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Evaluation of Changes in Cadmium and Copper levels for Diabetes Mellitus

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Abstract:

Diabetes mellitus is a metabolic disorder in which disequilibrium of necessary and toxics trace elements may have an important role in pathogenesis and complications. This study aimed to assess the alterations in serum concentrations of cadmium (Cd) and copper (Cu) among diabetic patients compared with healthy subjects, as well as their correlation. A sample of 20 diabetics and 10 normal persons were recruited for a case control study. Serum Cd and Cu levels were determined by atomic absorption spectrophotometry. The results showed that the average blood Cd concentration in diabetic patients (7.67 ± 2.51 µg/L) was significantly higher than that of healthy controls (2.53 ± 0.56 µg/L; $P < 0.001$), indicating a heavy accumulation of cadmium among the diabetics with respect to the control subjects. By contrast, the average Cu concentration was weakly higher, not significant difference (1.17 ± 0.065 vs 1.13 ± 0.12 µg/L; $P = 0.734$) in diabetic patients than control subjects were recorded (Table I). Further logistic regression showed a significant positive relationship between serum Cd and Cu levels in diabetes patients, which indicates that an increased cadmium exposure was able to affect copper metabolism, potentially leading to the occurrence of oxidative stress or enzyme perturbation with respect to diabetes. These results indicate that alteration of trace metal homoeostasis, especially the increase in cadmium and its association with copper, could be involved in metabolic and biochemical changes associated with diabetes mellitus. More research in larger cohorts is suggested to elucidate the mechanistic role of these factors in diabetic pathophysiology.

Keywords: Diabetes Mellitus; Cadmium; Copper; Heavy Metals; Oxidative Stress; Metal Interaction.

1. Introduction

Diabetes mellitus (DM) is a chronic condition characterized by high blood sugar levels due to defects in insulin action or secretion. It can lead to oxidative stress and inflammation, impacting trace element levels. Heavy metals, especially cadmium (Cd) and transition metals such as copper (Cu), are linked to diabetes and its complications. Cadmium, a harmful metal without a physiological role, causes oxidative stress and impairs pancreatic β-cell function and insulin signaling from long-term exposure to contaminated sources[1]. High cadmium exposure is positively correlated with the prevalence of diabetes and exacerbation of diabetic complications, such as nephropathy and cardiovascular disease[2].

This is supported also by experimental studies that show the endocrine disrupting effects of cadmium, which acts like zinc, inhibits antioxidant enzymes and promotes lipid peroxidation and tissue fibrosis[3]. Copper, in contrast is an indispensable trace element associated with a variety of enzymatic functions such as those related to mitochondrial respiration and antioxidant defense. However, impairment of copper homeostasis may result in oxidative stress and tissue injury. Serum copper level is elevated in the patients with type 2 diabetes mellitus (T2DM), and its level is related to poor glycemic control, as well as higher levels of glycosylated hemoglobin (HbA1c)[4]. Alteration of copper transport proteins and free Cu ions could lead to oxidative stress as was described in molecular studies investigating failure of copper ATPase in diabetes states[5]. In addition, an imbalance of toxic (for example, Cd) and essential metals (for example, Cu, Zn) is associated with renal tubules dysfunction and metabolic disruption in chronically exposed population[6].

In this regard, study of the levels of cadmium and copper in diabetic patients may offer useful information on mechanisms involved in trace metal imbalances and oxidative stress during diabetes. Therefore,

we designed the current study to assess the differences in serum Cd and Cu levels between diabetes mellitus and healthy subjects, as well as to investigate the potential relationship of these two elements. To the best of our knowledge, this is first time a positive correlation between serum Cd and Cu levels has been reported in diabetic subjects, implying that increased dietary cadmium exposure and concomitant perturbations of copper metabolism may synergistically play a role in the development of metabolic/biochemical alteration characteristics of diabetes.

2. Material and Methods

2.1 Collection Sample

This case–control study comprised 30 subjects, comprising 20 cases of DM, and 10 apparently healthy people. All the cases in this study were recruited from Al-Marjan and Al-Sadiq Teaching Hospitals, two governmental hospitals in Al-Hilla City, Iraq. Approval of ethics was granted prior to the sample collection, and informed consent was provided by all the participants. Fasting venous blood (5 mL) samples were drawn from all subjects in fasting after an overnight fast of at least 8 h in the morning. Blood samples were collected in clean, metal-free plain tubes to avoid contamination with trace metals. Serum was then separated by centrifugation at 3000 rpm for 10 min after allowing the sheet of this blood to clot at room temperature. The serums were transferred into tightly closed polyethylene tubes and stored at –20°C for analysis. All samples were processed under controlled conditions from collection to analysis in order to avoid contamination or loss of analyte and cadmium, copper concentrations were determined by Atomic Absorption Spectrophotometry (AAS).

2.2 Sampling Preparation

Serum aliquots from all subjects were analysed for heavy metals according to analytical protocol of the laboratory. All serum samples were thawed to room temperature, mixed slightly on a vortex mixer and homogenized prior to digestion. 1.0 mL of serum was dispensed to the digestion tube and measured. To each of these tubes, 5 mL of fresh prepared acid mixture of concentrated nitric acid (HNO_3, 65%) and perchloric acid ($HClO_4$, 70%) mixed in a ratio of 3:1 was added. The samples were covered with watch glasses and left on a hot plate at 120 °C for digestions until the solution was clear and colourless revealing total organic matter degradation.

Subsequently, after cooling to room temperature the digested solution was filtered with Whatman No.42 filter paper and transferred quantitatively into 10 mL volumetric flasks. The volume of the last tube was made with double-distilled deionized water. All digestions and dilutions were made in acid-cleaned glassware to avoid contamination. Assay blanks were treated in parallel under the same conditions to account for background levels of metal.

Concentrations of Cd and Cu in the digested serum samples were measured by an Atomic Absorption Spectrophotometer (AAS, Shimadzu AA-7000; Japan) with deuterium background correction and element-specific hollow cathode lamps. The wavelengths employed were 228.8 nm for Cd and 324.8 nm for Cu. Standard solutions prepared from analytical-reagent stock standard (1000 mg/L) were used to establish calibration curves for each metal. The readings were taken in triplcate for both standards and samples to ensure accuracy, and reproducibility. The quality control (QC) was guaranteed by analyzing reference materials and blanks in parallel with each batch of samples.

Heavy metals contents were presented as μg/L. The accuracy and precision of the analytical method were confirmed with replicates and recovery tests, resulted recoveries in 95 ~ 104 % for recovery test, indicating that it is applicable to determine heavy metal in biological samples.

2.3 Atomic Absorption Spectrometer

The Cd and Cu contents in serum samples were measured with an Atomic Absorption Spectrophotometer (AAS, Shimadzu Model AA-7000, Japan) attached with deuterium background correction and element-specific hollow cathode lamps[7]. The instrument was used under the recommended analytical working conditions for accurate and sensitive heavy metal determination.

The analytical wavelength for cadmium was 228.8 nm, and the lamp current was at 8 mA; measures for copper were carried out at 324.8 nm with a lamp current of 10 mA. The slit openings of both monochromated was 0.7 nm. The samples were atomized with an air–acetylene flame (C_2H_2–air) as the combustion source. The height of burner, gas flow rate, and aspiration speed were adjusted and then optimized for maximum absorbance and sensitivity[8].

Calibration curves for Cd and Cu were prepared with standard solutions of certified grade in a series from stock concentrations of 1000 mg/L (Fluka Analytical, Switzerland). Working standards (at concentration intervals of 0.1-2.0 mg/L) were newly prepared by serial dilution using double distilled deionized H(2)O. The absorbance of

each standard was read in triplicate and a mean value was used to plot the standard curve. Linearity of each calibration curve was checked ($R^2 \geq 0.998$) and results were considered reliable through the stated analytical range.

The serum samples were aspirated directly into the flame and the absorbance was automatically transformed into concentrations by means of software supplied with the instrument. BLank samples were analyzed concurrently to remove the drift of baseline and for potential contaminants. The system performance was assured by the analysis of reference standards and quality control samples daily[9]. The limits of detection (LOD) were 0.002 mg/L for Cd and 0.005 mg/L for Cu, while the quantification limits (LOQ) were 0.007 mg/ L and 0.02 mg/L, respectively.

All measurements were performed in triplicate and averaged concentrations of the extracts are given in µg/L. The precision and accuracy of the AAS method was verified by recovery values in the range 95-104% for three quality control samples at low, medium and high concentrations; these results are within acceptable criteria for a biological matrix assay[10].

2. 4 Statistical analysis

Statistical analysis All data were analyzed with the Statistical Package for the Social Sciences (SPSS) software, version 25.0 (IBM Corp., Armonk, NY, USA). Values represent mean ± SD of experiments. The mean concentrations of Cd and Cu were compared by an independent samples t-test in DM patients (study group) and healthy subjects (control).

Differences between groups in categorial variables, e.g. sex distribution were tested by chi-square (χ^2) tests. The linear correlation between the Cd and Cu concentrations in each group was further determined using Pearson's correlation coefficient(r).

Furthermore, binary logistic regression analysis was conducted to estimate the relationship between cadmium and copper concentrations and the presence of diabetes mellitus while controlling for potential confounders. The association was indicated as OR with a 95% confidence interval (CI). A p-value < 0.05 was judged to be statistically significant, and for the $p < 0.001$ a very high significance was considered. All analyses were thoroughly reviewed to ensure precision and uniformity before interpretation.

3. Results and Discussions

The mean serum concentrations of cadmium (Cd) and copper (Cu) in diabetic and control groups are presented in **Table (1)** and illustrated in **Figures (3-1)**. Statistical analysis revealed significant differences in Cd levels between the two groups, while Cu levels showed a slight, non-significant increase in diabetic patients.

Table (3-1): Demographic characteristics of study group and healthy control subjects

Characteristic	**Patients with DM** ***n*** **= 20**	**Healthy control** ***n*** **= 10**	***P***
Age (years)			
Mean ±SD	39.60 ± 6.37	36.90 ± 5.30	0.663 † NS
Range	15 –72 years	15– 62 years	
< 30, *n* (%)	6 (30.0%)	4 (40.0%)	0.853 ¥ NS
30-39, *n* (%)	2 (10.0%)	1 (10.0%)	
≥ 40, *n* (%)	12 (60.0%)	5(50.0%)	
Gender			
Male, *n* (%)	12 (60.0%)	4 (40.0%)	0.301 ¥ NS
Female, *n* (%)	8 (40.0%)	6 (60.0%)	

n: number of cases; **SD**: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at $P > 0.05$.

As shown in Table (1), serum Cd levels were markedly higher in diabetic patients compared to healthy individuals ($P < 0.001$), whereas Cu concentrations exhibited no statistically significant difference ($P = 0.734$). This indicates a selective disruption in heavy metal balance associated with diabetes mellitus.

Table (3-2): Cadmium (Cd) level in study group and healthy controls.

	Cases –control comparison		
	patients with DM ***n*** **= 20**	**Healthy control** ***n*** **= 10**	***P***
Cadmium (Cd) level			
Mean± SE	**7.67 ± 2.51**	**2.53 ± 0.56**	**< 0.001 †**
Range	0.33 – 49.84	0.86 – 5.96	**HS**

n: number of cases; **SE**: standard error; †: independent samples t-test; HS: Highly significant at P ≤ 0.001.

Table (3-3): Cuprum (Cu) level in patients with DM and healthy controls.

	Cases –control comparison		
	patients with DM ***n*** **= 20**	**Healthy control** ***n*** **= 10**	***P***
Cuprum (Cu) level			
Mean± SE	**1.17 ± 0.065**	**1.13 ± 0.12**	**0.734**
Range	0.75 – 1.72	0.56 – 1.58	**†** **NS**

n: number of cases; **SE**: standard error; †: independent samples t-test; NS: Non-significant at P >0.05.

Figure (1) displays a clear increase in the mean concentration of Cd in diabetic patients as compared with controls. This discrepancy was very statistically significant (P < 0.001). High Cd values may also indicate increased environmental exposure to, or impaired excretion of, Cd in diabetes.

Cadmium is also considered to induce oxidative stress, impairing pancreatic β-cells and decreasing insulin synthesis[1], which leads to metabolic derangement[2].

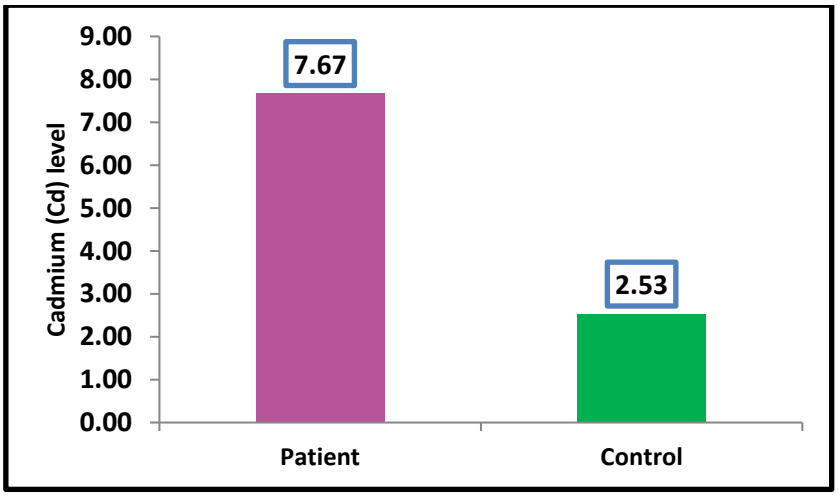

Figure (3.1): Mean Cadmium (Cd) levels of patients with DM and healthy controls

Figure 2 shows that the difference in Cu content in diabetic patients was also negligible, and P was 0.734. Thus, the content of Cu in the serum, for the most part, remains at an acceptable level with controlled diabetes. However, free Cu can itself act as a catalyst for the formation of free radicals and promote increased levels of oxidative stress. According to Lowe et al., the results showing an increase in Cu in uncontrolled diabetes argue that it might happen due to the dysfunction of Cu-binding protein in the affected cells.

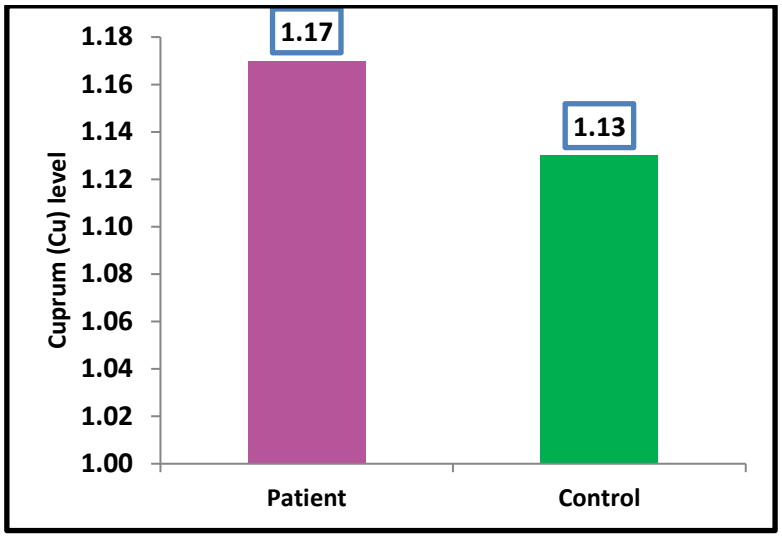

Figure (3.2): Mean Cuprum (Cu) levels of patients with DM and healthy controls

A positive significant correlation between Cd and Cu in diabetic patients was featured in Figure (3). This interaction, established by logistic regression analysis, suggests a biochemical interaction of these metals in the presence of hyperglycemic stress. Cadmium can interfere in Cu transportation by binding to metallothionein and ceruloplasmin, that modifies the amount of available Cu, and has effects on its transport or oxidative stress imbalance[6]. This both metallawellness disturbance can increase lipid peroxidation and decrease antioxidant enzymes[3].

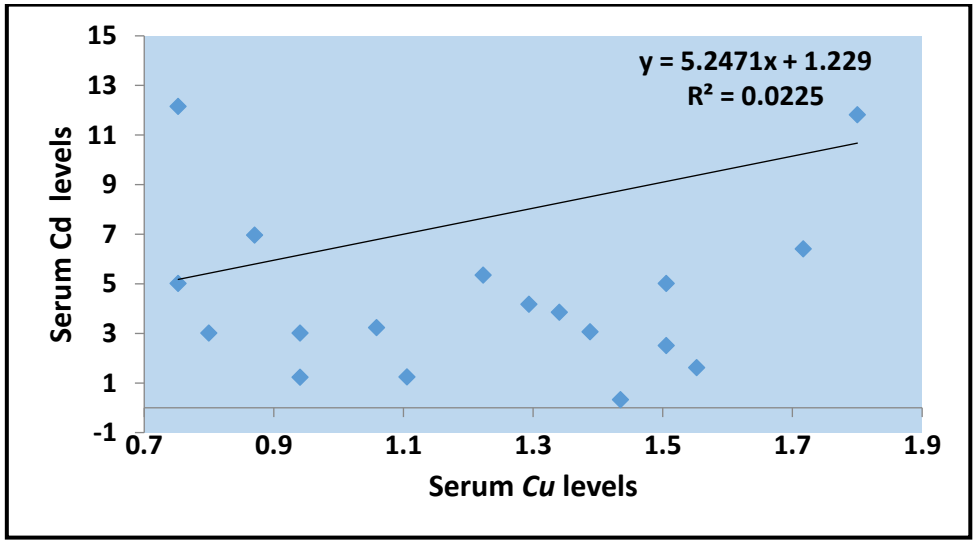

Figure (3-3): The Logestic scatter blot of Cadmium and Cuprum among patients with DM.

The present observations emphasize cadmium as a predominant mediator of biochemical changes in diabetes mellitus. The elevated Cd may further exacerbate oxidative stress and inflammation, resulting in β-cell dysfunctional and insulin resistance. Such activity may be enhanced by free copper, which despite being necessary, can react in redox cycles that promote oxidative damage. This correlation between Cd and Cu may

have indicated commonalities of mechanism in the disruption of metal regulation by which Cd induced oxidative reactions interfered with Cu homeostasis, possibly through shared metalloproteins and overproduction of pro-oxidants.

The current results are in accordance with the earlier reports showing that concurrent rise of toxic (Cd) and essential metals (Cu) disrupt metabolic homeostasis and enhances oxidative load among diabetic subjects. The heterogeneity among published studies might be due to variation in exposure dose or duration of disease, and methods of analysis.

Conclusions

Our current study has established substantial elevation in serum cadmium levels and significant positive association between Cd and Cu levels among diabetic subjects when compared to healthy controls. This finding imply that disproportionate heavy metal, especially Cd deposition, might contribute to metabolic and oxidative disruption in DM. Future studies with larger sample sizes and environmental exposure measurements should be undertaken to unravel the mechanism of heavy metals on diabetic pathophysiology.

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