Pak. j. life soc. Sci. (2024), 22(1): 1679-1687 E-ISSN: 2221-7630;P-ISSN: 1727-4915

Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

https://doi.org/10.57239/PJLSS-2024-22.1.00117

RESEARCH ARTICLE

Quantitative Determination of Trace Elements (Zn, Cu, And Mg) In Serum for Chronic Renal Failure Using Graphite Furnace Atomic Absorption GFAAS

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ARTICLE INFO

Received: May 25, 2024

Accepted: Jun 27, 2024

Keywords

Chronic kidney disease

Zinc

Magnesium

Atomic absorption

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ABSTRACT

Chronic kidney disease (CKD) is defined as a decrease in glomerular filtration rate, increased urinary albumin excretion, and, ultimately, complete loss of kidney function or kidney damage. This study was conducted in Kirkuk City from August 2023 to December 2023. The study involved collecting blood samples from 80 individuals with chronic kidney disease, aged 20-70 years, and 40 healthy individuals without any chronic diseases, aged 25-40 years The trace elements zinc, copper, and magnesium were quantitatively estimated in the blood serum of patients with kidney failure and the blood serum of healthy individuals using graphite non-flame atomic absorption spectroscopy (GFAAS). It was found that the concentration of zinc in the blood serum of patients with kidney failure was significantly lower ($P < 0.0001$) compared to the healthy group. The study also revealed that the copper concentration in patients with kidney failure was significantly higher ($P < 0.0001$) compared to the healthy group. Moreover, the study found that the concentration of magnesium in patients with kidney failure is significantly lower ($P < 0.0001$) compared to the healthy group. To investigate the effect of chronic kidney failure on the normal levels of trace elements zinc, copper, and magnesium, some of which were not previously measured in these patients at the dialysis center in Kirkuk.

INTRODUCTION

The existence of a defect in the structure or function of the kidneys is the defining feature of chronic kidney disease (CKD)[1,2] . Diabetes and high blood pressure are the two primary causes of CKD, and they are also prevalent causes of the condition[3]. Kidney cancer[4], kidney stones[5], or chronic urinary tract infections (UTIs)[6], hydronephrosis are other illnesses and causes that can impair kidney function and result in chronic kidney disease (CKD)[7,8]. A personal history of acute kidney injury (AKI)[9], a family history of renal failure or chronic kidney disease (CKD)[10], and tobacco product usage or smoking are additional risk factors for CKD[11]. In addition, CKD can be brought on by a wide range of different illnesses or situations, and it can strike anyone at any age. Since chronic kidney disease (CKD) sometimes starts without any obvious symptoms, early identification is crucial [12].

The search results make it clear that one common consequence of CKD is an imbalance of important trace elements, which is linked to a higher risk of cardiovascular events and death. It is underscored that tracking the levels of vital trace elements in patients with CKD is crucial for therapeutic care and may help halt the course of the illness and enhance patient outcomes[13,14,40]. Using blood concentration levels of trace elements, cross-sectional research has examined the potential use of trace element profiling in CKD. By evaluating the relationship between trace elements and CKD, the study set the stage for future investigations in this field[15]. The effects of certain trace metals on individuals with CKD, including Zn, Cu, Mn, Cd, and other trace elements, have been covered by the findings of earlier research. It has been shown that some trace elements can have an impact on the health and outcomes of CKD patients when they are out of balance[16,38]. The findings of the research also touched on uncharted ground regarding trace elements in end-stage renal disease (ESRD). Although some trace elements have been linked to undesirable findings in the general population, there has been no systematic investigation or regular testing of these elements in patients undergoing hemodialysis[17,18,39].

Essential trace metals like zinc and copper are involved in hematopoiesis, immune system function, and the control of catecholamine metabolism, among other physiological functions. When CKD patients take zinc supplements, they run the danger of developing a copper deficit, which can result in serious conditions, including anemia and myelopathy[19]. Individuals with CKD are at risk for zinc insufficiency, which can hinder immune response, development, and glucose metabolism, among other physiological functions. Concerns about zinc-induced copper deficit arise in CKD patients using zinc supplements[19,20]. Another study has looked at the blood concentrations of selenium, copper, iron, and zinc in individuals with various stages of chronic kidney disease. According to the results of the study, the levels of copper, iron, zinc, and selenium in patients in Stages 1-4 of CKD were not significantly different[21]. Manganese, selenium, and zinc are the three main elements that are markedly deficient in dialysis and TGBXSV patients compared to healthy individuals. Managing trace element shortages in individuals with chronic kidney disease and dialysis is important[22].

Commonly employed indicators of chronic kidney disease (CKD) are urea and creatinine. As CKD worsens, compromised kidney function causes less urea to be cleared from the body, which raises blood urea nitrogen (BUN) levels[23]. Moreover, when the glomerular filtration rate (GFR) is significantly reduced, serum creatinine levels rise. Serum creatinine is a late indication of acute renal damage; a rise in creatinine level requires approximately 50% of kidney function to be lost before it is noticeable. Elevated blood creatinine levels are caused by the kidneys' decreased clearance of creatinine as CKD advances and GFR decreases[23]

An atomic absorption spectrometer was used, which is intended to measure and estimate the concentration of elements. It is used for quantitative analysis of about 66 elements. The device is characterized by high measurement sensitivity for low concentrations of elements, high individual specificity and sensitivity for each element, simple operation and maintenance, and optical interference can also be controlled, which increases measurement sensitivity[24].

Atomic absorption spectrometry is an analytical method used to determine trace elements in solution or the solid state. The method is not affected by the presence of metals in the sample, whether it is in a free state or a molecular state. This depends on the ability of the free and neutral atoms. The resonance beam of the element is absorbed when it passes through the atomic vapor for the item[25,26].

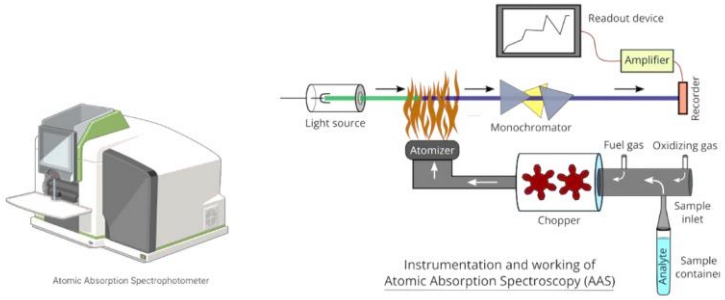

Figure 1: Atomic absorption spectrometry diagram[27].

MATERIALS AND METHODS

The trace element magnesium was found utilizing the Flameless Atomic Absorption Spectrophotometer method using the Graphite Furnace (GFAAS) technology. This element was determined using an Atomic Absorption Spectrophotometer (SHIMADZU AA7000). One of the most significant methods in atomic absorption spectrometry is GFAAS, which is renowned for its great sensitivity and ability to detect at very low detection limits (in parts per billion).

This method, also called Electrothermal Atomic Absorption Spectrometry (ETAAS), vaporizes the material in three steps: drying, ashing, and atomizing. It does this using a graphite furnace tube. This method's basic idea is based on the observation that unbound atoms of an element absorb light at wavelengths specific to the element when it comes from a particular cathode lamp. The amount of light absorbed can be linearly associated with the concentration of the analyte present, within specific bounds. The majority of elements can apply high heat to samples to make free atoms.

In GFAAS, a tiny volume of sample (10µL–20µL) is injected into a small graphite or graphite tube coated with paralytic carbon. The analyte is then heated to a variety of temperatures to evaporate and atomize it. When ultraviolet or visible electromagnetic radiation is absorbed by the atoms, it causes electrons to transition to higher electronic energy levels in the excited state and subsequently back to the ground state by releasing a particular type of light. To find the concentrations of the sample, this light can be detected. Depending on the element being examined, the graphite tube's temperature can rise to up to 3000ºC in a matter of seconds.

Standard Solutions Preparation:

The initial reference solutions for the relevant components were created with a 1000µg/ml concentration in 2% HNO3. The original standard stock solution was then diluted using the usual dilution law (C1 V1 = C2 V2) to create four standard solutions. Starting with the highest concentration and progressively working down to the levels needed for the calibration curve to behave as intended, a range of concentrations was produced.

"(1000µg/ml → 100µg/ml → 10µg/ml → 1µg/ml)".

A value of 1000ng/ml (1000 ppb) is equivalent to 1µg/ml (1 ppm). The original standard solution can be diluted in the following ways to get the necessary concentrations for creating the standard calibration curve: -

'(1000ng/ml → 100ng/ml → prepare the needed concentration of any element)'.

Determination of Zinc in Samples:

As was said and described earlier, four standard solutions of zinc were made. As seen in figure (2), the four standards for the calibration curve were (2, 4, 6, and 8 µg/ml). Using a calibration curve as

a guide, the zinc concentrations in samples were measured constantly and directly in addition to the standard solution analyses.

Conditions for Zinc Determination: Listed in table (1)

Table (1): Ideal conditions for zinc determination

The Variables	Units	The ideal condition
(Wavelength)	nm	213.9
(Lamp current)	mA	8
(Lighting mode)	……	BGC-D 2
(Sample size)	µL	20
(Slit width)	nm	5×10^{-1}
(Replicates)	…….	6

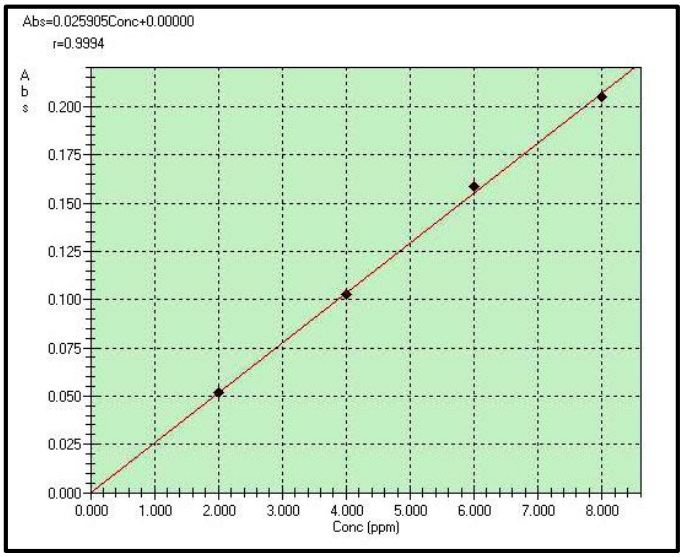

Fig (2): The standard curve for calculating Zinc.

Determination of Copper in Serum:

As stated earlier and detailed in the paragraph, four standard solutions of copper were made. As seen in figure (3), the four standards for the calibration curve were 5, 10, 20, and 30 ng/ml. Using a calibration curve as a guide, the concentrations of copper in samples were measured directly and continuously beyond the range of standard solutions.

Conditions for Copper Determination: Listed in table (2)

Table (2): Ideal conditions for copper determination.

The Variables	Units	The ideal condition
(Wavelength)	nm	324.8
(Lamp current)	mA	6
(Lighting mode)	……	BGC-D 2
(Sample size)	µL	20
(Slit width)	nm	5×10^{-1}
(Replicates)	…….	6

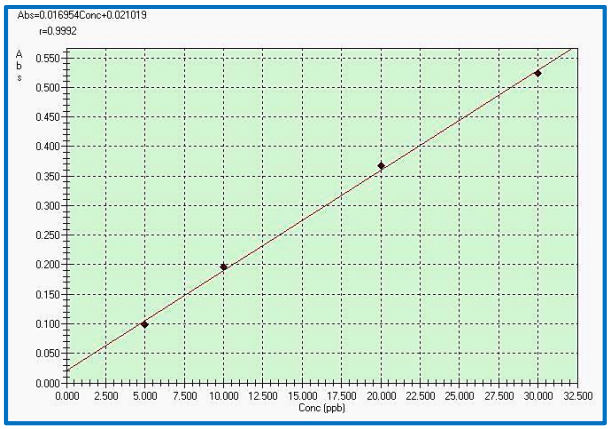

Fig (3): The standard curve for calculating Copper.

Determination of Magnesium in serum:

As was previously noted, four standard solutions for the element were produced. According to figure (4), the concentrations of these standards were 5, 10, 20, and 30 ng/ml for the calibration curve. Using the calibration curve as a guide, the magnesium concentrations in the samples were continually and directly measured.

Conditions for Magnesium Determination: Listed in table (3)

Table (3): Ideal conditions for magnesium determination.

The Variables	Units	The ideal condition
(Wavelength)	nm	285.2
(Lamp current)	mA	8
(Lighting mode)	.....	BGC-D 2
(Sample size)	µL	20
(Slit width)	nm	5×10^{-1}
(Replicates)	.....	6

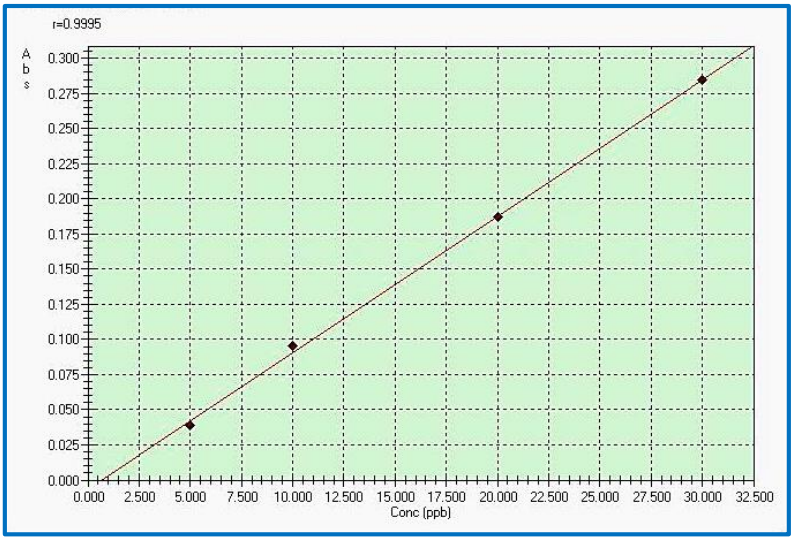

Fig (4): The standard curve for calculating magnesium.

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Statistical Analysis

The results of the statistical analysis were presented using the mean standard deviation (SD) and GraphPad Prism v8.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was considered as P≤0.05, and the correlation between the parameters was also evaluated.

RESULTS

Table (4) Concentration of trace elements in the blood serum of kidney failure compared to the healthy group.

Elements	Mean ± S. D Health	Mean ± S. D Patients	P-Value
Zn(ppb)	**898.8± 62.63**	**382.1±99.29**	**0.0001**
Cu(ppb)	**90.15±6.818**	**444.6±58.58**	**0.0001**
Mg(ppm)	**0.2800±0.1067**	**0.9060±0.1192**	**0.0001**

The results indicated that the concentration of zinc in the blood serum of patients with chronic kidney failure was lower compared to the healthy group, with a statistically significant difference ($P < 0.0001$), as shown in Table 4. Similarly, the concentration of copper in the blood serum of patients with chronic kidney failure was higher compared to the healthy group, with a statistically significant difference ($P < 0.0001$), as shown in Table 4. Additionally, the results revealed that the concentration of magnesium in the blood serum of chronic kidney failure patients was higher compared to the healthy group, with a statistically significant difference ($P < 0.0001$), as shown in Table 4.

DISCUSSION

Zinc: The results, as shown in Table 4, indicate that the average deviation rate in blood serum for individuals with kidney failure is (99.29±382.1), compared to (62.63±898.8) in the healthy group. This suggests a decrease in the concentration of zinc in the blood serum of individuals with kidney failure compared to the healthy group. This study is consistent with previous findings that patients with renal failure have lower blood serum zinc concentrations[25,28]. Zinc deficiency in kidney failure patients can have various causes, including inadequate dietary intake. Individuals with kidney disease may not be able to consume sufficient amounts of zinc-rich foods due to dietary restrictions. Additionally, in addition to zinc loss related to dialysis, these patients may have malabsorption of zinc from their digestive systems, leading to a zinc deficit. Some researchers suggest that because zinc in blood serum binds with plasma proteins, a decrease in albumin and protein concentrations in the blood could lead to excessive loss of zinc. Proteinuria, especially for albumin, causes individuals with renal failure to excrete a large amount of zinc along with protein. As a result, the constant excretion of urine eventually leads to a decrease in the blood serum concentration of zinc. Furthermore, although their pH levels are often acidic, individuals with kidney failure also experience an acid-base imbalance. Thus, it is believed that the conversion of zinc into red cells in acidic conditions contributes to a reduction in zinc concentration in the serum of kidney failure patients[29,30,31]

Copper: The results of the study, as shown in Table 4, indicate that the average deviation rate in the blood serum of patients with kidney failure is (444.6±58.58), while in the healthy group, it is (90.15±6.818). Research has shown that the blood serum copper levels in patients with chronic kidney failure are higher than those in healthy individuals, which is consistent with other studies[32]. Excessive intake of copper-containing foods can lead to nephrotoxicity, characterized by proximal tubule necrosis caused by oxidative stress, cellular damage, and reduced kidney function[33]. Conversely, there is a reciprocal relationship between kidney disease and copper. Patients with chronic renal disease may also have disorders in protein metabolism and inadequate kidney excretion, leading to copper imbalances. Regulating copper levels in patients with chronic kidney failure is crucial in preventing complications, as high copper levels have been associated with chronic

renal disease in previous studies[34,35]. Imbalances in copper concentration may result from various factors, such as changes in dietary habits or reduced ability to absorb copper from food. Medications used to treat kidney failure and infections associated with kidney failure or other health issues can also impact blood copper levels. Elevated blood copper levels may be a primary risk factor for chronic renal disease. High levels of copper in the blood have also been linked to rapid deterioration in kidney function and a decrease in glomerular filtration rate.

Magnesium: The results of the study, as shown in Table (4), indicated that the average deviation rate for the blood serum of patients with kidney failure was (0.9060 ±0.1192), while for the healthy group, it was (0.2800±0.1067). It was observed that there was a significant increase in the magnesium concentration in the blood serum of patients with kidney failure compared to the healthy group[36].

In chronic kidney disease (CKD), renal regulatory mechanisms may not be adequate to maintain a balance in the intestinal tract absorption of magnesium. Magnesium levels usually remain normal; however, variations in serum magnesium concentration are noted when the glomerular filtration rate falls. Dialysate magnesium levels are crucial for maintaining magnesium balance in the serum of patients receiving dialysis who have end-stage renal failure. Increased magnesium is less typical, but study results are contradictory and suggest other factors, such as a poor diet and the use of drugs (such as phosphate binders containing magnesium, sevelamer, and proton pump inhibitors), may impact magnesium concentrations.

Dialysis prescriptions can impact both the magnesium concentration and the flow of magnesium through the dialysis machine. For instance, the pH of the dialysate bicarbonate solution can impact the number of anionic sites in albumin and the degree to which magnesium binds to albumin. Using dialysate citrate may also impact magnesium concentration, as citrate and magnesium can form a combination that is dialyzable and promotes the body's elimination of magnesium. Modifications in dialysate glucose or dialysate glucose delivery can stimulate insulin in insulin-sensitive tissues, which may enhance cellular magnesium uptake[37]**.**

CONCLUSION

The present study, employing non-flame atomic absorption spectrometry technology under specific conditions for each element in the graphite device, revealed that the concentration of zinc in the blood serum of patients with chronic kidney failure was significantly lower than in the healthy group. Furthermore, the study demonstrated that the concentration of copper in the blood serum of patients with chronic kidney failure was notably higher compared to the healthy group. Lastly, the investigation highlighted that the concentration of magnesium in the blood serum of patients with chronic kidney failure was markedly lower than in the healthy group.

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