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Evaluation of Some Trace Elements (Zn, Cu, Mg and Mn), Among Patients with Kidney Stones

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Abstract: Some elements may take part in the initiation of stone crystallization or inhibitor in urine, in this study; we estimated some of trace elements in patients and control groups to correlate between the distributions of trace elements in the serum with kidney stone formation in Sudanese's patient. This is descriptive cross-sectional study correlated during 1st May to 30ty September 2014 and was conducted in Khartoum State hospitals**.** Sixty blood samples were collected from the patients at Urology unit along with forty blood samples as a control, the mean ± SD of the age in patients were 41.75 ± 2.03 and for the control were 42.95±1.67, this study included both gender. Biochemical analysis of serum samples was carried out for the patients and the control group to estimate some of the trace elements (Zn, Cu, Mg and Mn) concentrations. It was found that there is a statistical significant difference in Zn and Mn concentration between the two groups with P value in Zn (0.003) and in Mn (0.000), but not in Mg and Cu concentration, P value in Mg (0.074) and in Cu (0.273). Our study suggested that increase of Zn and decrease of Mn associated with increased risk factor of kidney stone disease.

Keywords: Trace Element, Kidney Stones, Biochemical Analysis, Statistical Analysis

1. Introduction

A kidney stone is a solid piece of material that forms in a kidney when substances that are normally found in the urine become highly concentrated. [1]. Stones can be composed of either single substances or salts, which are two or more substances that join together. Uric acid and cystine are single substances. On the other hand, calcium stones and struvite stones are each composed of salts. Seventy five percent of the kidney stones that reach the stone analysis laboratory contain calcium oxalate as the predominant mineral. [2]. Nucleation is the formation of a solid crystal phase in a solution. It is an essential step in renal stone formation the term super saturation refers to a solution that contains more of the dissolved material than could be dissolved by the solvent under normal circumstances. Crystal aggregation and attachment of crystals or aggregates to an alternative nidus such as renal epithelial cells are critical processes in stone formation. [3]. The contribution of trace constituents to the pathogenesis of kidney stones remains unclear and under debate. The findings of some studies seem to support a role for some major and trace elements in the initiation of stone crystallization. [4]. The process of crystallization in the urinary tract occurs when the equilibrium between promoting and inhibiting factors is broken, some studies may support the thesis that some major and trace elements may take part in the initiation of stone crystallization for instance as a nucleus or nidus for the formation of the stone, or simply contaminate the stone structure. [5]. Specific chemical elements that were either involved in the crystallisation process of kidney stones, The concentrations of Zn, Cu, Fe, Pb and Cr were greater than that of ingested from a standard diet, [6]. [7], suggests that higher DZI is associated with increased risk of kidney stone disease. Magnesium is considered as a one of the most important inhibitors of lithogenesis in urinary tract, but its real role in this process has never been fully explained. [8], found urinary Mg level to be higher in healthy controls and no differences in serum levels this may support the thesis about its role as a potential inhibitor of lithogenesis. [9], reported that contrary to zinc excretion, the copper and manganese urinary levels were lower in stone formers than in normal subjects, Mn concentration in the serum and urine of active stone patients

is shown to be lower than healthy people. [5, 10], reported that low level of manganese might interfere with the fragility of urinary stones in ESWL (extracorporeal shockwave lithotripsy) therapy.

2. Materials and Methods

This research was conducted in Khartoum state hospitals (urology unit; Alribat hospital, Military hospital and Ibn Sina hospital). Hundred subjects were included in the study, sixty patients with kidney stone, and forty healthy appearances considered control group.

2.1. Ethical Consideration

This study was approved term the committed of Al Neelain University Faculty of post Graduate Studies Department of Biochemistry and Molecular Biology, all individual shared in the study was informed by the aim of the study and verbal informed console was obtained from each subject.

2.2. Blood Sample and Processing

Three ml venous blood was taken from nephrolithiasis patient, another three ml from controls group. The samples were collected in tube (plain tube). Serum was separated from RBCs by centrifugation at 2000 rpm for 10 minutes. Two ml serum was using for estimation some of the trace elements (Zn, Mg, Cu and Mn), the samples were stored at -20oc. The process of measurement was conducted in biochemistry lab by using Atomic Absorption Spectrophotometer in Suba Center for Veterinary Research.

2.3. Principle of Phoenix-986 AAS Atomic Absorption Spectrophotometer Instrument

The Phoenix-986AAS is an easy to use atomic absorption spectrophotometer dedicated to the laboratories that are after practical operation rather than extravagant sophistication which may never be required to the extent of 70% sometimes, it uses a personal computer to control via dedicated applications software the instrument and relevant accessories, the automatic 8-lamp turret provides a multi-sequential AA analysis whereas eight different elements may be selected at one time with their respective measurement parameters and then loaded automatically in the respective order provided by the operator. The settings and adjustments of the instrument's conditions for measurement are completed automatically.

A complete data report of single element or multi element measurements is offered for display and print-out. The spectra of element lamps, standard curves, signal curves, operating parameters and operator's own-defined texts may be displayed and printed out upon demand.

Apparatus:

A motorised flame / furnace switchov.

Place of Origin: United Kingdom

Brand Name: Biotech Engineering Management Co limited.

Model Number Phoenix-986AA

Wavelength Range 190 - 900 nm

2.4. Sample Preparation of Zinc, Manganese, Magnesium and Copper

Diluted the samples by adding 4.5 ml distilled water to 0.5 ml of samples (serum).

2.5. Zinc Measurement

Procedure

All reagents and standards were of analytical graded. Stock solutions of Zn were prepared, samples brought to room temperature, samples were made directly on each of the final solutions, the absorbance of standard and samples were measured at 213.9 nm against blank using biotech engineering Phoenix - 986 atomic absorption spectroscopy (AAS).

Concentration of Zinc

The concentration (ppm) x 10 (dilution factor) = concentration of sample.

2.6. Manganese Measurement

Procedure

All reagents and standards were of analytical graded. Stock solutions of Mn were prepared, samples brought to room temperature, samples were made directly on each of the final solutions, the absorbance of standard and samples were measured at 279.5 nm against blank using biotech engineering Phoenix - 986 atomic absorption spectroscopy (AAS).

Concentration of Manganese

The concentration (ppm) x 10 (dilution factor) = concentration of sample.

2.7. Magnesium Measurement

Procedure

All reagents and standards were of analytical graded. Stock solutions of Mg were prepared, samples brought to room temperature, samples were made directly on each of the final solutions, the absorbance of standard and samples were measured at 285.2 nm against blank using biotech engineering Phoenix - 986 atomic absorption spectroscopy (AAS)**.**

Concentration of Magnesium

The concentration (ppm) x 10 (dilution factor) = concentration of sample.

2.8. Copper Measurement

Procedure

All reagents and standards were of analytical graded. Stock solutions of Cu were prepared, samples brought to room temperature, samples were made directly on each of the final solutions, the absorbance of standard and samples were measured at 324.7 nm against blank using biotech

engineering Phoenix - 986 atomic absorption spectroscopy (AAS).

Concentration of copper

The concentration (ppm) x 10 (dilution factor) = concentration of sample.

3. Results

A total of hundred subject were enrolled in this study 60 of them were patients diagnosed with kidney stone disease and 40 were healthy individual (control group), in this study included both gender, the male and the female in the case group reported (63%) and (37%) respectively. Whereas the control group includes (58%) male and (43%) female, the minimum and the maximum age of the patients were (4-93) years old of patient's ages and the mean ± SD was 41.75 ± 2.03 and minimum and the maximum for the control were (5-70) and the mean ± SD was 42.95±1.67.

Table 1. *Descriptive analysis of the trace elements.*

		Case	Control
Zn mg/L	Mean ± SD	3.51 ± 2.27	2.36 ± 0.96
	Median (Minimum-Maximum)	(0.08 - 9.70)	(0.59 - 4.17)
Cu mg/L	Mean ± SD	10.94 ± 8.42	9.20 ± 6.55
	Median (Minimum- Maximum)	(0.00 -33.70)	(0.00- 23.70)
Mn mg/L	Mean ± SD	0.64 ± 0.40	1.18 ± 0.54
	Median (Minimum- Maximum)	(0.00 – 2.14)	(0.30 - 2.99)
Mg mg/L	Mean ± SD	64.31 ± 10.84	60.48 ± 9.62
	Median (Minimum- Maximum)	(30.74 - 99.22)	(34.49 – 8-.34)

*SD (Stander Deviation)

Table 2. *Comparison of the mean, stander error of mean and significant differences between the cases and the controls of serum trace elements.*

Parameters	Control group 40 individuals	Case group 60 individuals	*P* value
Copper	9.20± 1.04	10.94± 1.09	0.273
Zinc	2.36±0.15	3.51± 0.29*	0.003
Manganese	1.18± 0.09	0.64±.051*	0.000
Magnesium	60.48±1.52	64.31± 1.4	0.074

Mean ± Std. Error;* = Significant differences; $P< 0.05$ consider to be significant.

Table 2. Shows that the patients have higher means of zinc, copper and magnesium than controls, and lower means of manganese compare with controls.

Used spss 16. Independet t-test there are significal diffrences in zinc, manganese between the patients with kidney stone disease and controls, but there no significal diffrence in magnesium and copper.

4. Discussion

In this study it found may be their relation between distribution of some trace element like (Zn and Mn) in the serum but it found their no effect of Mg serum level and no significant difference of copper but they had high concentration compeer with control, their significant differences in (Zn and Mn) and it found high concentration of Zn in patient compared with the controls, and low level of Mn in patients of kidney stone disease than the healthy people.

[4] Suggested that some of studies seem to support a role for some major and trace elements in the initiation of stone crystallization process in the urinary tract and promoting or inhibiting factors is broken. [7] Suggests that higher DZI is associated with increased risk of kidney stone disease. In this study we found that the Mg level in serum has no effect of kidney stone risk but [5] reviewed several studies demonstrate that a low level of magnesium in urine is a risk factor for lithogenesis. On the other hand [11] reported that urinary Mg excretion is not significantly different in stone patients and healthy and no difference in urinary magnesium level in recurrent stone disease when compared to control group [8] also found urinary Mg level to be higher in healthy controls and no differences in serum levels.

Also in this study we found the low level of serum Mn concentration in kidney stone patents compeer with the control and this compatible with [5] who he reported that the Mn concentration in the serum and urine of stone patients is shown to be lower than healthy people, [10], reported that low level of manganese might interfere with the fragility of urinary stones in ESWL therapy, [12], suggested that manganese, could be of significance in the pathological mechanism of stone formation, not from mineralogical or crystallographic viewpoints but for the smooth flow of enzymatic reactions in biological systems**.**

5. Conclusions

This study was designed to investigate the differences between serum (Zn, Mg, Mn and Cu) in 60 patients suffering from kidney stone disease and 40 healthy individual, the indepented sample t-test showed significal difference Zn and Mn concentration ($P < 0.05$). the analysis of serum trace elements levels may be helpful in understanding the reaction of promoters and inhibitors that affect the crystallization or the kidney stone formation, it may be the Zn have role of formation the stone by enters the structural composition of the stone and the Mn make the stone more fragile.

Recommendation

Increase dietary intake of manganese (I delete the word and) can be helpful to prevent stone formation and also decrease dietary intake of zinc.

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