evaluate the concentrations/ levels of toxic metals (Cd, Pb and As) and antioxidant element (Se) in type 2 diabetes mellitus and to establish possible correlation between these elements and type 2 diabetes mellitus.

## MATERIALS AND METHODS

### Study population

The study population is known diabetic patients attending Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Osogbo (Western Nigeria) and non-diabetic individuals. The non-diabetic individuals were selected following medical examination and laboratory test that determined fasting blood glucose level.

### Study design

This was a cross-sectional randomized study designed to investigate the levels of toxic metals in diabetic patients.

### Exclusion criteria

These included pregnant women, lactating mothers, smoking and alcoholic individuals, anyone on medications that could affect exposure to measured metals. Women with other chronic illnesses or taking any other medications that could potentially affect levels of metals were also excluded.

### Subject population

The subjects were recruited from the Department of Medicine, Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Osogbo. Of the 120 participants recruited, 90 patients participated in this study. The subjects were grouped into two categories comprising 50 type 2 diabetic patients and 40 non-diabetic healthy individuals (control group). The study received an approval from the Ethical Committee of LAUTECH, Osogbo.

### Determination of body mass index (BMI)

### Measurement of height

Measurement of height was taken in standing position using a stadiometer. The height was measured to the nearest 0.01 m. When measuring height, the subjects and controls stood straight with the head positioned such that the Frankfurt plane was horizontal, feet together, knees straight and heels, buttocks and shoulder blades in contact with the vertical surface of the wall, arms hanging freely at the sides with the palm facing the thighs.

### Measurement of weight

A beam balance with non-detachable weights was used. The body weight was recorded to the nearest kg. The balance was situated on a hard flat surface and checked for zero balance before each measurement. The subject stood unassisted in the center of the platform and was asked to look straight ahead, standing relaxed and in light clothing. The BMI was calculated from the average height and weight (weight/height) in square metres.

### Blood sample collection

After an overnight fasting (from 8 pm to 8 am), blood was drawn from the cubital vein of each participant using a sterile needle and syringe into appropriate specimen tubes (lithium heparin for lead,

**Table 1.** BMI and biochemical parameters of diabetic and control groups.

| Parameters | Diabetic group N = 50 (mean ± SD) | Control group N = 40 (mean ± SD) | T-test | P-value |
|---|---|---|---|---|
| BMI (kg/m²) | 27.13 ± 1.97 | 22.59 ± 2.05 | 10.70 | $p < 0.01$ |
| FBS (mmol/L) | 15.51±2.39 | 4.91 ± 0.98 | 26.27 | $p < 0.01$ |
| Cd (µmol/L) | $7.1 \times 10^{-3} \pm 1.4 \times 10^{-3}$ | $5.7 \times 10^{-3} \pm 1.6 \times 10^{-3}$ | 4.26 | $p < 0.01$ |
| Pb (µmol/L) | 0.26 ± 0.054 | 0.21 ± 0.060 | 4.43 | $p < 0.01$ |
| As (µmol/L) | 0.058 ± 0.019 | 0.057 ± 0.018 | 0.078 | $p > 0.05$ |
| Se (µmol/L) | 0.93 ± 0.13 | 1.15 ± 0.16 | 7.34 | $p < 0.01$ |

BMI = Body mass index, FBS = Fasting blood sugar.

cadmium and arsenic estimation, fluoride oxalate for fasting blood sugar and plain tube for selenium estimation). The samples in plain specimen tubes were allowed to clot undisturbed and serum were separated by centrifugation for 10 min at 4000 rpm into plain specimen tubes and stored at -20°C until analysis was performed. The whole blood in lithium heparin was centrifuged, plasma separated, stored at -20°C until analysis was performed.

#### Biochemical analysis

The plasma concentration of glucose was measured using standard enzymatic spectrophotometric method (Trinder, 1969). Lead, cadmium, arsenic ions and selenium ions were determined by flame atomic absorption spectrophotometry.

#### Determination of plasma glucose

Glucose is converted by glucose oxidase (GOD) into gluconic acid and hydrogen peroxide which in the presence of peroxidase, react with chromogen (4 amino phenazone) to form a red colour complex pyryl-quinomines and the absorbance was read at 500 nm.

#### Trace metals determination

Levels of the selected toxic metals and selenium were estimated by atomic absorption spectrophotometry. The principle is based on dissociation of the element (from the flame) from its chemical bonds. This is then placed in unexcited or ground state (neutral atom).Thus, the neutral atom is at a low energy level in which it is capable of absorbing radiation at a very narrow bandwidth corresponding to its own line spectrum (Kaneko, 1999). The amount of radiant energy absorbed is proportional to the concentration of trace metals present. Blood (2 ml) was taken from the thawed samples after ensuring thorough mixing, added to a clean 10 ml centrifuge tube and diluted to 10 ml with hydrochloric acid. The diluted blood sample was then centrifuged (30 s, 3000 rev/min) to remove cellular debris and aspirated directly into the flame.

#### Statistical analysis

The SPSS software package was used for statistical analysis and significant was set at $p < 0.05$. Values obtained from this study were expressed as mean and standard deviation. Pearson correlation coefficient was used to measure the level of association between variables.

## RESULTS

The mean body mass index (BMI) was significantly higher in diabetic patients when compared with that of control subjects ($p < 0.01$). The fasting plasma sugar of the diabetic patients was significantly higher than the control group ($p < 001$) (Table 1).

### Biochemical parameters

The mean serum cadmium and lead concentrations were significantly higher ($p < 0.01$) in diabetic patients in comparison to the control subjects (Table 1). The mean serum concentration of selenium was significantly lower ($p < 0.01$) in diabetic patients when compared to the control group ($p < 0.01$). However, there was no significant difference in the mean serum concentration of arsenic in both diabetic and control groups (Table 1). The distribution of cadmium in diabetic and control groups is represented in Figure 1, the distribution of lead in diabetic and control groups is represented in Figure 2, the distribution of arsenic acid in diabetic and control groups can be found in Figure 3 and the distribution of selenium in diabetic and control groups is represented in Figure 4.

Pearson correlation analysis was carried out to determine the relationship between variables (Table 2).There was a positive relationship between fasting blood sugar and cadmium ($r = 0.378$, $p < 0.01$), fasting blood sugar and lead ($r = 0.425$, $p < 0.01$) and cadmium and lead ($r = 0.247$, $p < 0.05$). In contrast, there was an inverse relationship between fasting blood sugar and selenium ($r = -0.599$, $p < 0.01$), cadmium and selenium ($r = -0.306$, $p < 0.01$) and lead and selenium ($r = -0.253$, $p < 0.05$).

## DISCUSSION

Some trace elements act as antioxidants and prevent membrane peroxidation while others act directly as co-factors in metabolism of macromolecules such as glucose. It is commonly suggested that disturbed concentration of

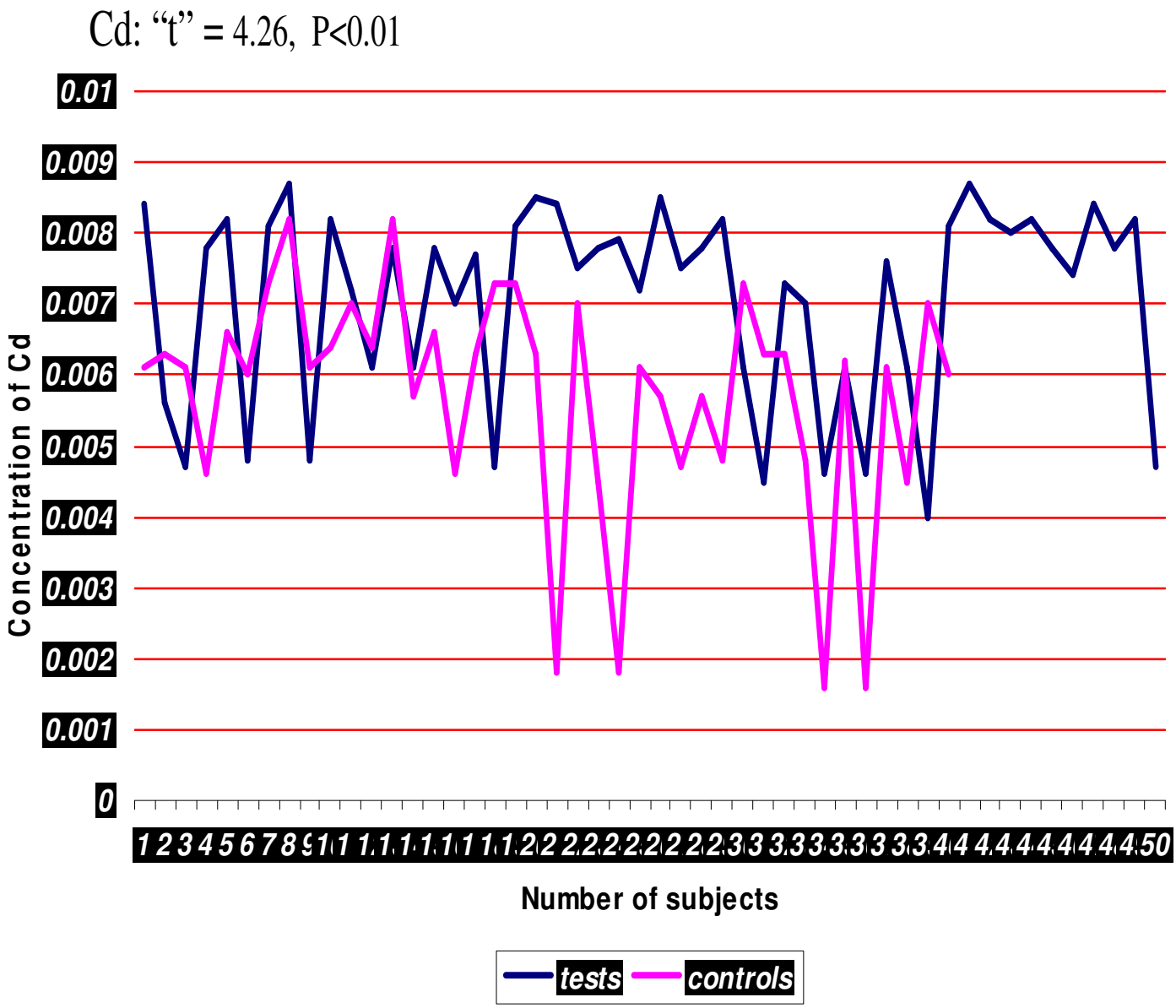


**Figure 1.** Distribution of cadmium in diabetic and control groups. While few patients have Cd values comparable to control group, the majority of the patients have significantly higher concentrations of cd than the control group.

toxic metals (such as Cd, Pb and As) and antioxidants (such as Se) in the body are often found in patients with diabetes mellitus (Tripathy et al., 2004). In this study, Cd levels were found to be significantly higher ($p < 0.01$) in diabetic patients when compared to healthy control group. This result agrees with that of Schwartz and Reis (2000) who reported a significantly higher level of cadmium in experimental rats than in a control group. It is important to emphasize that the results of our study were obtained in a cross-sectional study. Therefore, a prospective study, in which cadmium levels are determined before the development of type 2 diabetes will be required to establish a stronger association. However, our data suggest that increase cadmium concentration may be a contributing factor to the pathogenesis of diabetes mellitus.

There was no significant difference in serum arsenic levels between diabetic and control groups. Accumulation of arsenic (As) in diabetic patients has been described in other studies (Chong et al., 2006). Also, in populations exposed to high arsenic levels via drinking water in Taiwan and Bangladesh, diabetes risk was consistently increased. Our study suggests that our population is not exposed to high arsenic. It could be said that accumulation of arsenic in diabetes mellitus patients as reported in China (Chong et al., 2006) is not a likely

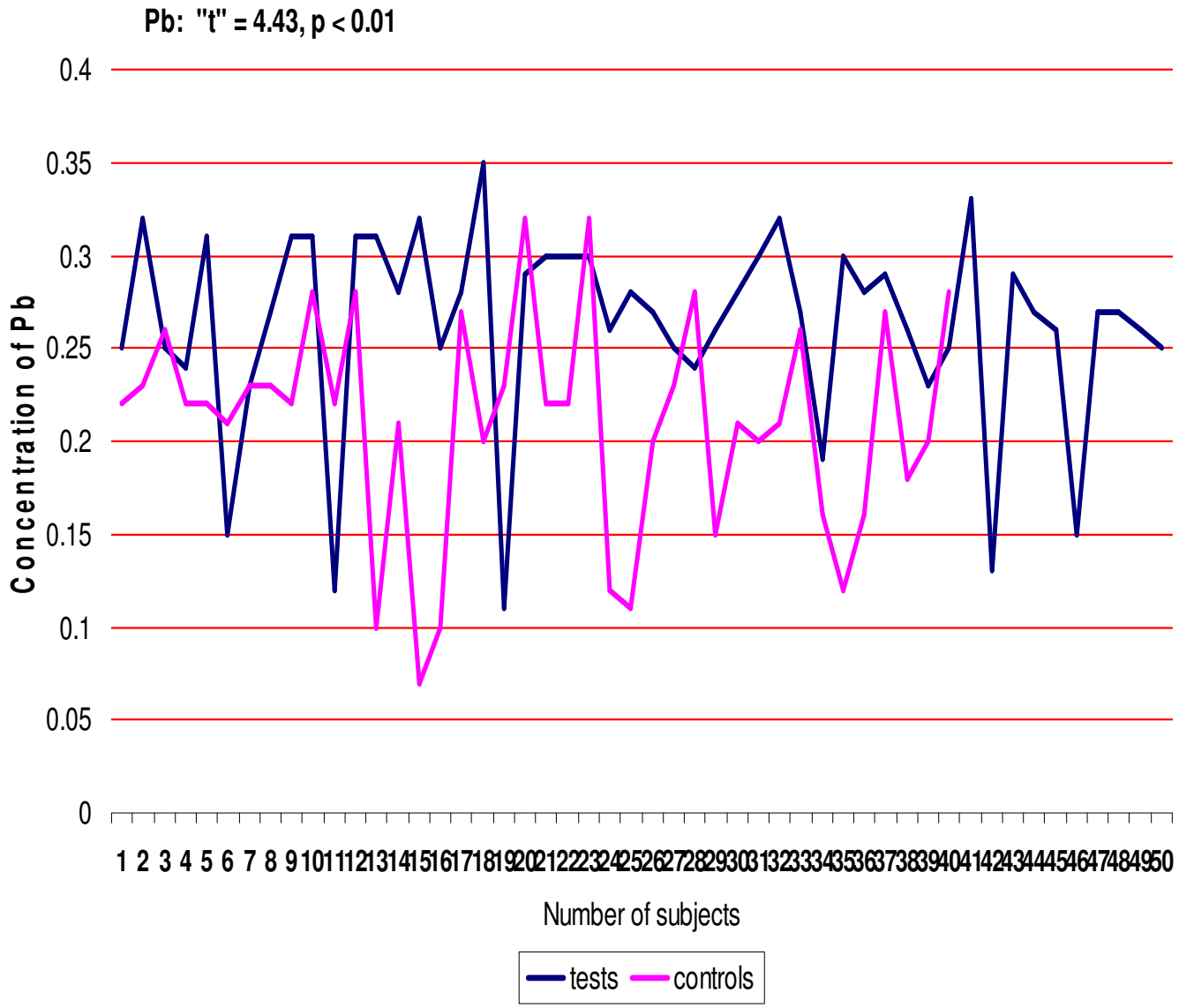


**Figure 2.** Distribution of lead in diabetic and control groups. This distribution showed a disparity between patient and control groups. Few diabetic patients have lead levels comparable to the control group. However, the majority of the patients had significantly higher levels of lead.

phenomenon in our population.

Lead levels were found to be significantly higher in diabetic patients when compared to healthy control group. This is in support of a recent report by the Center for Disease Control (2008) which observed a significant association between higher serum lead levels in diabetic patients.

Selenium (Se) levels were found to be significantly lower in diabetic patients when compared to healthy control group. This decrease in serum Se levels has been described in an earlier study (Burt, 2007). Selenium is known to act as an antioxidant and peroxynitrite scavenger when incorporated into selenoproteins (Gramm et al., 1995; Beytut and Akasakal, 2003). It is the main element in glutathione peroxidase (an active enzyme against oxidative stress) that reduces formation of free radicals and peroxidation of lipoproteins. The low concentration of selenium in serum could potentially expose the subject to oxidative stress which is known to be associated with the pathogenesis of diseases such as diabetes mellitus (Schwartz and Reis, 2000). On the other hand, low concentration of this element in blood might be an indication of active production of free radical and increased scavenging activity of either selenium or glutathione peroxidase. This decrease in serum selenium levels could contribute to oxidative stress and low selenium level has

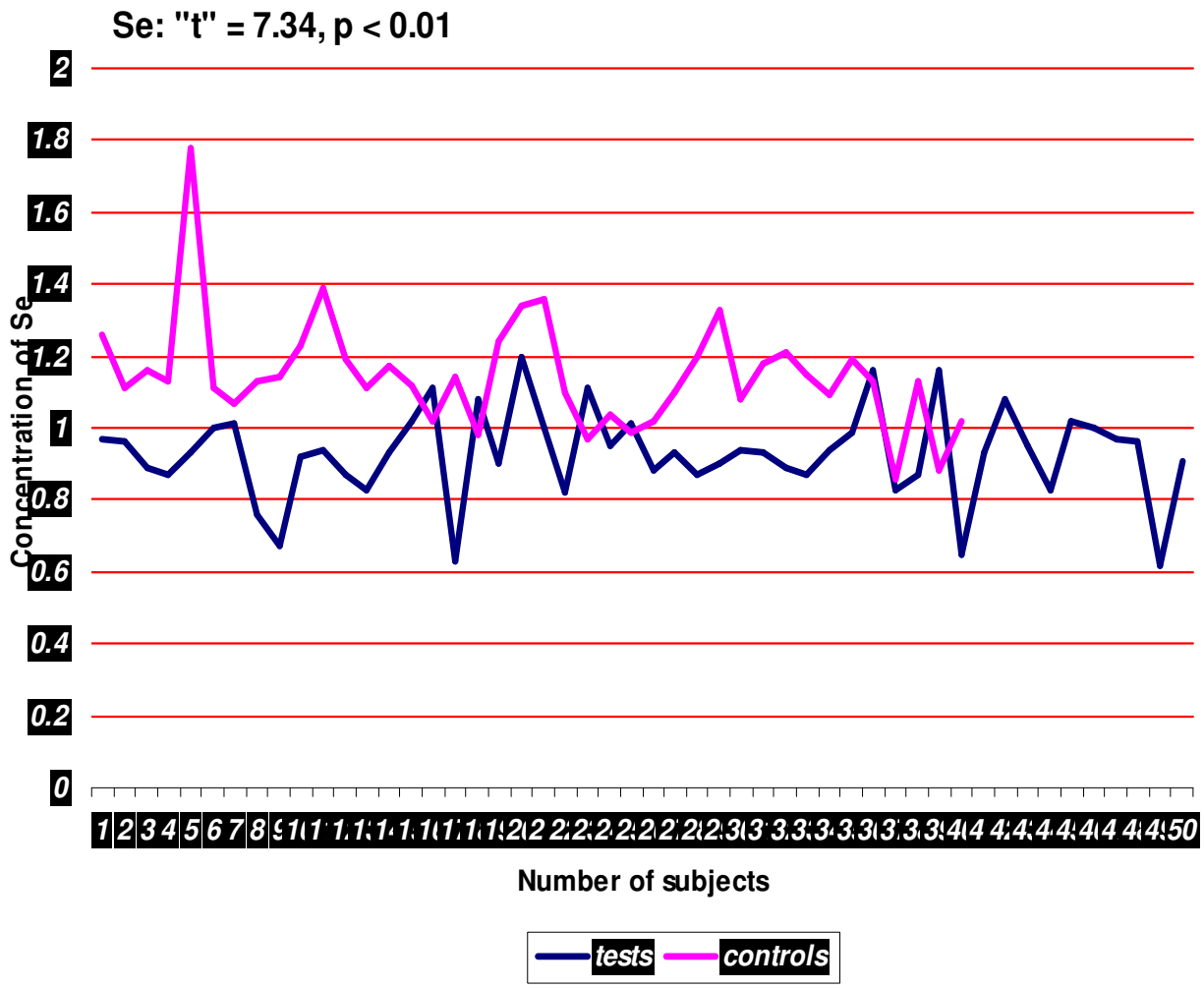


**Figure 4.** Distribution of selenium in diabetic and control groups. There is a significantly lower concentration of selenium in the serum of all diabetic patients when compared to the control group.

**Table 2.** Pearson correlation showing level of association in the BMI and biochemical parameters among diabetic and control subjects.

| | Sex | BMI | FBS | Cd | Pb | As | Se |
|---|---|---|---|---|---|---|---|
| Sex | 1 | 0.064 | 0.066 | -0.21 | -0.067 | -0.131 | -0.135 |
| BMI | 0.064 | 1 | 0.730** | 0.365** | 0.224* | 0.23 | -0.463** |
| FBS | 0.066 | 0.730** | 1 | 0.378** | 0.425** | -0.046 | -0.599** |
| Cd | -0.21 | 0.365** | 0.378** | 1 | 0.247* | 0.066 | -0.306** |
| Pb | -0.067 | 0.224* | 0.425** | 0.247* | 1 | 0.080 | -0.253* |
| As | -0.131 | 0.23 | -0.46 | 0.066 | 0.080 | 1 | -0.38 |
| Se | -0.135 | -0.463** | -0.599** | -0.306** | -0.253* | -0.38 | 1 |

*Correlation is significant at the 0.05 level (2-tailed), ** correlation is significant at the 0.01 level (2-tailed).

been shown to reduce insulin secretion and increased insulin resistance in some experimental models, thereby possibly playing a causal role in the development and pathogenesis of type 2 diabetes (Evans et al., 2005).

## Conclusion

This study shows that blood cadmium and lead levels were significantly higher in the diabetic group when compared

with the control group while serum arsenic levels in diabetic patients were not found to be signifi-cantly different from that of apparently healthy control group. Serum selenium levels were lower in the diabetic group when compared to the control group. This result suggests that increase concentration of toxic metals and reduced antioxidant concentration may be a contributing factor to the pathogenesis of diabetes mellitus.

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