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Haptoglobin Characterization and Evaluation of Some Selected Trace Elements and Toxic Metals in Type 2 Diabetes mellitus Patients in Ado-Ekiti, Ekiti State

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Abstract
Alterations in serum levels of trace elements reported in type 2 diabetes mellitus (T2DM) have been linked with induction of T2DM and associated complications. This study was carried out to evaluate the levels of Zinc (Zn), Copper (Cu), Selenium (Se), Lead (Pb) and Cadmium (Cd) in different haptoglobin phenotypes of complicated and uncomplicated diabetic patients. A total of 100 samples comprising of 30 apparently healthy subjects (control), 39 treated and untreated uncomplicated diabetic patients and 31 hypertensive type 2 diabetic patients were recruited for this study. Characterization of serum haptoglobin phenotypes was determined using protein electrophoresis, while Zn, Cu, Se, Pb and Cd were estimated using Atomic Absorption Spectrometry. Results obtained showed that Pb and Cd levels were significantly higher ($p<0.05$) in treated complicated diabetes subjects compared to control, while glucose, Zn, Se and Cu levels were non-significant ($p>0.05$). BMI, glucose, Pb and Cd were significantly higher ($p<0.05$), while Se and Zn were significantly lower ($p<0.05$) in untreated complicated diabetes subjects compared to control. Cu was non-significantly lower ($p>0.05$) in untreated complicated diabetes subjects compared to control. The results also showed that Cu, Se, Pb and Cd levels were significantly higher ($p<0.05$) in treated diabetes subjects compared to control subjects, while BMI, glucose and Zn levels were non-significant. Conclusively, the results of this study showed significant alterations in Zn, Se, Cu, Pb and Cd of complicated and uncomplicated diabetes subjects which suggests that increase concentration of toxic metals and reduced trace elements concentration may be a contributing factor to the pathogenesis of diabetes mellitus.

Key words: Diabetes mellitus, hypertension, trace element, haptoglobulin, toxic metal.

Introduction
Diabetes mellitus is one of the prevalent metabolic diseases characterized by hyperglycaemia caused by a disorder in insulin secretion, insulin action, or both (Dandekar *et al*., 2021). Diabetes mellitus affects millions of people worldwide and is a chronic disorder of carbohydrates, fats and protein metabolism. Diabetes is caused by a combination of pathogenic mechanisms such as autoimmune death of pancreatic-cells resulting in insulin insufficiency and anomalies that result in insulin resistance (Sameer *et al*., 2020). Diabetes mellitus can also lead to loss of vision (retinopathy), renal failure (nephropathy), foot ulcers and Charcot joints, autonomic neuropathy causing gastrointestinal, sexual dysfunction, genitourinary and cardiovascular symptoms respectively. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes (Wang *et al*., 2022). Diabetes is of two types; Type 1 diabetes mellitus (T1DM) and Type 2 Diabetes mellitus (T2DM). T1DM is a chronic autoimmune disease characterized by insulin deficiency (Insulin-dependent diabetes) which is caused by autoimmune destruction of β-cells of the endocrine pancreas (Paschou *et al*., 2018). T2DM on the other hand is a chronic and metabolic disease characterized by defects in pancreatic insulin secretion and insulin effect on target tissues, generating a persistent state of hyperglycemia

(Insulin resistance) (Pivari *et al*., 2019).

Hypertension is a substantial risk factor for other diseases such as Diabetes. Hypertension and T2DM appear to represent two components of the same pathophysiological process, particularly in persons with metabolic syndrome. It is believed that nearly two-thirds of people with T2DM also have hypertension. Elevated arterial blood pressure (BP) contributes to an elevated risk of micro and macro-vascular problems in T2DM patients. Furthermore, the coexistence of these two major risk factors increases the risk of cardiovascular disease (CVD) by four fold when compared to normotensive non-diabetic individuals. The extent of the concurrent prevalence of T2DM and hypertension is affected by age, BMI and ethnicity (Pavlou *et al*., 2018). Diabetes can be complicated with hypertension, meaning the hypertension is secondary to diabetes mellitus, hypertension affects around 70% of diabetic patients and is roughly twice as common in diabetics as in non-diabetics (Lara-Esqueda *et al.,* 2021).

Trace elements are vital essential micronutrients which play important roles in body metabolism and homeostasis. They can also be beneficial and/or toxic to the body depending of the amount con. Some of the essential trace minerals are Chromium (Cr), Calcium (Ca), Copper (Cu), Zinc (Zn), Selenium (Se) among others (Mehri, 2020). Minerals only forms about 5% of the typical human diet but are essential for normal healthy function. Chromium potentiates insulin which influences carbohydrate, lipid and protein metabolism. Calcium homeostasis influences insulin resistance and secretion. Diabetes impairs calcium homeostasis, which contributes to abnormal cell regulation in erythrocytes, cardiac muscles, platelets and skeletal muscles. Impaired homeostasis is problematic since it may be a substantial contributory element in the regulation of normal insulin secretion and action, as well as independently affecting numerous vascular problems (Dubey *et al*., 2020). Selenium functions as cofactor in the functioning of the thyroid gland and in every cell that uses thyroid hormone. It is an antagonist of mercury, arsenic and also it can protect the body from cadmium, lead, thallium and silver (Schomburg, 2019).

The Haptoglobin (Hp) is an abundant human plasma protein that binds to haemoglobin during haemolysis. Haptoglobulin are plasma glycoproteins synthesized in the liver, white adipose tissue and the kidney (Buehler *et al*., 2020). The genetic polymorphisms and phenotypes of haptoglobulin serve several biological purposes. Haptoglobin contains antibacterial, antioxidant and angiogenic properties and has been linked to a variety of disorders, including simple obesity, vascular complications of diabetes, non-alcoholic fatty liver disease, hypertension, blood diseases, autoimmune diseases and malignant tumors (Wan *et al*., 2021). The haemoglobin-binding function of Hp serves as an important protection against the haemoglobin toxic properties which escape the intracellular compartment of red cells during haemolysis. The human gene encoding Hp exists in two major allelic forms; Hp1 and Hp2 respectively. These genes combine to give three major phenotypes which are Hp1/Hp1, Hp2/Hp1 and Hp2/Hp2 respectively (Ricotti *et al*., 2020).

Type 2 diabetes mellitus affects more than 90% of diabetic patients, causing micro-vascular and macro-vascular problems that cause substantial psychological and physical anguish in patients (Chatterjee *et al.,* 2017). Recent research has shown a close relationship between some specific micronutrients and this disease, with implications for the pathogenesis of this disease and its vascular complications (Chiu *et al.,* 2021). There are accumulating evidences 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 (Dubey *et al.,* 2020). Hence, this study was carried out to determine the levels of some essential trace metals (Zinc, Copper and Selenium) and toxic metals (Lead and Cadmium) in different hapatoglobin phenotypes of complicated and uncomplicated diabetic patients.

Methods

Study design

A case-control research design using stratified random sampling techniques was employed in this study.

Study area
The study was carried out in Ido-Ekiti and its environs (like Otun, Aiyee, Ifaki). Ido-Ekiti is a town in Ido-Osi Local Government Area of Ekiti State in South-west Nigeria. The state is mainly an upland zone, rising over 250 meters above sea level. Its coordinates are 7^0 40'N 5^0 15'E. The study involved both outpatients and admitted patients at Federal Teaching Hospital, Ido-Ekiti, Ekiti State.

Sample size
The minimum sample size (N) was calculated using the formula: $N = Z^2p(1-p)/w^2$
Where Z = confidence level at 95, N=Minimum sample size, w= allowance for error=0.05, P= estimated prevalence of diabetes patients at 6.28% (Adeloye *et al.*, 2021).

$q = 1, p = 1 - 0.0628 = 0.9372$

$$N = \frac{1.96^2 \times 0.0628 \times 0.9372}{0.05^2} = 90.44$$

A total of 100 samples comprising of 30 apparently healthy subjects (control), 39 treated and untreated uncomplicated diabetic patients and 31 hypertensive type 2 diabetic patients were recruited for this study.

Inclusion criteria
Men and women who are type 2 diabetic and hypertensive type 2 diabetic patients on treatment, those not on treatment who gave their consent were included in this study.

Exclusion criteria
Subjects below the age of 18 years, pregnant women, nursing mothers, and those with chronic kidney disease and/or any other metabolic disorders were excluded from this study.

Ethical clearance
Ethical approval was sought from Ethics and Health Research Committee of Afe Babalola University, Ado-Ekiti, Ekiti State. Informed consent was obtained from each subject who participated in the study before sample collection.

Sample collection
Venous blood sample of about 5mls was collected from the cubital fossa using 22G needle and syringe from each subject after overnight fasting. The sample was dispensed into plain non-anticoagulated sample bottle and was allowed to clot first before centrifuging at 5000rpm for 5 minutes to separate the serum from cells and dispensed into another plain non-anticoagulated sample bottle. The serum samples were stored at temperature of -20 degree Celsius until analysis.

Anthropometric analysis
Blood Pressure: Blood pressure was determined with digital sphygmomanometer.

Height and weight were obtained using a meter gauge and a bathroom scale respectively.

Body Mass Index (BMI): BMI was derived from the height and weight using the formula:

$$BMI = \frac{\text{Weight (kg)}}{\text{Height}^2\,(m^2)}$$

It was expressed in kg/m^2 (Kuang *et al.*, 2022)

Sample analysis
Plasma glucose was estimated spectrophotometrically using Glucose-Oxidase Peroxidase method (Randox Laboratories Ltd. County Antrim, United Kingdom).

Principle: Glucose is oxidized in the presence of gluconic acid and hydrogen-peroxide. Peroxidase catalyzed the breakdown of hydrogen peroxide to atmospheric oxygen and water. The oxygen then reacts with phenol and 4-aminophenazone to give a pink colour which is read spectrophotometrically at 505nm.

Characterization of serum haptoglobin phenotypes was determined using protein electrophoresis method in polyacrylamide gel and determined using specific peroxidase staining (Simon *et al.*, 2020).

Principle: When proteins are separated by electrophoresis through a gel matrix, smaller proteins migrates faster due to less resistance from the gel matrix.

Zinc (Zn), Copper (Cu), Selenium (Se), Lead (Pb) and Cadmium (Cd) were estimated using Atomic Absorption Spectrometry (AAS).

Principle: A hollow cathode lamp containing a coated cathode of the element that is to be analyzed is used as a light source. The light source emits a beam of a specific wavelength across the burner and into the monochromator. The sample is aspirated into the flame at the burner which converts the aerosol into energy at a specific wavelength and as the atoms increase the amount of light absorbed will also increase. The amount of light absorbed can be measured and used for a quantitative determination of the amount of analyte in a sample.

Statistical analysis
The results were presented using tables. Data was presented as mean ± S.D (standard deviation). Comparison was made between subjects and control groups using one-way analysis of variance (ANOVA) and the student t-test. Significant difference was accepted at $p<0.05$.

Results
Table 1 showed the mean values of blood pressure, BMI, Zinc (Zn), Copper (Cu), Selenium (Se), Lead (Pb) and Cadmium (Cd) in treated and untreated hypertensive diabetic subjects. Results obtained showed that Systolic (mmHg), Diastolic (mmHg), BMI (Kg/m^2) Pb and Cd levels were significantly higher ($p<0.05$) in treated complicated diabetes subjects compared to control. Glucose concentration (mmol/L), Zn, Se and Cu levels were non-significantly higher ($p>0.05$) in treated complicated diabetes subjects compared to control. Results obtained from untreated hypertensive patients showed that Systolic, Diastolic, BMI, glucose concentration, Pb and Cd were significantly higher ($p<0.05$), while Se and Zn were significantly lower ($p<0.05$) in untreated complicated diabetes subjects compared to control. Cu was non-significantly lower ($p>0.05$) in untreated complicated diabetes subjects compared to control.

Table 2 showed the mean values of blood pressure, BMI, Zn, Cu, Se, Pb and Cd in treated and untreated diabetic subjects. The results obtained showed that Cu, Se, Pb and Cd levels were significantly higher ($p<0.05$) in treated diabetes subjects compared to control subjects, while systolic, diastolic, BMI, glucose concentration and Zn levels were non-significantly higher in treated diabetes subjects compared to control subjects. Results obtained from untreated diabetic patients showed that glucose concentration, BMI, Pb and Cd were significantly higher ($p<0.05$), while Zn was significantly lower ($p<0.05$) in untreated diabetes subjects compared to control subjects. The values for systolic and diastolic blood pressures were non-significantly higher ($p>0.05$), while Se and Cu were non-significantly lower ($p>0.05$) in untreated diabetes subjects compare to control subjects.

Table 3 showed the Haptoglobin phenotypes in treated and treatment naïve diabetic and hypertensive diabetic subjects. The results obtained showed that haptoglobin phenotype 1-1 was higher in subjects with uncomplicated diabetes (23.1%) compared to subjects with complicated diabetes (12.9%). Furthermore, haptoglobin phenotype 2-1 was higher in subjects with complicated diabetics (51.6%) compared to subjects with uncomplicated diabetes (46.2%). In complicated diabetes, haptoglobin phenotype 2-2 was higher (35.5%) compared to subjects with uncomplicated diabetes (30.8%).

Figures 1-8 shows that trace essential elements are higher in haptoglobin 1 phenotypes than haptoglobin 2. The reverse was observed as regards the concentration of toxic metals as they appear to be higher in the haptoglobin 2 phenotypes.

Table 1: Mean values of all parameters in treated and treatment naive hypertensive diabetic subjects and control

Parameters	Treated Mean ± SD (n = 18)	Treatment Naive Mean ± SD (n = 12)	Control Mean ± SD (n = 30)
BMI (kg/m^2)	31.11±4.98^a	30.69±5.37^a	25.64±1.01^b
SBP (mmHg)	141.01±8.32^a	151.97±10.01^a	108.19±4.88^c
DBP (mmHg)	92.05±5.41^a	97.03±6.61^a	71.99±7.71^c
Glucose (mmol/l)	4.51±1.23^a	10.55±34.11^b	4.67±0.82^a
Zinc (µg/dL)	15.31±2.60^a	10.44±1.61^b	18.82±2.41^a
Selenium (µg/dL)	0.93±0.50^a	0.62±0.32^b	1.74±0.41^a
Copper (µg/ml)	18.93±8.30^a	14.78±6.67^b	22.38±5.80^a
Lead (µg/L)	1.26±0.41^a	2.01±1.10^a	0.85±0.24^b
Cadmium (µg/L)	4.43±1.74^a	4.95±2.87^a	1.99±1.34^b

***Values with different superscript are significantly different at p<0.05**
Keys: **n** = number of subjects, **BMI**: Body Mass Index, **DBP**: Diastolic Blood Pressure
SBP: Systolic Blood Pressure

Table 2: Mean values of all parameters in treated and treatment naïve diabetic subjects and control

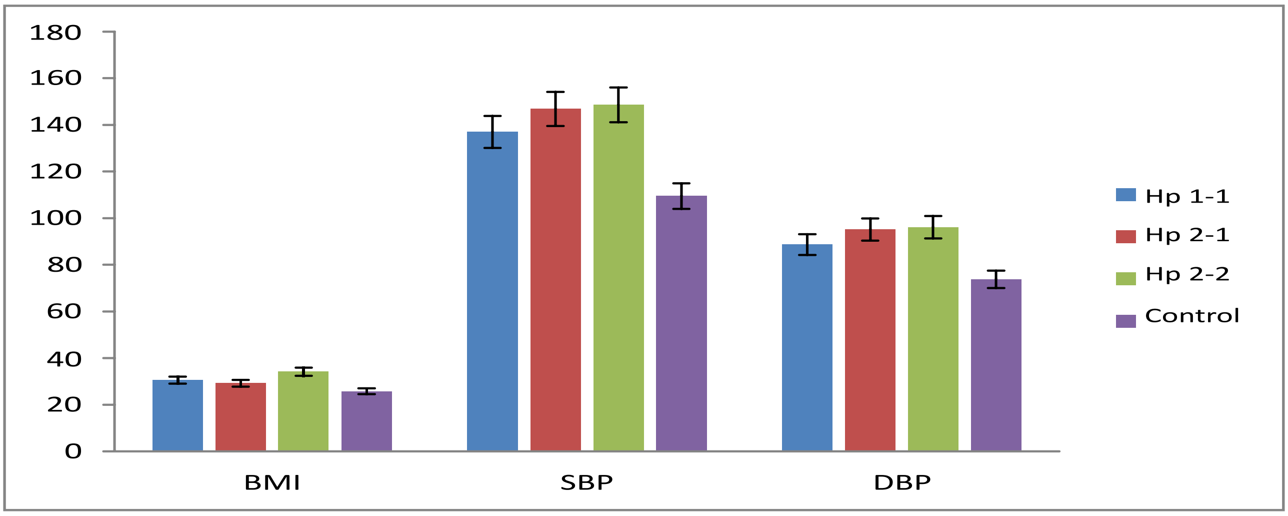
Parameters	Treated Mean ± SD (n = 24)	Treatment Naïve Mean ± SD (n = 16)	Control Mean ± SD (n = 30)
BMI (kg/m^2)	26.55±6.11	27.64±3.90^a	25.64±1.01^b
SBP (mmHg)	105.51±7.47^a	123.11±8.92^a	108.19±4.88^c
DBP (mmHg)	71.03±2.97^a	78.19±5.08^a	71.99±7.71^c
Glucose (mmol/l)	5.21±0.77^a	8.89±1.14^b	4.67±0.82^a
Zinc (µg/dL)	17.19±3.13^a	11.62±1.97^b	18.8±2±2.41^a
Selenium (µg/dL)	1.07±0.60^a	0.78±0.31^a	1.74±0.41^b
Copper (µg/ml)	16.47±7.71^a	13.8±3.69^a	22.38±5.80^b
Lead (µg/L)	1.13±0.39^a	1.69±1.04^a	0.85±0.24^b
Cadmium (µg/L)	4.10±1.72^a	4.41±2.83^a	1.99±1.34^b

***Values with different superscript are significantly different at p<0.05**
Keys: **n** = number of subjects, **BMI**: Body Mass Index, **DBP**: Diastolic Blood Pressure,
SBP: Systolic Blood Pressure

Table 3: Haptoglobin phenotypes in treated and treatment naive diabetics subjects and treated and treatment naive hypertensive diabetic subjects

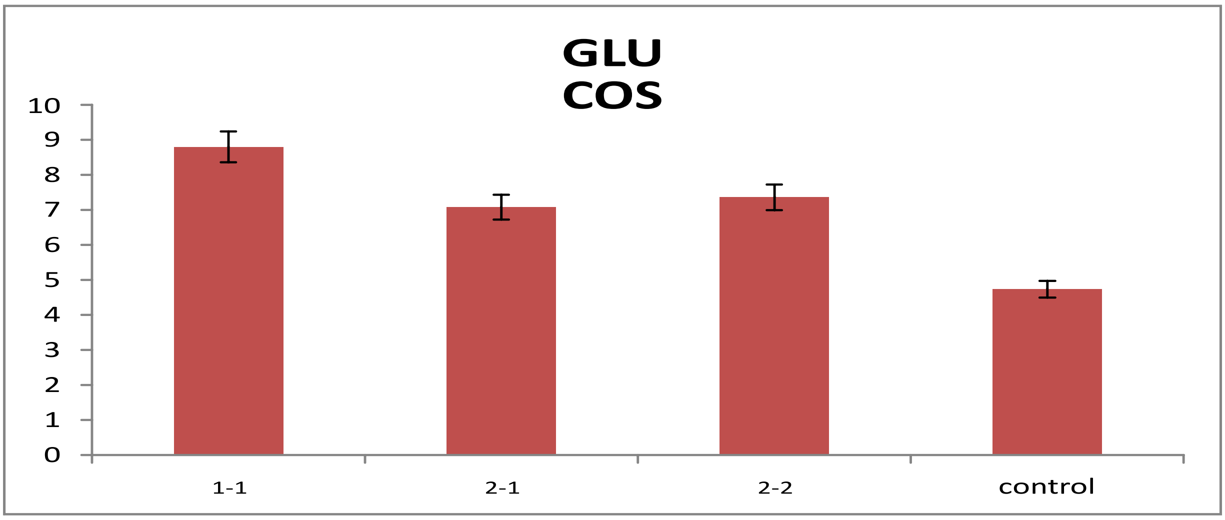
Group (n)	Frequency (%)		
	1-1	2-1	2-2
DM-HTN (n = 31)	4/ 31 (12.9)	16/31 (51.6)	11/31 (35.5)
DM (n = 39)	9/39 (23.1)	18/39 (46.2)	12/30 (30.8)
Control (n = 30)	14/30 (46.7)	14/30 (46.7)	2/30 (6.7)

Keys: DM-HTN: Diabetes complicated with hypertension; **DM**: Diabetes mellitus.



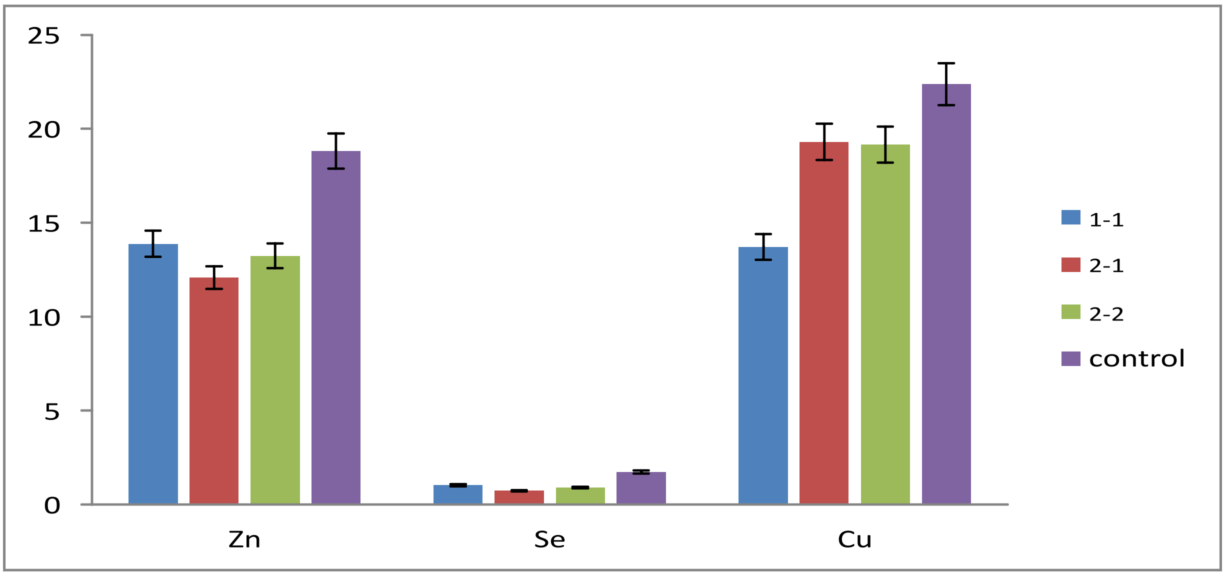
Keys: **BMI**: Body Mass Index; **DBP**: Diastolic Blood Pressure; **SBP**: Systolic Blood Pressure

Fig 1: BMI, SBP and DBP in treated and untreated complicated diabetic subjects and control subjects based on haptoglobin phenotypes



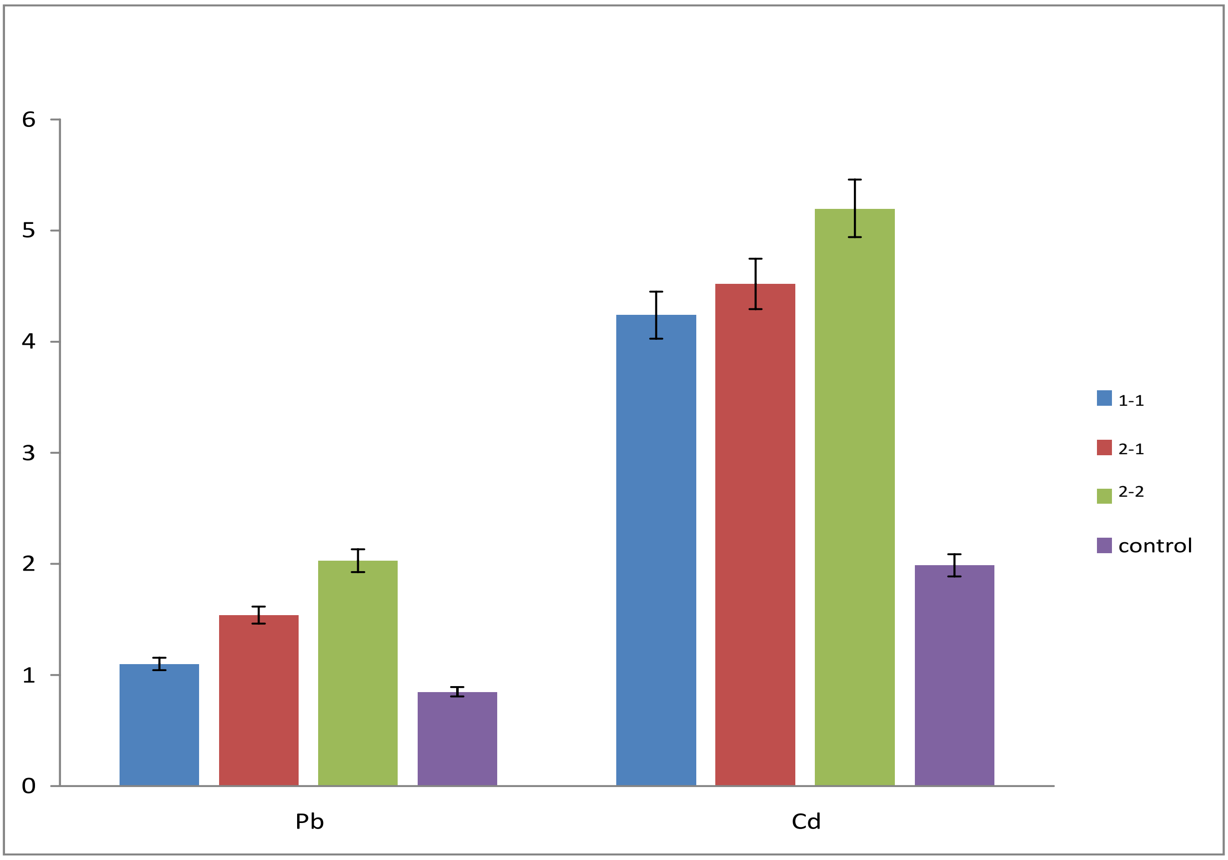
Key: GLU – Glucose

Fig 2: Glucose concentration in treated and untreated complicated diabetic subjects and control subjects based on haptoglobin phenotypes



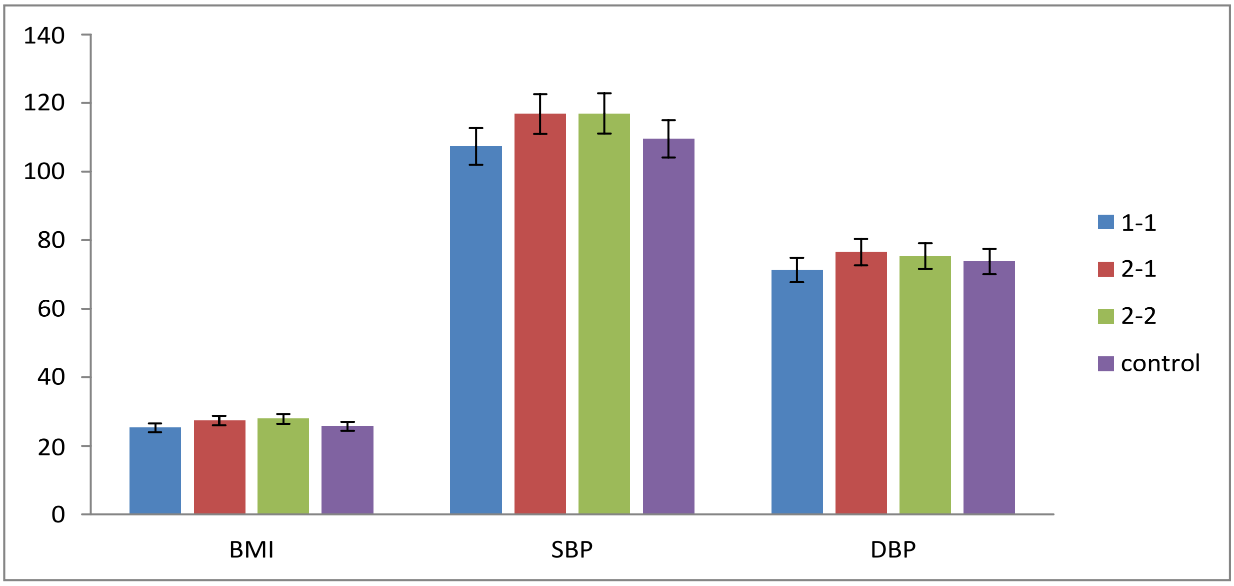
Keys: Zn – Zinc; Se – Selenium; Cu – Copper

Fig 3. Zn, Se and Cu in treated and untreated complicated diabetic subjects and control subjects based on haptoglobin phenotypes



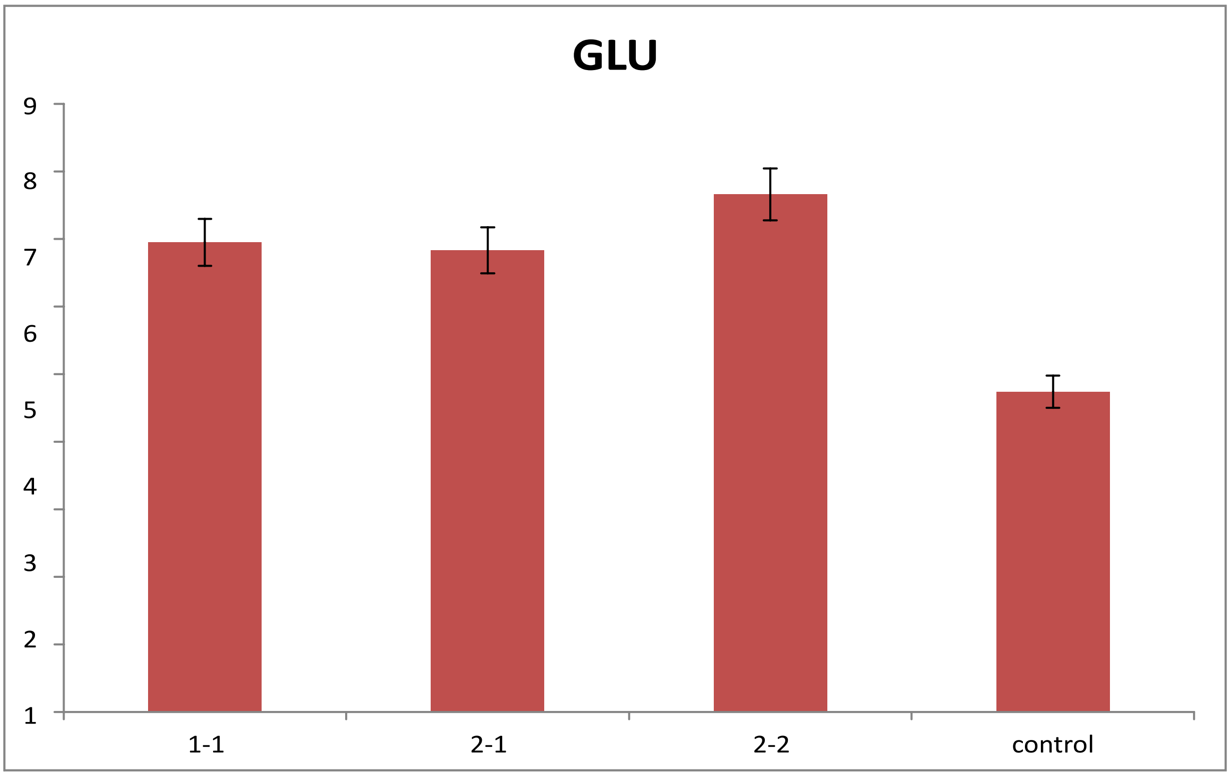
Keys: Pb – Lead; **Cd** – Cadmium

Fig 4. Pb and Cd in treated and untreated complicated diabetic subjects and control subjects based on haptoglobin phenotypes



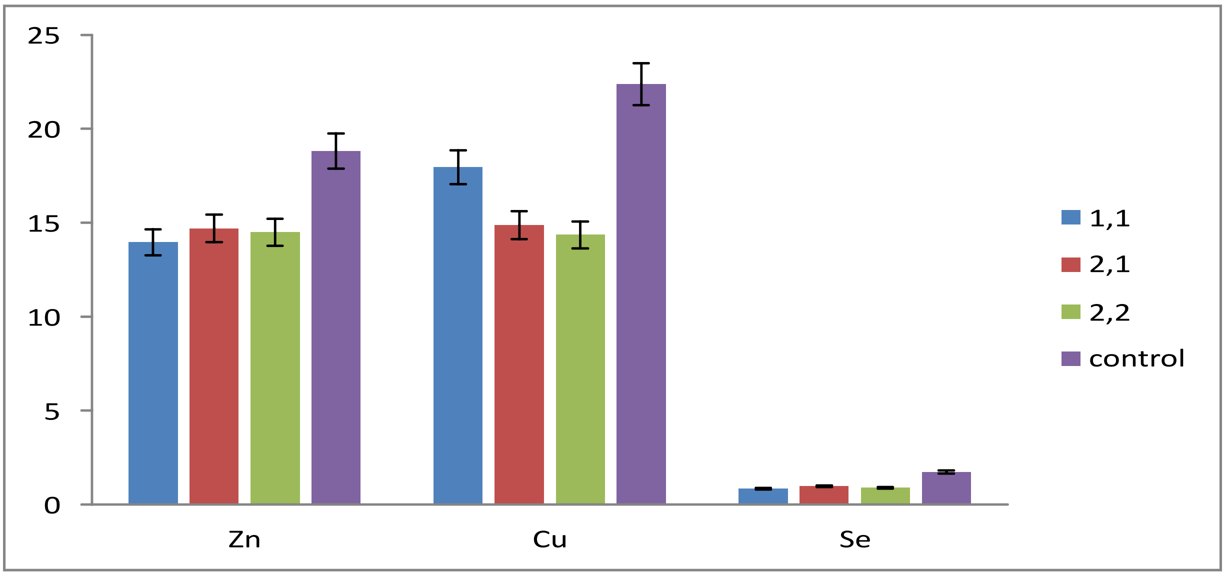
Key: BMI – Body Mass Index; **DBP** – Diastolic Blood Pressure; **SBP** – Systolic Blood Pressure

Fig 5. BMI, SBP and DBP in treated and untreated uncomplicated diabetics subjects and control subjects based on haptoglobin phenotypes



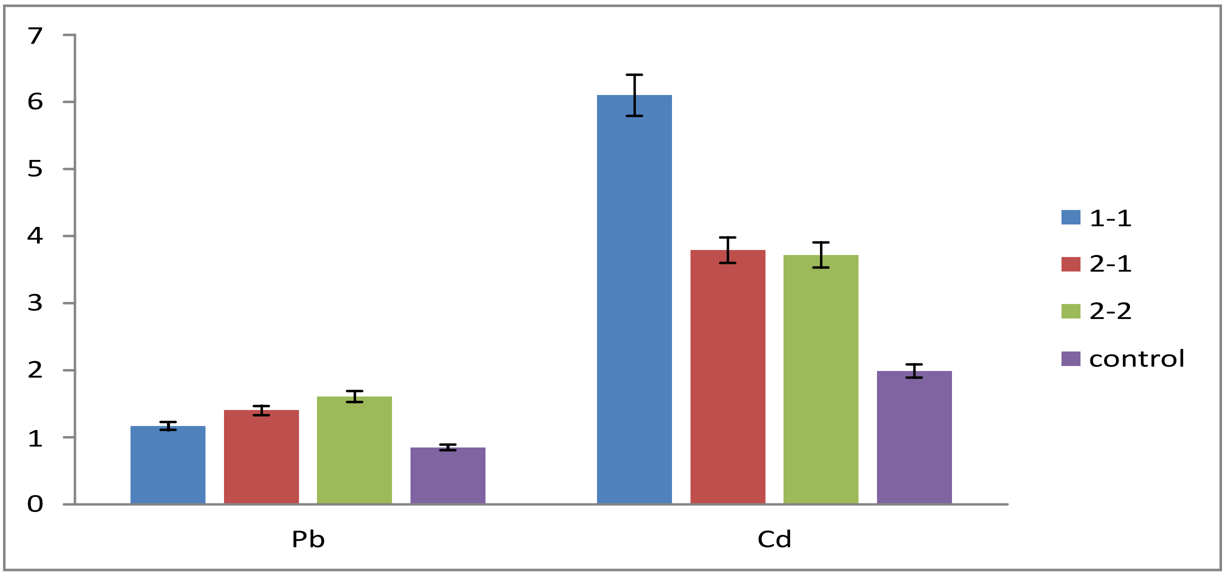
Key: GLU: Glucose

Fig 6. Glucose concentration in treated and untreated uncomplicated diabetic subjects and control subjects based on haptoglobin phenotypes



Key: Zn – Zinc; **Cu** – Copper; **Se** – Selenium

Fig 7: Zn, Cu, and Se in treated and untreated uncomplicated diabetic subjects and control subjects based on haptoglobin phenotypes



Keys: Pb – Lead; **Cd** – Cadmium

Fig 8: Pb and Cd in treated and untreated uncomplicated diabetic subjects and control subjects based on haptoglobin phenotypes

Discussion

Diabetes mellitus have been shown to be complicated with hypertension, the frequency of which varies by ethnic, racial and social group (Bijelic *et al.,* 2020). Diabetes mellitus has been reported to cause a significant percentage of mortality annually and it has been linked with plasma protein abnormalities due to an increase in oxidative stress markers, the effect of which worsens with different haptoglobin phenotype (Sapra & Bhandari, 2022). Some trace metals act as antioxidants preventing the deleterious activities of oxidants on membranes while others act directly as co-factors in metabolism of macro molecules such as glucose. Diabetes has been associated with abnormalities in the metabolism of these trace metals (Kyrou *et al*., 2020). This study was carried out to determine the levels of some essential trace metals and toxic metals in different hapatoglobin phenotypes of complicated and uncomplicated diabetic patients.

In this study, BMI was found to be significantly higher in treated and treatment naïve complicated diabetes subjects compared to control. Increased BMI has been shown to be associated with type 2 DM and indicates an excess of body fat distribution. Significantly higher level of BMI in treated and untreated complicated diabetes mellitus subjects could be due to adipose tissue release of excess circulating fatty acids, glycerol, hormones and pro-inflammatory cytokines which impairs cellular insulin signaling and increases insulin resistance. Chronically raised lipid levels results in impaired islet beta-cell function and lower levels of insulin production which are all characteristic of T2DM (Grant *et al.,* 2021). Insignificantly higher level of BMI in treated complicated and uncomplicated diabetes mellitus compared to control could be an indicator of treatment compliance and lifestyle modification. The finding of this study is in line with previous studies (Naryzny & Legina, 2021; Shi *et al.,* 2022; Odewusi *et al*., 2023) who reported significantly higher BMI level in treated and untreated complicated diabetes mellitus subject compared to control subjects.

The results of this study showed that SBP and DBP in treated and untreated complicated diabetes subjects was significantly higher compared to control ($p<0.05$). This is due to the fact that increased systolic and diastolic blood pressure is characteristic of hypertension which is a complication of T2DM. Significantly raised SBP and DBP reflect the impact of the underlying insulin resistance on the vasculature and kidney (Tsimihodimos *et al*., 2018). This finding is in line with previous studies (Chen *et al*., 2020; Li *et al.,* 2021; Merino *et al*., 2021) which reported significant increase in both SBP and DBP levels of treated and untreated complicated diabetes subjects compared with control subjects. SBP and DBP were found to be insignificantly higher when treated and untreated uncomplicated diabetes was compared to control. This findings disagrees with (Chen *et al*., 2020; Li *et al.,* 2021) which showed that DBP and SBP levels was significantly increased when treated and untreated uncomplicated diabetes was compared to control.

Glucose concentration in this study was found to be significantly higher in untreated complicated and uncomplicated diabetes subjects compared to control subjects ($p<0.05$). This is due to impaired glucose metabolism which is characteristic of diabetes mellitus. T2DM is caused by a combination of two primary factors: defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond appropriately to insulin. Because insulin release and activity are essential processes for glucose homeostasis, the molecular mechanisms involved in the synthesis and release of insulin, as well as in its detection are tightly regulated. Defects in any of the mechanisms involved in these processes can lead to a metabolic imbalance responsible for the development of the disease (Galicia-Garcia *et al.,* 2020). This finding is in tandem with previous findings (Khardori, 2019; Hasanato *et al.,* 2020; Odewusi *et al.,* 2023) which reported significantly higher glucose concentration in untreated complicated diabetes subjects compared with control subjects. Glucose concentration was found to be insignificantly higher in treated complicated and uncomplicated diabetes subjects compared to control, which could be as a result of response to therapy in diabetic subjects. This finding also agrees with previous studies (Khardori, 2019; Odewusi *et al.,* 2023).

In this study, zinc level was significantly lower in untreated complicated and uncomplicated diabetic subjects compared to control subjects. Low blood levels of Zn in T2DM patients observed in the present study are consistent with the previous findings that patients with T2DM tend to have hypozincemia along with the depletion of tissue zinc stores (Hasanato, 2020). Increased zincuria observed in diabetes is believed to be secondary to osmotic diuresis and diabetes related polyuria may also contribute to hypozincemia (Farooq *et al.,* 2020). Zn deficiency has been associated with diabetic complications such as hypertension, thrombosis, ocular involvement, insulin production and resistance to insulin. The main reason for the wide ranging effects of Zn deficiency particularly in diabetes mellitus is due to Zn being a co-factor for over three hundred enzymes involved in various metabolic pathways (Hasanato, 2020). This finding is agreement with previous studies in which significant lower zinc have been reported in complicated and uncomplicated diabetes mellitus subjects (Farooq *et al.,* 2020; Hasanato, 2020). Zinc level was found to be insignificantly higher in treated complicated and uncomplicated diabetic subjects were compared to control subjects. This could be as a result of response to treatment by the subjects.

The results of this study showed that selenium (Se) was significantly lower in untreated complicated and uncomplicated diabetic subjects compared to control subjects. Data regarding serum levels of Se among patients with T2DM are inconsistent however low serum level of Se among patients with T2DM has been reported (Hasanato, 2020). Selenium is an anti-oxidant and provides protection against oxidative stress that has been implicated in the etiology, pathogenesis and complications of T2DM. Deficiency of Se may therefore be a predisposition for development of diabetes (Tarif and AL-Hellawi, 2023). Low Se has 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. This finding is in agreement with previous studies in which a significant reduction in Se in diabetes mellitus subjects have been reported (Hasanato, 2020; Ositadinma and Martina, 2020; Tarif and AL-Hellawi, 2023). Selenium level was found to be insignificantly higher in treated complicated and uncomplicated diabetic subjects compared to control subjects. This could be as a result of response to treatment by the subjects. This is in agreement with previous study (Ositadinma and Martina, 2020).

The results of this study showed that the concentration of copper was insignificantly higher in treated complicated diabetic subjects were compared to control. This could be as a result of response to treatment by complicated diabetic subjects. An imbalance of copper can lead to the progression of diabetes-related complications and impaired antioxidant homeostasis (Gembillo *et al.,* 2023). Furthermore, serum Cu level was significantly higher in treated uncomplicated diabetic subjects compared to control subjects. Increased Cu level among T2DM patients reported previously has been linked with the development of diabetes (Gembillo *et al.,* 2023). Significantly higher serum concentrations of copper may be as a result of hyperglycemia that may stimulate glycation and release of copper ions and this accelerates the oxidative stress, so that advanced glycation end products are formed, that are involved in the pathogenesis of diabetic complication (Oguntibeju, 2019). This finding is in agreement with previous studies (Shenoy Belle *et al*., 2020; Sharifi-Rad *et al*., 2020). Copper level was insignificantly lower in untreated uncomplicated and complicated diabetic subjects compared to control subjects. The lower Cu levels found in this study agrees with the study of Rana (2020) who reported lower levels of Cu in untreated uncomplicated diabetes subjects, but disagrees with findings of Gorini & Vassalle, (2022) who reported significant increase in untreated uncomplicated diabetes subjects.

In this study, Lead (Pb) and Cadmium (Cd) levels were found to be significantly higher ($p<0.05$) in treated and untreated complicated and uncomplicated diabetic subjects compared to control subjects. Lead and Cadmium are toxic metals associated with oxidative stress, increased blood pressure and atherosclerosis (Lamas *et al*., 2021). Pb has been discovered to have a negative effect on the immune system as a major element involved in inflammation and also defensive reactions to cause damage in a living cell (Metryka *et al*., 2018). Therefore, the high concentration of lead and cadmium in diabetic patients might be an indicator of DM and glucose elevations (Ji *et al*., 2021). Our data suggest that increase Pb and Cd concentrations may be a contributing factor to the pathogenesis of diabetes mellitus. This finding agrees with previous studies (Balali-Mood *et al*., 2021; Goyal and Jialal, 2022) which reported significantly higher serum concentration of lead and cadmium in diabetes mellitus subject compared to control subjects.

In this study, Hp 2-1 (51.6%) and Hp 2-2 (35.5%) were higher in complicated diabetes subjects compared to uncomplicated diabetes subjects, while Hp 1-1 (23.1%) was higher in uncomplicated diabetes subjects compared to complicated diabetes subjects (12.9%). This may be as a result of antioxidant capacity of Hp 1-1, Hp 2-1, and Hp 2-2 in decreasing order. Haptoglobin is likely to exert its oxidative protective role through the differences in the phenotypic molecular size and shape of the protein products encoded by the two different Hp alleles. Smaller Hp complexes are believed to quickly mobilize into the extracellular space and undergo glomerular serving better than the larger Hp. This agrees with previous finding (Olaniyan *et al*., 2020) which reported that Hp 2 is highest in complicated diabetes subjects.

Conclusion

The results of this study showed significant alterations in Zn, Se, Cu, Pb and Cd of complicated and uncomplicated diabetes subjects. This result suggests that increase concentration of toxic metals and reduced trace elements concentration may be a contributing factor to the pathogenesis of diabetes mellitus. Alteration in the level of one metal may also influence the normal levels of other metals. The high serum Pb and Cd level of diabetic patients in this study may also be related to the kidney damage complication associated with the disease. Based on these findings, it is recommended that health-care providers consider testing diabetic patients for complications due to Pb and Cd toxicity as part of the treatment regimen.

Conflict of Interest: The authors declare no conflict of interest.

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