Change in Serum Trace Elements Concentration before and after Removal of Gallbladder with Gallstone

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Citation

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Abstract

Epidemiological evidence among the human population has shown a significantly increased incidence of gallstone disease worldwide. The levels of nutrients are represented by different physiological and pathological processes. The aim of this work is to analyze the change in trace element (Al, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Ni, Se, and Zn) concentrations of human serum 48h before and after the removal of gallbladder with gallstone in 15 patients by using Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP-AES). The results showed increased post operative serum Fe, Ni and Se levels and decreased level of other elements significantly. The results were statistically compared ($p < 0.05$). This study indicates the complexity of interpreting changes in trace elements in serum after surgery.

INTRODUCTION

Choleolianthisis has been found among the human race. Ever since the early days of civilization and it is one of the common causes of disease of the gallbladder stones. Gallstone formation is predominant in all countries. Gallstone disease represents a national health care problem, resulting in more than 600,000 cholecystomies per year [1]. Despite decades of research, the mechanism of gallstone formation remains incompletely understood. The trace elements are believed to play an important role in gallstone formation [2-3].There are several types of gallstones; they are (calcium- bilirubinate and pure pigments stone), pure cholesterol, a mixture of bilirubin and cholesterol and inorganic stone [4]. Gallstone studies from south India have highlighted the common occurrence of pigment and mixed variety of gallstone [5-8]. The determination of trace elements in human serum not only leads to discoveries of their role in human metabolism but also provides an important basis for the diagnosis of clinical disorders and intoxication [9]. There are some reports on the bile acid in serum and bile of patients with gallstone [10]. Unfortunately, the study on this issue is still rather limited. ICP –AES is one of the most versatile analytical techniques for determination of biological fluid [11]. The present study was undertaken to investigate the sequential change of trace elements such as Al, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Ni, Se, and Zn in human serum in patients 48h before and after surgery.

MATERIAL AND METHODS

The study population of 15 patients with gallbladder diseases admitted to the surgical unit of the Rajah Muthiah Medical College and Hospital (RMMCH), Annamalai University, Annamalaingar was randomly selected. All these patients were from the same geographical area, mean age of the patients was 43.60 ± 12.36 years (mean ± SD), weight 60.2 ± 12.01 kg and height 152.4 ± 11.8 cm. 7 patients were male and 8 female, aged between 18 and 65 years . They have clinical symptoms or signs of biliary disease, or a diagnosis of a biliary disease screened by abdominal ultrasonography (USG)[12], which is highly accurate method in establishing diagnosis of gallbladder stone and carcinoma in symptomatic patients [13]. Children below 16 years and individual with clinically overt heamolysis, chronic alcoholics and subjects with obvious asocial background were excluded from the study. All these cases underwent open cholecystectomy surgery. Common bile duct was not damaged in any patients during surgery. Patients were healthy and without medication. All patients were in normal nutritional status as estimated by pre-operatively measured anthropometry and biochemical indices (serum albumin, prealbumin and transfetrin).

6 ml of blood samples were obtained by vein puncture after 8 -9h (over night) of fasting and drug withdrawal. Specimens were kept in metal free container for trace element analysis, without adding any anti coagulant. Blood samples were left

to clot in sterile place to avoid contamination and then centrifuged for 15 min at 3000 rpm. The transparent serums were obtained by ultra filtration using 0.45 µm pore size filter. The serum was then separated and stored at cryo vials in deep freezer at -20o C until analysis. Surgical procedure was removal of gallbladder.

REAGENTS AND STANDARD SOLUTIONS

Natural element standard solutions (1000 µg/ml) of Al, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Ni, Se, and Zn were obtained from Merck, USA. The standard solutions were kept at 4 oC in dark room to reduce the risk of contamination. All works were carried out under clear room condition. High pure nitric acid, perchloric acid and hydrochloric acid, Ultra pure de-ionized water (18 M ohm) used were used for sample preparation. Standard solutions were prepared freshly from the stocks, with dilute nitric acid (3% v/v). In order to obtain appropriate ICP-AES responses, the experiments were performed using different concentration levels. Instrument was calibrated using aqueous standards of elements 10, 20, 30, and 40 g/L. Accuracy and precision of the method for elements were checked by spiking the samples with known amount of standard.

ICP-AES

Inductively coupled plasma spectrometer has been extensively used in the analysis of major, minor and trace elements in biological material because of its high sensitivity, accuracy, low matrix effect and simpler operation. The ultra-trace elements in such biological fluids could be determined by ICP – AES [12]. A comparative study on the analytical performance of flame atomic absorption, flame atomic emission and inductively coupled plasma (ICP) emission for biological strontium assays, especially in blood serum was carried out [11]. The present work was performed using ISA JOBIN YNON – 24.

Operating conditions were

R.F Generator : 40.68 MHz, 1000 Watts

Power required : 220 ± 10 V, 50/60 Hz, Single phase 5 kV

Flame Temp : 11000 K

Plasma : Argon

Spectra range : 189 – 800 nm

Sensitivity : ppb level of detection (±.002 ppm)

Presence of various elements in the sample was identified by determining the wave length of the emitted radiation (Al: 394.401 nm, Cd: 226.502 nm, Co: 236.379 nm, Cr: 284.325, Cu: 324.754 nm, Fe: 238.204 nm, Mg: 383.826, Mn: 293.930 nm, Ni: 231.604 nm, Pb:220.353 nm, Se: 196.090 nm, Zn: 213.856 nm) and their concentrations can be calculated by intensity of radiation, which may be sufficiently low for certain applications with a simple matrix. The commercially available liquid chromatography (LC) system was directly connected to the pheumatic nebulizer. All samples were injected directly without any dilution through the 100 µl injection loop while deionized water continuously flowed at 1 ml min-1 via LC pump. The emitted light from the ICP was collected by the monochromator and polychromator simultaneously. The dynamic viscosity of the sample is measured by fluid spectrometer, which requires 1 to 2 ml of digested sample. The temperature was set at 20o C with the stability of ±0.5o C. Standard solutions were prepared by diluting single element solution (1000 mg/L) in the matrix matching solution. Data acquisition and processing were performed using ICP JY version 5.2 software.

SAMPLE DIGESTION FOR ICP- AES ANALYSIS

1 ml of sample was transferred to a Teflon beaker and 7.5 ml of concentrated nitric acid, 2.5 ml of concentrated percholoric acid were added [14], then brought out very slowly to boiling on a hot plate and heated to dryness. If blackening of samples occurs during the fuming stage, nitric acid was added drop wise then the sample was cooled, 10 ml of deionized water and a drop of concentrated HCl was added. Then the solution was made up to 100 ml with addition of deionized water and analyzed against calibration curve established.

STATISTICAL EVALUATION

The results of monitoring gallstone patient's serum metal level before and after surgery are described in terms of mean, standard deviation (SD), and statistically compared using paired sample t test at 95% confident level. p value less than or equal to 0.05 is considered as significant. The statistical package used was SPSS for windows 12.0 version (SPSS, Chicago, IL,USA).

RESULTS AND DISCUSSION

RESULTS

All blood serum /plasma samples of 15 patients 48h before and after gallstone surgery were analyzed using ICP-AES. From the results, serum Al level (Pre 0.0438±0.0252 ppm, post 0.0217 ± 0.0132 ppm), Cd level (Pre 0.0058 ± 0.0021

ppm, post 0.0022 ± 0.0011 ppm); Co level (Pre 0.0176 ± 0.0045 ppm post 0.0060 ± 0.0020 ppm); Cr level (Pre 0.0177 ± 0.0083 ppm, post 0.0061 ± 0.0037 ppm); Cu level (Pre 0.5683 ± 0.0735 ppm, post 0.4663 ± 0.0503 ppm); Fe level (Pre 0.6491 ± 0.1405 ppm, post 0.9396 ± 0.3509 ppm); Mg level (Pre 8.2880 ± 1.1033 ppm, post 6.6320 ± 1.1657 ppm); Mn level (Pre 0.0208 ± 0.0104 ppm, post 0.0061 ± 0.0031 ppm); Ni level (Pre 0.0400 ± 0.0310 ppm, post 0.0925 ± 0.0374 ppm); Pb level (Pre 0.0529± 0.0131 ppm, post 0.0269 ± 0.0105 ppm); Se level (Pre 0.2220 ± 0.0624 ppm, post 0.2971 ± 0.0384 ppm) and Zn level (Pre 0.4407 ± 0.1102 ppm, post 0.3731 ± 0.1126 ppm) were obtained. Fig.1 reports elemental concentrations serum before and after 48h of gallstone surgery.

Figure 1

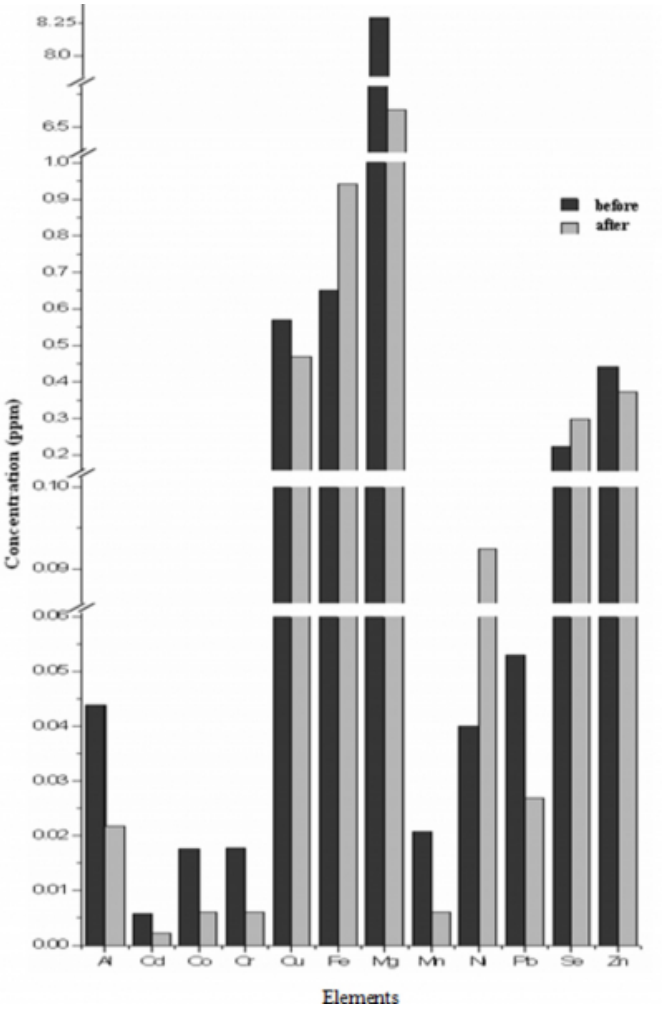
Table 1. Changes in trace element levels in patients (n=15) at 48h before and after surgery

Element	Pre operative (Mean ± SD)	Post operative (Mean ± SD)
Al#ns	0.0438 ± 0.0252	0.0217 ± 0.0132
Cd$	0.0058 ± 0.0021	0.0022 ± 0.0011
Co$	0.0176 ± 0.0045	0.0060 ± 0.0020
Cr$	0.0177 ± 0.0083	0.0061 ± 0.0037
Cu*$	0.5683 ± 0.0735	0.4663 ± 0.0503
Fe*$	0.6491 ± 0.1405	0.9396 ± 0.3509
Mg$	8.2880 ± 1.1033	6.6320 ± 1.1657
Mn$	0.0208 ± 0.0104	0.0061 ± 0.0031
Ni$	0.0400 ± 0.0310	0.0925 ± 0.0374
Pb$	0.0529 ± 0.0131	0.0269 ± 0.0105
Se$	0.2220 ± 0.0624	0.2971 ± 0.0384
Zn*$	0.4407 ± 0.1102	0.3731 ± 0.1126

* - highly positive correlation (r>0.80), # negative correlation (r<0.00); **s** –significant, **ns**- not significant SD –Standard deviation

Figure 2

Fig 1. Changes in trace element levels in patients (n=15) at 48h before and after surgery



DISCUSSION

Both analytical techniques developed, ICP - AES and ICP – MS, proved fully satisfactory for the purpose of present investigation. Many minerals and trace elements are required as cofactors, coenzymes or metalloenzymes in metabolic pathways and participated in biological functions, oxygen transport, free radical scavenging, structural organization of macromolecular and hormonal activity. The essential function of trace elements is maintaining the physiological condition in many organs.

The elemental concentration of Al, Cu, Cd, Cr, Co, Pb, Mg, Mn and Zn in pre operative serum was higher than the post operative serum concentrations, whereas elevated serum Fe, Ni and Se concentrations were found in 48h after surgery.

Patients undergoing surgery are at risk of increased oxidative stress. The trauma of a surgical procedure is known to promote a pro-oxidative state, due to ischaemia –

reperfusion processes [15]. Many of these associations have been documented in various forms of liver disease in other species, including the elevation of copper and decrease in zinc and their concurrent association with elevations of ALT and glutamic pyruvic transaminase (GGT) or decreases in albumin. Cu is an essential trace element for blood formation, most concentrated in the liver, heart , kidneys, brain, bones and muscles and is essential to blood. Statistically significant decrease in Cu concentration was found in post operative patient's serum. Cu in excess is not common because only a small percentage is assimilated and in those rare instances can present in diseased stages. An elevated level of serum Cu is documented in liver diseases like cirrhosis, obstructive jaundice and cholestasis. Decrease in post operative serum Cu concentration maybe due to the formation of new blood.

Elements such as Fe and Se are needed to maintain biological activity. Fe is necessary for healthy blood chemistry and is essential for recovery from illness. Without sufficient Fe body cannot manufacture enough new hemoglobin. The statistically significant increase in serum Fe concentration was found in post operative patients. The increase in serum Fe level is due to damage of erythrocytes in the extracorporeal circuit damage not evident as visible hemolysis but sufficient to cause an increases in serum Fe [16]. Fe is also needed for proper muscle and organ function. Fe from these sources is readily absorbed in the intestines. The increase in serum Se reduces the cancer mortality. Low level of Se imports liver necrosis which causes jaundice. Pigmented stones are mainly due to hemolysis. Serum Zn concentration is not statistically significant in the study. More than 300 enzymes in the human body requires Zn for proper functioning that is vital to many biological functions such as immune resistance, wound healing, digestion, reproduction, physical growth etc. The decrease in serum Zn may also be related to redistribution of Zn to the site of tissue injury. It has been shown that radio labeled Zn localizes in actively healing wounds, reaching maximal concentration in tissue within 24 to 48h after injury [17] and nonspecific reaction to stress [16]. Sixty percentage of Cu in the blood is tightly bound to a Cu-Zn-dependent enzyme known as superoxide dismutase (CuZnSOD) which is a powerful anti- oxidant enzyme. A similar antioxidative enzyme is dependent on the trace mineral manganese (MnSOD). As with glutathione peroxidases(GSH-Px) the role of superoxide dismutases is to protect cells against free-radical injury [18]. Addition of single or multiple trace elements as antioxidants (specifically Se) during parental nutrition in human studies was associated with a significant decrease in mortality. Se is a vital component of GSH-Px which acts as a powerful antioxidant enzyme. The patient recovering from surgery may be in a state of negative anti-oxidant balance, which can only be corrected by nutritional input. Se with other trace elements play a role in disease-condition. The highest GSP-Px activity has been found to occur in the liver, moderately high in erythrocytes, heart muscle, lung and kidneys, and lesser in the intestinal tract and skeletal muscles [19]. Decrease in Cr is linked with various effects and diseases like arteriosclerosis, ischaemic heart disease, lipid metabolism, hyperglycaemia and protein synthesis. Some of the trace elements fall in the group of non-essential or toxic (Pb, Cd, Hg, Al, Co, Cr) trace elements because their biological significance is confined and the change is mainly due to environmental factors. Antioxidants such as MnSOD can neutralize free radicals and may reduce or even help to prevent some of the damage of infected body organ. Mn acts as a catalyst and cofactor in many enzymatic processes involved in the synthesis of fatty acids and cholesterol. Low levels of Mn have also been associated with muscle disorders. Several minerals, such as Ca, Fe and possibly Zn can reduce the absorption of Mn. Cu is needed to help the body to use iron whereas Mg is used by the body to maintain muscles, nerves, and bones. Mn is required to manufacture enzymes necessary for the metabolism of proteins and fat. Lower hepatic Cr is reported diabetic patients. Decrease in serum Cr maybe associated with temporary body metabolic disorder. Cr is essential for maintaining the structural stability of proteins and nucleic acids and studies on animals have found that this element is also vital for healthy foetal growth and development. Change in serum trace elements like Cd, Zn concentration may associate with elevated Se and Fe respectively. Increase in Fe is also linked with a decrease in Mn. Mn also supports the immune system, blood sugar balance, and is involved in the production of cellular energy, reproduction and bone growth. Closely associated with Cu, Zn and Mn. Low levels of Mn in blood or tissue have been associated with several chronic diseases and muscle disorders. Mn is a natural tranquilizer. Change in serum Mn level due to loss of antioxidant property of blood in cardiac heart disease and carcinogenesis [20] Changes in other elemental concentrations are not known that may also be the reason of malnutrition, it disturbs cellular and organ functions.

CONCLUSION

Through the reproducible pattern of serum metal concentration measured in surgical trauma, the functions of

trace elements in the human body have not been fully understood. Their importance in the development and maintenance of physiological condition has been clarified with the advancement in technology to analyze trace elements. The magnitude of change in metal concentration in two cases (pre and postoperative serum elements) were investigated and interpreted in the study. Determination of elemental concentrations in biological fluids is considered as a very routine and necessary procedure in the clinical laboratories of today. Serum levels of nutrients represent different physiological and pathological processes. The interpretation of the result could help to understand the vital role of trace elements in the human and animal metabolism. These results are used as a guide to nutritional supply of essential metals in patients during the post operative method. Further studies are required to confirm the roles of trace elements in blood serum in gallbladder surgery.

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