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Evaluation of Selected Trace Elements in Chronic Hemodialysis Patients in Nnamdi Azikiwe Unviersity Teaching Hospital, Nnewi Southeastern Nigeria

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Abstract

Background: Hemodialysis is the most common form of treatment for end stage renal disease (ESRD), and is associated with considerable morbidity and mortality which might be as a result of imbalance in essential minerals. **Objective**: This study is aimed at determining the levels of some trace elements (zinc, copper, selenium and cobalt) in chronic hemodialysis patients before dialysis, immediately after dialysis and one hour after dialysis. **Methods**: A total of 100 participants were used for this study. This includes fifty chronic hemodialysis subjects and 50 aged matched apparently normal subjects. The trace elements were assayed by atomic absorption spectrophotometric method. **Results**: The mean levels of Zn and Se were significantly lower in dialysis subjects 79.33±16.27 μg/dl, P = 0.02 and 6.67±1.43 μg/dl, P<0.001 compared with the control 88.97±9.77 μg/dl and 9.00±2.48 μg/dl respectively. Cu showed a significantly high mean value in the dialysis 117.54±14.69 μg/dl, P = 0.01 when compared with the control subjects 108.50±15.49 μg/dl. There was no significant difference in the mean level of Co 0.03±0.01 μg/dl, P = 0.26 when compared with the control 0.04±0.00. Comparison of the mean levels of these minerals between male and female counterparts among the dialysis and control groups showed no significant differences. **Conclusion**: This study showed a marked reduction in zinc and selenium levels, and increased copper levels in hemodialysis patients than in controls. This shows an alteration in trace elements levels in chronic hemodialysis patients.

Keywords: *Trace elements, Hemodialysis, Infection, Supplementation, Oxidative stress*

Introduction

Hemodialysis is the most common form of treatment for end stage renal disease (ESRD), and is associated with considerable morbidity and mortality due to accelerated cardiovascular disease and infection [1]. Patients on long-term dialysis tend to have high rates of morbidity and mortality [2]. Risk factors associated with outcome include dialysis modality, age, gender, duration of dialysis, and presence of complications and inflammation [3-5]. Despite the well-documented burden of disease much remains to be learned about how best to prevent these complications of hemodialysis.

Many studies have reported that patients with chronic kidney disease on hemodialysis have disturbed levels of trace elements [6, 7]. Although these elements constitute a relatively small amount of total body tissues, they are very essential in many physiological and biochemical processes. For instance, copper performs vital roles in hemoglobin synthesis and immune

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function. It is also a cofactor for superoxide dismutase, cytochrome c oxidase and ceruloplasmin [8].

Selenium is a potent antioxidant that acts as an anti-inflammatory agent and is required for immune system function [9]. The biological functions associated with selenium include male fertility, prevention of cancer, cardiovascular disease, viral mutation, endocrine and immune function as well as modulating inflammatory response [10]. Zinc plays a critical structural role for antioxidant enzyme superoxide dismutase and can stabilize biological membranes to decrease their susceptibility to oxidative damage that can impair cell functions [11].

In ESRD patients different factors affect serum concentration of trace elements like increased oral intake, failure of renal excretion, degree of renal failure, use of medications, contamination of dialysate, quality of water used for dialysis and metabolic alterations associated with renal failure [12-14].

This study is aimed at determining the levels of zinc, selenium, copper and cobalt in chronic dialysis patients before, immediately after and one hour after hemodialysis. The levels of these trace elements especially zinc and selenium may affect the dialysis outcome of these patients considering the role they play in immunity and oxidative stress. So supplementation of these minerals may prove beneficial to these hemodialysis patients. In our environment, especially Nnewi, South Eastern Nigeria, the number of works relating to this topic is very scanty, thus the significant of this work.

Material and Methods

Study population

The study population is known hemodialysis patients attending Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi (Eastern Nigeria) (both in-patients and out-patients) and normal individuals without kidney disease. The control individuals are laboratory workers in NAUTH that were selected following laboratory test that determined their kidney function. A total of 100 participants (male = 60, female = 40) participated in this study. Fifty patients (grouped as chronic hemodialysis subjects) and 50 age matched apparently normal subjects (grouped as control subjects) were recruited.

Study design

This is a cross-sectional study designed to investigate the levels of zinc, selenium, copper and cobalt in chronic hemodialysis patients and in aged-matched controls. The hemodialysis subjects include both the out-patients and the in-patients of the hospital that have undergone hemodialysis for five or more times. The dialysis was carried out twice per week on each patient and the duration of each dialysis was four hours. This study was carried out from September to December, 2010. All the participants gave their consent before they were recruited and the study was approved by the ethics committee of NAUTH, Nnewi.

Blood sample collection

The sample collection was carried out immediately before dialysis (Pre-D), 0-hour after dialysis (0-HAD), and 1-hour after dialysis (1-HAD) on either of the two sessions per week. 5mls each of venous blood was collected for Pre-D, 0-HAD, and 1-HAD into sterile plain containers, allowed to clot, retracted and spun. The serum was separated from the red cells into small vials and stored frozen at -20°C in a sterile plain container until analysis.

Trace Elements Analysis

The analysis of trace elements was done by atomic absorption spectrophotometric method.

A. Principle

The principle is based on dissociation of the element from its chemical bonds by the heat of the flame to form unexcited or ground state atoms. These unexcited atoms are at a low energy level in which they are capable of absorbing radiation at a very narrow bandwidth corresponding to its own line spectrum. The amount of radiant energy from the hollow-cathode lamp absorbed by the ground-state atoms in the flame is proportional to the concentration of trace metals present. Generally, a separate lamp is required for each metal (e.g. a copper hollow-cathode lamp is used to measure copper) [15].

B. Assay procedure

A three point standard curve was used for the determination of zinc in the serum. The series of zinc standards (0.1ppm, 0.2ppm, and 0.4ppm) were prepared from 1000ppm commercial zinc standard stock (analytical grade). A 1:10 dilution of sample or controls to diluents is first carried out in order to prepare the samples and controls for analyses. This was done by adding 0.25 ml of sample or control to 2.25 ml of sample diluents into the Sarstedt trace-element free tube.

For copper, 1000 ppm commercial copper stock standard solution was diluted down into 1.2 ppm, 2.4 ppm, 3.6 ppm, 4.8 ppm and 6.0 ppm for standard curve. Each 1 ml of serum sample or control was then diluted with 0.5 % nitric acid to a total volume of at least 2.5ml.

A series of selenium and cobalt standard solutions were also prepared from the 1000 ppm commercial stock Se and Co standards respectively for the preparation of standard curve.
1ml of the serum was deproteinised with 9ml of 10% trichloroacetic acid in 0.1% lanthanum solution for the analysis of selenium and cobalt. The resulting supernatant was diluted with 0.11% lanthanum depending on concentration of individual elements and aspirated to the atomic absorption spectrophotometer (AAS).

Statistical analysis

The version 17 of Statistical Package for Social Sciences (SPSS) (IBM, Los Angeles information Technology Services), was used in statistical analysis. All data were expressed as the mean ±SD. Comparisons between groups were performed by using a one-way ANOVA. Student's t-test was used when comparing two groups. Differences with $P < 0.05$ were considered significant.

Results

Comparison of zinc, copper, selenium, and cobalt in hemodialysis patients and control subjects.

The mean levels of zinc and selenium are significantly lower in hemodialysis subjects when compared with the control subjects (**See Table 1**), while the mean levels of copper is significantly higher in hemodialysis subjects than in control subjects (**See Table 1**). However, there was no significant difference between the mean levels of cobalt in hemodialysis and control subjects (See **Table 1**).

Comparison of zinc, copper, selenium, and cobalt in Pre-D, 0-HAD, 1-HAD, and control subjects.

Mean levels of Zn in Pre-D, 0-HAD and 1-HAD are significantly lower when compared with the control subjects, but there was no significant difference between Pre-D and 1-HAD (See **Table 2**). A significant lower concentration of selenium was observed in Pre-D, 0-HAD, and 1-HAD when compared with the controls. But there was a significant higher Pre-D Selenium concentration than 1-HAD selenium concentration (**See Table 2**).

There were significant higher mean levels of copper between Pre-D and control subjects, and between Pre-D and 1-HAD (**See Table 2**). In case of cobalt, there was no significant difference observed between the Pre-D, 0-HAD, and control subjects.

Comparison of zinc, copper, selenium, and cobalt in male and female subjects.

The mean levels of zinc, copper, selenium, and cobalt in Pre-D, O-HAD, and 1-HAD are independent of sex as shown in **Tables** 3.

Discussion

This study showed a significant higher zinc levels in chronic hemodialysis subjects when compared with the normal control subjects. This work agreed completely with other findings elsewhere [16-19]. Decreased zinc levels in HD patients may be as a result of restricted food intake because all the HD patients were receiving diet with limited protein content. Similar observation was made by Sen et al. [20]. According to them; rich sources of Zinc like meat, fish, cheese, chicken, nuts, and almond were restricted in dialysis patients. Zinc deficiency is associated with delayed wound healing [21] and immune deficiency characterized by impaired cell proliferation, abnormal T-cell function, defective phagocytosis and abnormal cytokine expression [22]. This might contribute to the risk of infection observed in hemodialysis patients. [23] Zinc deficiency may also cause or contribute to a number of relatively non-specific conditions commonly observed in hemodialysis patients, including anorexia [24] dysgeusia, and impaired cognitive function [25].

This study showed decreased levels of selenium in dialysis patients than in normal control. This agreed with the previous studies [26-28]. Reduce level may be attributed to reduced intake of selenium in food due to food restriction, [29] and increased inflammation which affect the absorption of selenium.[30]

Table 1: Zinc, Copper, Selenium, Cobalt in haemodialysis and control groups.

Parameters	Hemodialysis Mean (SD) (n=50)	Control Mean (SD) (n=50)	P- value
Zn (µg/dl)	79.33 (16.27)	88.97 (9.77)	<0.001
Cu (µg/dl)	117.54 (14.69)	108.50 (15.49)	0.01
Se (µg/dl)	6.67 (1.45)	9.00 (2.48)	<0.001
Co (µg/dl)	0.03 (0.01)	0.04 (0.00)	0.25

Table 2: zinc, copper, selenium, cobalt in pre-d, 0-had, 1-had and controls.

Parameters	Pre-D N=50	0-HAD N=50	1-HAD N=50	Control N=50	F-value	P-value
Zn(µg/dl)	79.33(16.27)[a]	76.92(20.19[a]	75.12(17.68[a]	88.97(9.77)	7.037	<0.001
Cu(µg/dl)	117.54(14.69)[c]	113.03(14.5)	109.55(14.7)	108.50(15.9)	3.83	0.01
Se(µg/dl)	6.67(1.43)[a,c]	6.38(1.20)[a]	5.60(1.13)[a]	9.00(2.48)	39.43	<0.001
Co(µg/dl)	0.03(0.01)	0.04(0.00)	0.04(0.00)	0.04(0.00)	1.37	0.25

a: $P \leq 0.02$ compared with control; c: $P \leq 0.04$ compared with 1-HAD

Table 3: Zinc, Copper, Selenium, and Cobalt Levels in Male And Female Subjects.

Parameter	Male Mean (SD), (N=30)			Female Mean (SD) (N=20)		
	Pre-D	0-HAD	1-HAD	Pre-D	0-HAD	1-HAD
Zn (µg/dl)	79.3(16.0)	75.9(21.5)	88.2(10.0)	79.4(17.2)	78.4(18.5)	89.8(9.6)
Cu (µg/dl)	117.6(16.1)	113.6(16)	106.9(16.2)	117.5(12.6)	112.3(12.)	110.3(14.7)
Se (µg/dl)	6.4(1.4)	6.4(1.2)	8.3(2.4)	7.1(1.3)	6.4(1.2)	8.9(2.4)
Co (µg/dl)	0.03(0.01)	0.04(0.0)	0.04(0.0)	0.03(0.01)	0.04(0.0)	0.04(0.0)

There was no significant difference in all the male groups when compared with female groups; P>0.05

Lower levels of serum selenium may increase the risk of anaemia and certain cancers [31]. Heart failure, and coronary disease [32] and cardiomyopathy among dialysis patients [33]. Fujishima et al [34] have also shown that low blood selenium status may contribute to immune system dysfunction and an increased risk of death especially from infectious diseases. Finally, mild selenium deficiency appears to increase susceptibility to oxidative stress, [35] which may be relevant to hemodialysis patient in whom oxidative stress is markedly increased [36].

It was also found that the level of copper is significantly higher in the hemodialysis patients than in the controls. This is in line with many previous works [1, 18]. Guo et al [18] explained that raised Cu level in HD may be as a result of the release of Cu during inflammatory tissue damage. Our work did not agree with the works of Anees et al, [16] and Tetiker et al, [6] who found lower Cu level in HD than in normal control. Also shouman et al [37] reported an insignificant Cu level in HD patient and control subjects. An increased Cu level in blood is accompanied by an increased oxidative stress in HD patients [38].

Comparison of the levels of these elements between male and female counterparts in different dialysis groups studied was not significant. This shows that observed levels of these minerals in the hemodialysis patients is independent of sex. Furthermore, there was no significant difference between 0-HAD and 1-HAD in all the trace elements in this study. This indirectly shows that similar biochemical changes take place in both 0-HAD and 1-HAD.

Conclusion

Though with relatively lower sample size, this study showed a marked reduction in zinc and selenium levels, and increased copper levels in hemodialysis patients than in controls. This shows an alteration in trace elements levels in chronic hemodialysis patients. Thus we recommend zinc and selenium supplementation and also suggest future research on the association of low trace elements with mortality in Chronic HD patients.

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