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Original Research Article

Concentration of Selenium, Copper, and Zinc in Nephrolithiasis Patients

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Abstract

One of the most popular disease worldwide is nephrolithiasis (kidney stone). Its distributed in many countries specially in the middle east. It may occurred due to changes in electrolytes and minerals. Trace elements are the most important cofactors that contribute in many enzymatic reactions in the body. The most common and important elements are selenium, copper and zinc. The aim of this study is estimate the concentration of the selenium, copper and zinc and compare them between healthy subjects and patients with kidney stones. The flame atomic absorption spectroscopy is the most accurate method to detect the trace elements. The study was conducted on 40 patients and 40 healthy subjects in Hilla city. The results of this study showed that the selenium and zinc levels decreased and copper increased in patients group when compared with the healthy subjects. Selenium and zinc has an inhibitory role to renal stones prevent nucleation of the stones in kidneys.

Key Words : Renal stones, copper, zinc, selenium.

الخلاصة

يعد مرض تحصي الكلية (حصى الكلية) واحدا" من الامراض الأكثر شيوعاً في جميع أنحاء العالم ويتوزع في العديد من البلدان وخاصة في منطقة الشرق الأوسط. قد يحدث نتيجة للتغيرات في الشوارد والمعادن . العناصر النزرة هي من العوامل المساعدة الهامة التي تساهم في العديد من التفاعلات الإنزيمية في الجسم. العناصر الأكثر شيوعا وأهمية هي السيلينيوم والنحاس والزنك.

الهدف من هذه الدراسة هو تقدير تركيز السيلينيوم والنحاس والزنك ومقارنتها بين الأصحاء والمرضى الذين يعانون من حصى الكلى . مطياف لهب الامتصاص الذري هو الأسلوب الأكثر دقة للكشف عن العناصر النزرة. أجريت الدراسة على ٤٠ مريضا و ٤٠ شخصا سليما في مدينة الحلة.

أظهرت نتائج هذه الدراسة أن مستويات السيلينيوم والزنك وانخفضت وزاد النحاس في مجموعة المرضى بالمقارنة مع الاشخاص الاصحاء . السيلينيوم والزنك له دور المثبط لحصيات الكلية.

الكلمات المفتاحية : حصيات كلوية، النحاس، الزنك، السيلينيوم.

Introduction

Kidney stone is one of the most common diseases expected to affect in a person life time. A study suggests that 1 in 11 people suffer from a kidney stone disease in the United States [1]. The main factors contribute to the formation of a kidney stone are age, sex, occupation, social class, climate and dietary habits. Kidney stone is a crystal aggregation formed in the kidney from super saturation of urine with salts. Therefore, formation of different type of kidney stones depend on the urinary pH and type of salt constituents involved in such case. The formation of stone primarily depends on major constituents. However, knowledge of minor and trace elements is also important for treatment and development of medicines for kidney stones [2].

Trace elements are defined as any element that comprises in little or very little amount in the body. Despite their small concentration, trace elements play a vital role in many different biological functions. The relationship of trace elements to different disease states has long been known [3]. The World Health Organization (WHO) has classified 19 trace elements that are vital to human health including Se, Zn, and Cu [4]. Although trace elements are essential components of biological structures or essential for enzymatic function, they can be toxic at higher concentrations beyond what is necessary for their biological functions [5]. The deficiency of trace elements from diet produces a structural and/or functional defects because of specific or general biochemical changes and can be reversed by supply the body with essential trace elements, so the deficiency of trace elements considers a critical for health [6]. Nutritional elements such as Zn, Cu, and Se are components of proteins, enzymes and hormones, which regulate many processes, including balancing redox reactions, immune response, and formation of connective tissue during development [5]. The deficiency (or excess) of essential trace elements can result in pathological disorders, such as infections, inflammatory reactions, cancers or even neurological diseases [5].

It is a trace element present everywhere, It is important for developing living organisms, so it's important for the body. It can be find in all cells and tissues of the body. In mammals, 85% of the all amount In the body is in muscle and bone, and 11% in skin and the liver with the remaining 4% in all the other tissues [7]. The Recommended Dietary Allowance (RDA) of Zn is 11 mg for males and 8 mg for females. Doses that are larger than 25 mg may cause anemia and Cu deficiency. Zinc is required for over 300 enzymes, and it plays three major biological roles: catalyst, a structural component, and regulatory ion. It is directly participated in catalysis and co-catalysis functions of enzymes, which hold many cell processes including normal growth, membrane stability, bone formation, fetal development and reproduction [8]. It also activates or inhibits both enzymatic activity and the stability of proteins. Zinc has been established to modulate cellular signal reception, second messenger metabolism, protein kinase and protein phosphatase activities [9].

Copper (Cu) is a trace element and essential for humans. Copper finds in two forms – the first and second oxidation form, as most of the copper in the human is in the second form. The copper able easily to attach and accept electrons and that is show its importance in oxidative reduction processes and in disposing and removing free radicals from the body [10]. Although scientists explorer copper compounds to treat diseases in 400 BC (during Hippocrates) [11], new information was discovered regarding the biochemistry, physiology, toxicology, many clinical, laboratory and other indicators of the impact of copper in the human. The human body consists of about 70 - 80 mg of copper [12]. The content of copper varies depending on gender and age [13,14]. Copper is found in all cells of the human body. The highest concentrations of copper are found in the brain and the liver; the central nervous system and the heart have high concentrations of copper as well [12]. The importance of copper nutritive is clear due to its requirement in enzyme systems. The adverse effects of copper toxicity are given more importance than copper deficiency. Copper balance is important specially in relationship to other nutrients. The consideration should be given to the possibility of copper to deficient as to copper be toxic [15].

Selenium (Se), is an essential nutrient of main importance to human. This is become more clear in recent years. The new research has revealed that the selenium until today unsuspected role for this element in areas of vital importance to human health. The selenocysteine is the 21st amino acid, it is a compound of selenoproteins, some of which act important enzymatic functions [16]. Selenium is important for selenium-dependent antioxidant enzymes such as Glutathione Peroxidase and other selenoproteins. Since the antioxidant characteristic of selenium and/ or selenoenzymes, it has been suggested that selenium may prevent Cardio-Vascular Diseases. Many observational studies investigating the relationship of low selenium concentrations with cardiovascular outcomes and randomized trials investigating whether selenium supplements prevent coronary heart disease (CHD) have been inconclusive [17].

Materials and Methods

Patients

A (40) samples of patient groups from (15) women and (25) men with renal stone. The patients' age were ranged from (30 - 65) years.

The samples were collected from Urology Center in Al Hilla Teaching Hospital in Hilla City, Iraq, from October 2015 to January 2016, according to the history: patients with positive history of renal stone, patients with radio-opaque stone or radio-lucent stone ultra sound, KUB and/or CT scan examination. The patients were free from chronic diseases except type 2 diabetes. Pregnancy women and patient under renal medical treatments were excluded. All patients underwent full history and physical examination.

Control groups (C) consist of (40) samples of healthy volunteers from (15) women and (25) men. They were collected from relatives who were free from signs and symptoms of renal stones, with age ranged from (30-65) years, the healthy control group were non-smokers, free from DM, hypertension and no family history of renal stones.

Samples Collection

Fasting blood samples were collected in plain tubes then to centrifuged at 4,000 rpm for 5 min at 4°C to obtain serum. Serum were stored at (-20°C) immediately after separation in multiple Eppendorf till analysis.

Procedure

The technique of Flameless Graphite Furnace (GFAAS) Atomic absorption Spectrophotometer method was used to determine the some essential trace elements (Copper, zinc and selenium). SHIMADZU AA7000 Spectrophotometer was used for determination of these elements. GFAAS is one of the most important of the five techniques of the atomic absorption spectrometry in which has the higher sensitivity which can be reached to the low detection limits (in ppb units).

In GFAAS, very small amount of samples (10µL-20µL) was injected in small graphite or paralytic carbon coated graphite tube, which heated by a wide range of temperature to vaporize and atomize the analyst. The atoms absorb the electro-magnetic radiation in the ultraviolet or visible region resulting in transitions of electrons to higher electronic energy levels to the excited state and then back to the ground state by emitting it's specific characteristic light which can be measured to determine the samples concentrations.

The heat of the Graphite tube increases over a matter of seconds and reach up to 3000ºC depending on the element being analyzed.

Original standard solutions were (1000µg/ml in 2% HNO_3) for interested elements. Four standard solutions were prepared by dilution from original standard stock solution using general dilution law ($N_1 V_1 = N_2 V_2$). A series of concentrations from the highest one reaching the values required for calibration curve must be prepared.

Samples were digested by transferring (20µL) of serum into Eppendorf tube, then (40µL) of 10% nitric acid was added and mixed well for 10 minutes, finally complete dilution volume to (100µL) with deionized water. Solution was filtered and appropriate solution volume of (20µL) was injected into the graphite tube for reading.

Determination of serum Copper:

The four standards were (5, 10, 20, 30 ng/ml) for calibration curve as shown in figure (1). The concentrations of copper in samples were measured directly and continuously beyond measuring of standard solutions depending on the calibration curve. Conditions for Copper Determination: Listed in table (1).

Determination of serum Zinc:

The four standards were (100, 200, 500, 1000 ng/ml) for calibration curve as shown in figure (2). The concentrations of Zinc in samples were measured directly and continuously beyond measuring of standard solutions depending on the calibration curve. Conditions for Zinc Determination: Listed in table (2).

Determination of serum Selenium:

The four standards were (5, 10, 20, 40 ng/ml) for calibration curve as shown in figure (3). The concentrations of Selenium in samples were measured directly and continuously beyond measuring of standard solutions depending on the calibration curve. Conditions for Selenium Determination: Listed in table (3).

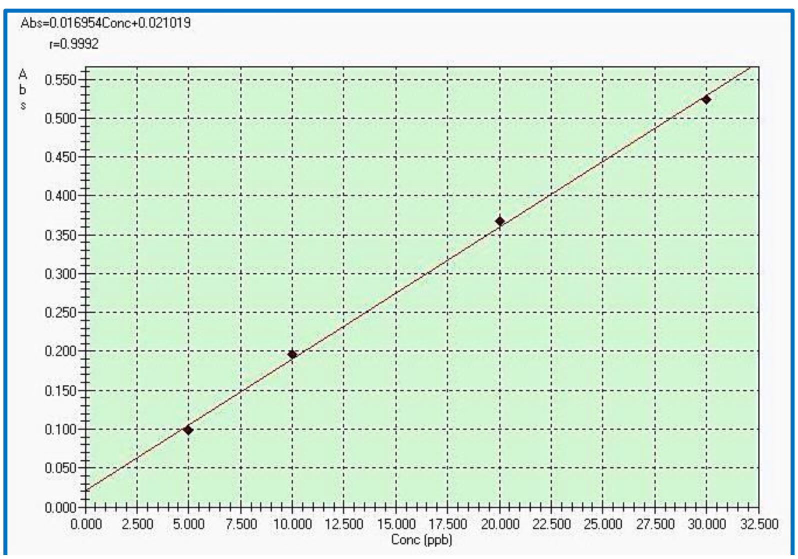

Figure 1: Standard curve for Copper determination

Table 1: Conditions for Copper Determination

Variable	Ideal condition
Lamp current	6 mA
Wavelength	324.8nm
Slit width	0.5nm
Lighting mode	BGC-D2
Sample Size	20 µl
Replicates	3

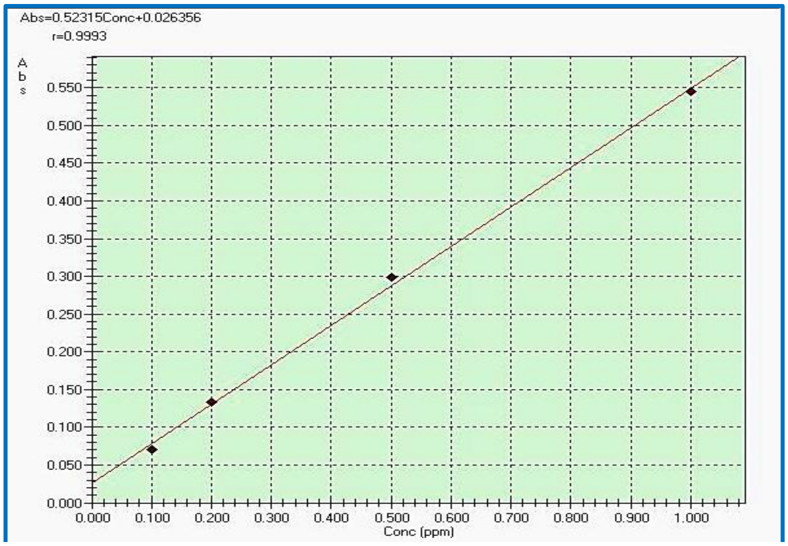

Figure 2 : Standard curve for Zinc determination

Table 2 : Conditions for Zinc Determination

Variable	Ideal condition
Lamp current	8 mA
Wavelength	213.9 nm
Slit width	0.5nm
Lighting mode	BGC-D2
Sample Size	20 µl
Replicates	3

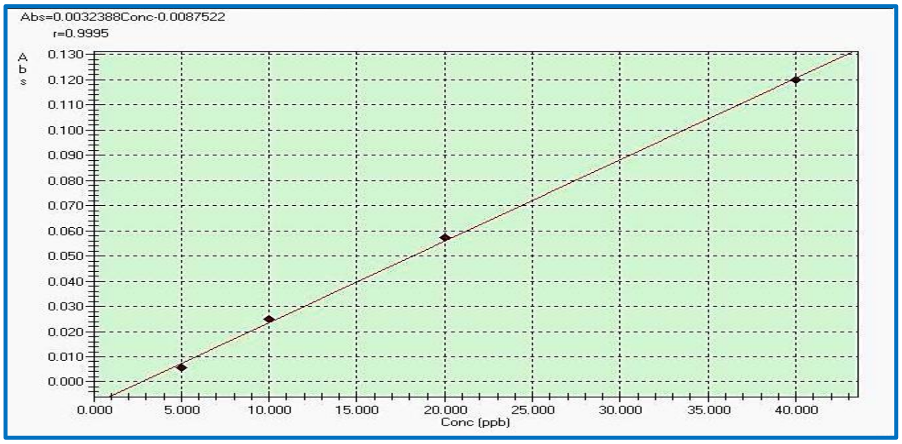

Figure 3 : Standard curve for Selenium determination

Table 3: Ideal Conditions for Selenium Determination

Variable	Ideal condition
Lamp current	23 mA
Wavelength	196.0nm
Slit width	0.5nm
Lighting mode	BGC-D2
Sample Size	20 µl
Replicates	3

Results

Selenium is found in control subjects with the average level of (71.29±4.2, 74.3±6.98) ng/ml male and female respectively while in nephrolithiasis patients group is found with average level of (53.05±3.1, 52.55±2.8) ng/ml male and female respectively, $p<0.05$.

Copper was found with increasing level in patients when compared with healthy subjects. In this study, the mean serum Cu levels in renal stone's patients was (190.6±15.3) ng/ml and was significantly higher than healthy controls which is (167.85±12.5) ng/ml, $p<0.05$.

In this study was found significantly higher serum zinc levels in the healthy controls with serum Zn level was 174.88±7.99 than renal stone patients with mean Zn. In this study, the patients had Zn level was 104.87 ± 10.65ng/ml, $p<0.05$.

Discussion

Selenium is one of stone formation inhibitors studied which could be stuck onto the crystal surface and would inhibit induction of new crystal growth and aggregation. The selenium concentration decreased in nephrolithiasis patients.

Some researchers found significantly higher amounts of copper in patients with renal stones [18-20]. Some studies showed that serum levels of copper do not differ between renal stone patients and healthy groups.

Zinc is a second important trace element in the human body . It has been considered as inhibitors of renal stone formations. In some studies, serum Zn is lower in renal stone patients than healthy controls [18, 21, 22].

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