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Study of Magnesium, Copper, Zinc, Iron and Ceruloplasmin in Liver Cirrhosis and Ovarian Cancer Ascitic Patients

Md. Khaleel Pasha[1*], Qamar Ayesha[1], M. Lakshmi Narasu[2], K. Pandu Ranga Rao[1], B. Prabhakar[1], K. Suseela[3], N. Balakrishna[4], M. Srinivasulu[5] and M. Gopal Reddy[6]

[1]Department of Gastroenterology, Osmania General Hospital, Hyderabad, A.P., India.
[2]Department of Biotechnology, JNTUH, Kukatpally, Hyderabad, A.P., India.
[3]Department of Pathology, Basavatarakam Indo-American Cancer Hospital & Research Institute, Banjara Hills, Hyderabad, A.P., India.
[4]Division of Biostatistics, National Institute of Nutrition, Hyderabad, A.P., India.
[5]Department of Surgical Oncology, M. N. J. Institute of Oncology and Regional Cancer Centre, Red Hills, Hyderabad, A.P., India.
[6]Department of Biochemistry, Osmania General Hospital, Hyderabad, A.P., India.

Authors' contributions

This work was carried out in collaboration between all authors. Author MKP has carried out and managed the analysis of the entire study, author QA has designed the study, helped in preparing the paper and overall editing with scientific suggestions, author MLN gave moral support, authors KPRR and BP helped in providing the patients with Liver cirrhosis, authors KS and MS helped in procuring ovarian cancer patients, author NB performed the statistical analysis, MGR for his constant guidance in carrying out the work. All authors have read and approved the final manuscript.

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ABSTRACT

Introduction: The excess accumulation of free fluid in the peritoneal cavity is due to its multitude causes. Certain cytological and biochemical markers are reported for the differential diagnosis of ascites. In the present study, ascitic patients with liver cirrhosis and ovarian carcinoma are investigated.
Aim: To study the concentrations of certain trace elements and ceruloplasmin levels in

**Corresponding author: Email: kpkhaleel6@gmail.com;*

ascitic fluid and in serum of patients with liver cirrhosis in comparison to ovarian cancer patients.
Study Design: The study includes 170 patients with liver cirrhosis, 95 patients with ovarian cancer and 100 serum controls.
Place and Duration of Study: The study is performed in the department of Gastroenterology, Osmania General Hospital Hyderabad, A.P, INDIA, between December 2011 to July 2013.
Methodology: The trace elements such as magnesium, copper, zinc, iron and ceruloplasmin were investigated in 170 patients with liver cirrhosis (120 males, 50 females, 22-75 years; mean age 46.2±11.2) and 95 ovarian cancer patients (19-84 years; mean age 50.2±12.2). All the analysts were measured in serum and ascitic fluid by using standard commercial kits. 100 serum samples from healthy controls were also included in the study.
Results: The mean serum concentrations of copper and ceruloplasmin were significantly increased in ovarian cancer patients when compared to that of liver cirrhosis and healthy controls (*P*<. 001, *P*<. 001) respectively. The mean zinc levels in serum samples were low in both the groups when compared to controls (*P*<. 001). The ascitic fluid zinc levels in ovarian cancer were higher when compared to that of liver cirrhosis (*P*<. 001). Similar results were noted in the serum iron levels in both the groups. The serum magnesium levels in liver cirrhosis are comparable to that of controls but the levels of magnesium in ovarian cancer ascitic patients are high when compared to that of controls.
Conclusion: Our results showed that there are differences in trace elements and ceruloplasmin levels in liver cirrhosis and ovarian cancer patients in both serum and ascitic fluid samples. The cutoff value of copper and ceruloplasmin in serum samples are 134.5 µg/dl and 43.5 mg/dl, while in ascitic fluid were 46.5 µg/dl and 21.0 mg/dl respectively. As the diagnostic efficiency of copper and ceruloplasmin in serum and ascitic fluid samples are 97% to 99% with highest sensitivity and specificity, analysis of trace elements with ceruloplasmin in depth with their ratios may be helpful in differentiating the cirrhotic and neoplastic illness.

Keywords: Ascites; Liver cirrhosis; ovarian cancer; trace elements and ceruloplasmin.

1. INTRODUCTION

The pathological accumulation of peritoneal fluid is due to multiple causes often associated either with non-peritoneal diseases such as sinusoidal portal hypertension, hypoalbuminemia, ovarian disease or primary peritoneal diseases such as peritoneal mesothelioma, peritoneal carcinomatosis, granulomatous peritonitis etc. [1]. In the process of ascites formation there is an increased capillary permeability, surface area, hydrostatic pressure and decreased oncotic pressure influenced by various factors such as VEGF, Interferon's, matrix metalloproteases which lead to imbalance in the fluid, electrolyte and metabolic homeostasis [2]. Runyon and colleagues reported that 80% of ascites is caused by parenchymal liver disease and then malignancy 10%, heart failure 5%, tuberculosis 2%, and other causes in 3% of cases [3]. The most common tumors that cause ascites are of gastrointestinal and pelvic organs; the single most common is ovarian cancer that develops ascites in the initial stages. The presence of portal hypertension is most accurately diagnosed by ultrasonography, CT scan, MRI, and also by obtaining a serum and ascitic levels of albumin, rather than traditional total protein concentrations [4]. The conventional tests such as serum ascitic albumin gradient (SAAG) - low and high status, cholesterol, fibronectin, CEA, CA-125, polymorphoneutrophilic count, and microbial identifications are

used for the differential diagnosis. Malignancy as a cause of ascites can be confirmed with the cytological presence of tumor cells in the ascitic fluid. Many studies showed certain reliable cytological and biochemical markers to solve the problem of differential diagnosis of ascites [4-7]. Runyon et al. [8] reported that approximately 97% of patients with peritoneal carcinomatosis had positive ascitic fluid cytology. Most aspects of intermediary metabolism require essential trace elements in the form of metalloenzymes that have a range of catalytic properties, required for the transport, safe storage of very reactive metal ions. Zinc serves as an integral part of biomembrane; where it may be involved in the control of membrane integrity, stability as cytoskeletal stabilizator and lipid per oxidation-related injuries. An important role of zinc was suggested in carcinogenesis since it has an inhibitory effect on phosphodiesterase as well as it has a role in RNA and DNA polymerase and has an activating effect on membrane bound adenyl cyclase [9]. Zinc is an important element in the preservation of immune resistance and both zinc and copper is required for numerous biochemical functions and for optimal activity of the immune system [10-12]. Copper is an essential trace element which participates in many enzymatic reactions and redox processes. Reactive copper damages the liver directly or indirectly through Kuppfer cells stimulations [13]. Ceruloplasmin is a copper containing major transporting protein, which indirectly influence in the bioavailability of copper and it oxidizes a variety of amines, including epinephrine, melatonin, serotonin, paraphenylene diamine [14]. It also converts ferrous iron to ferric iron. Magnesium is a divalent cation and is a cofactor of many enzymes acting on adenosine triphosphate and plays an essential role in the regulation of cellular permeability and neuromuscular excitability [15], and it is important for protein synthesis, enzyme activation, oxidative phosphorylation, renal potassium and hydrogen exchange etc. Iron is essential to most living organisms and participates in a variety of vital processes varying from cellular oxidative mechanisms to the transport of oxygen to the tissue. It is a constituent of the oxygen-carrying chromoproteins, hemoglobin and myoglobin, as well as various enzymes such as cytochrome oxidase, xanthine oxidase, peroxidase and catalase.

In the present study we have analyzed certain trace elements and ceruloplasmin levels in both serum and ascitic fluid samples. The cutoff values along with the sensitivity, specificity and diagnostic efficiency are calculated so as to evaluate these parameters for their role in the diagnosis of disease status.

2. MATERIALS AND METHODS

2.1 Patients and Samples

A total number of 265 ascitic patients' venous blood and ascitic fluid samples were collected from confirmed cases of liver cirrhosis and ovarian cancer from Liver care unit of Osmania General Hospital, MNJ Institute of Oncology and Regional Cancer Centre and Basavatarakam Indo-American Cancer Hospital & Research Institute Hyderabad, over a period of 1 year and 8 months from December 2011 to July 2013. Among them there were 95 females with ovarian cancer, 170 patients with liver cirrhosis (120 males and 50 females) and 100 blood samples are collected for healthy controls (59 males and 41 females). Serum was separated immediately and stored along with the corresponding ascitic fluid at -70°C for analysis of the proposed parameters.

2.2 Exclusion Criteria

Patients with kidney disease, pancreatitis and tuberculosis were excluded from this study. To rule out the involvement of these pathological conditions, certain parameters like urea, creatinine, amylase and adenosine deaminase were estimated in serum and ascitic fluid of all the cases and only the cases with normal levels were included in this study.

2.3 Methods

Biochemical analytes viz; magnesium, copper and zinc were estimated colorimetrically by using commercial kits from Crest Biosystems (Coral clinical systems) and iron by using a Human kit (Germany). Ceruloplasmin was measured by turbidometric immunoassays using kit from Agappe diagnostics India Ltd., in semiauto-analyzer (Microlab-300-Merck). Calibrator, normal and abnormal control sera were obtained from Randox (USA) and run for every batch for quality control and standardization purpose.

2.4 Statistical Analysis

The data in the serum samples of the two groups were analyzed separately and compared with that of the healthy controls, whereas the data in ascitic fluid were compared in two groups and expressed as Mean±S.D. Statistical analysis was performed by one-way (ANOVA) followed by post hoc analysis using Bonferroni's test for significance.

3. RESULTS AND DISCUSSION

3.1 Results

The ovarian cancer group covers patients with age 19-84 yrs (50.2±12.2), the age of the liver cirrhosis patients was 22-75 yrs, (46.2±11.2) and that of the healthy controls was 24-60 yrs (41.79±8.07). The results of the studied parameters in the serum and ascitic fluid are depicted in the Figs 1, 2 and 3 shows typical liver cirrhosis patient with ascites, admitted in Liver care unit, Osmania General Hospital, Hyderabad.

The serum concentration of magnesium, copper, zinc, iron and ceruloplasmin in ovarian cancer patients were 37±4.26 mg/L, 168 ±19 µg/dl, 32.7±8.4 µg/dl, 36.1±10.2 µg/dl and 66.4±11.8 mg/dl respectively, in the liver cirrhosis patients were 26.3 ±4.99 mg/L, 105 ± 18 µg/dl, 35±8.1 µg/dl, 44±12 µg/dl and 30±6.21 mg/dl respectively, and in healthy controls were 25.5±3.28 mg/L, 121±12 µg/dl, 108±15 µg/dl, 134±9 µg/dl and 36±5.67 mg/dl respectively. The ascitic fluid concentrations of respective parameters in ovarian cancer patients were 31±4.62 mg/L, 116±16.4 µg/dl, 19.6±6.7 µg/dl, 24.5±7.7 µg/dl and 32.9±6.5 mg/dl and in liver cirrhosis were 21±3.89 mg/L, 22±8.3 µg/dl, 16.8±4.95 µg/dl, 18±4.85 µg/dl and 10.6±3.02 mg/dl.

The mean serum and ascitic fluid concentrations of copper and ceruloplasmin were significantly increased in ovarian cancer when compared to that of liver cirrhosis patients (168±19 µg/dl; 66.4±11.8 mg/dl) vs (105±18 µg/dl; 30±6.21 mg/dl) with P<.001, while in ascitic fluid were (116±16.4 µg/dl; 32.9±6.5 mg/dl) vs (22±8.3 µg/dl;10.6±3.02 mg/dl) P<.001 respectively. The concentration of serum ceruloplasmin was decreased in liver cirrhosis when compared to that of healthy controls. The zinc levels in the serum samples were significantly decreased in both ovarian cancer and liver cirrhosis ascitic patients when

compared to healthy controls (32.7±8.4 µg/dl; 35.0±8.1 µg/dl vs 108.0±15 µg/dl), with *P*<.001, *P*<.001) respectively. The zinc levels in ascitic fluid samples in ovarian cancer were higher when compared to that of liver cirrhosis. Similar results were noted in serum iron levels as seen for zinc levels in both the groups (36.1±10.2 µg/dl, 44±12 µg/dl vs 134±9.0 µg/dl) *P*<.001, *P*<.001 respectively. The magnesium levels in serum samples of liver cirrhosis patients are comparable to that of healthy controls (26.3±4.9 mg/L vs 25.5±3.28 mg/L), but the levels in ovarian cancer are high when compared to that of controls (37.0±4.26 mg/L vs 25.5±3.28 mg/L). The magnesium level in ascitic fluid increases in ovarian cancer when compared to that of liver cirrhosis (31.0±4.6 mg/L vs 21.0±3.89 mg/L).
The results show that zinc and iron levels are decreased; ceruloplasmin was increased in serum samples of patients with ovarian cancer and decreased in liver cirrhosis when compared to that of healthy serum controls. Copper and magnesium levels are increased in ovarian cancer when compared to that of liver cirrhosis and healthy serum controls. The cutoff value, sensitivity, specificity and diagnostic efficiency are also calculated for all these parameters both in serum and ascitic fluid samples and are presented in Tables 1 and 2. The cut-off value for copper in serum sample is 134.5 µg/dl, while in ascitic fluid is 46.5 µg/dl. The cutoff value for ceruloplasmin in serum sample is 43.5 mg/dl, while in ascitic fluid is 21.0 mg/dl.

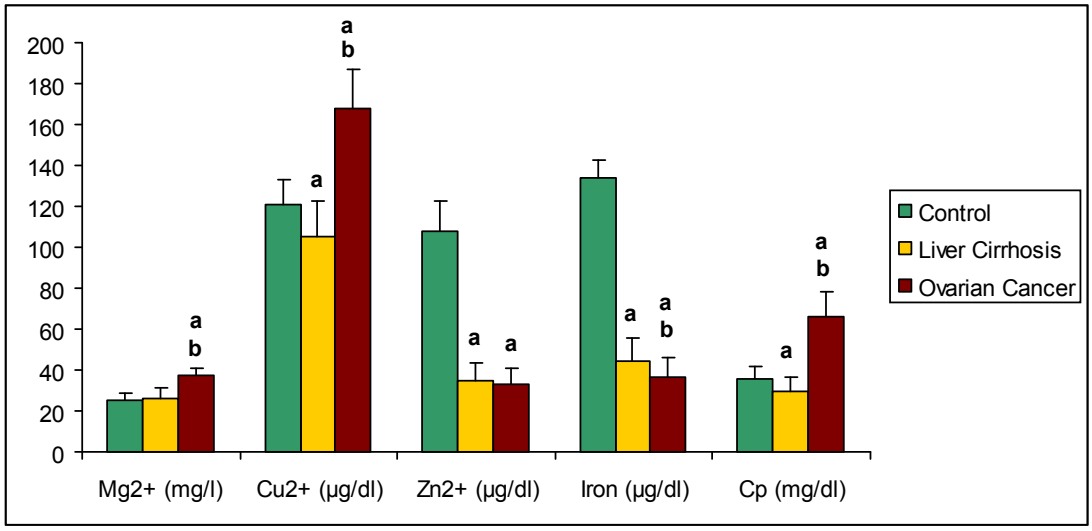

Fig. 1. Concentration of Magnesium (Mg^{2+}), Copper (Cu^{2+}), Zinc (Zn^{2+}), Iron and Ceruloplasmin (Cp) in serum samples of Ovarian cancer, Liver cirrhosis and control groups. Statistical analysis was done by one-way ANOVA followed by post hoc analysis using Bonferroni's test. Values are mean±S.D. [a]*P*<.001 control vs Liver cirrhosis and control vs Ovarian cancer; [b]*P*<.001 Liver cirrhosis vs Ovarian cancer

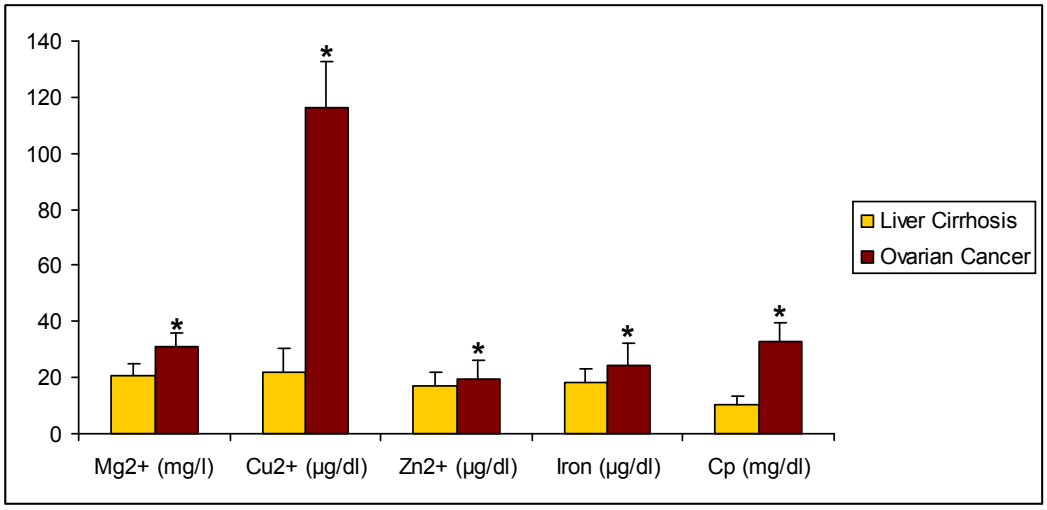

Fig. 2. Concentration of Magnesium (Mg^{2+}), Copper (Cu^{2+}), Zinc (Zn^{2+}), Iron and Ceruloplasmin (Cp) in the ascitic fluid samples of Ovarian cancer and Liver cirrhosis. Statistical analysis was done by one-way ANOVA followed by post hoc analysis using Bonferroni's test. Values are mean±S.D. $^{*}P<.001$ Ovarian cancer vs Liver cirrhosis.

Fig. 3. Patient showing typical Liver cirrhosis with ascites, admitted in Liver care unit, Osmania General Hospital, Hyderabad

Table 1. The Diagnostic value of individual parameters in differentiating ovarian cancer from liver cirrhosis ascitic patients in serum samples

Parameter	Cutoff	Sensitivity (%)	Specificity (%)	Diagnostic Efficiency (%)
Magnesium	26.5 mg/L	87	88	88
Copper	134.5 µg/dl	98	97	97
Zinc	31.5 µg/dl	61	49	55
Iron	36.5 µg/dl	73	60	67
Ceruloplasmin	43.5 mg/dl	100	99	99

Table 2. The Diagnostic value of individual parameters in differentiating ovarian cancer from liver cirrhosis ascitic patients in the ascitic fluid samples

Parameter	Cutoff	Sensitivity (%)	Specificity (%)	Diagnostic Efficiency (%)
Magnesium	20.5 mg/L	86	85	86
Copper	46.5 µg/dl	100	99	99
Zinc	17.5 µg/dl	61	59	60
Iron	19.5 µg/dl	75	61	68
Ceruloplasmin	21.0mg/dl	98	100	99

3.2 Discussion

Our results show elevated levels of serum and ascitic fluid copper and ceruloplasmin in ovarian cancer patients, while the zinc level was found to be decreased in both liver cirrhosis and ovarian cancer patients. These data confirm the earlier studies in other cancers [9,16,17]. Nayak et al. [18] found significantly increased levels of copper, ceruloplasmin and their ratios in serum samples of ovarian cancer, when compared to healthy controls. They suggested that the trace element copper may be useful for the early diagnosis of cancer. Swamynathan et al. [19] explained the positive correlation of copper with the tumor marker CEA and CA-125, and serum copper levels have also been found to be positively associated with the disease; there was a decline in the levels of serum copper as the disease was responding to the treatment. They suggested it may be the result of the increased liver's production of copper containing enzyme, ceruloplasmin as an inflammatory response to the cancer. It was suggested that the measurement of copper, which is an inexpensive assay could be carried out along with the tumor markers; further the measurement of copper should become a routine test for cancer patients. Nazmus Sadat et al. [20] observed increased levels of serum copper and decreased levels of zinc in the patients with lung cancer. They suggested that zinc acts as a cellular growth protector including growth of the cancer cells, and its deficiency was involved in several stages of malignant transformation [21]. Talat, [22] also found increased levels of copper and decreased levels of zinc in serum samples of ovarian cancer patients when compared to controls, and suggested that the ovarian cancer patients might be at the risk from oxidative cell damage, because increased levels of copper in ovarian cancer patients may lead to increased catalytic activity of copper, therefore there is an increased formation of reactive oxygen species which inturn lead to increased lipid peroxidation on cell membrane. Alessandra Buico et al. [23] found increased levels of ceruloplasmin in the serum samples of ovarian cancer patients when compared to that of control group (57±9 µmol/l vs 44±6 µmol/l) and explained that the increased levels of ceruloplasmin, often accompany neoplastic processes and may be caused by interleukin-1 and tumor necrosis factor released by macrophages.

In our study the serum ceruloplasmin in ovarian cancer patients was found to be elevated when compared to the level in liver cirrhosis and control group; some studies support the possibility of an increase of oxidative stress reduction in the nature of the cancer related to a ceruloplasmin levels [24] and ceruloplasmin has been found as a diagnostic factor in some studies [24,25]. Hulya Aksoy et al. [26] found decreased levels of ceruloplasmin in patients with chronic liver disease, which is in correlation with our present study. Ching-Chiang Lin et al. [27] at different stages of viral hepatitis patients, found decreased levels of iron and zinc in serum samples of hepatic cirrhosis patients when compared to that of controls. Significantly decreased level of serum zinc was observed in liver cirrhosis patients when compared to that of controls. The result confirms the previous studies of Kugelmas research [28]. The decreased levels of zinc in serum and ascitic fluid samples in liver cirrhosis patients, is an agreement with the previous studies of Celik's research [29]. As zinc is bound to albumin in serum, it has been thought that the serum zinc concentration would decrease as the disease advances clinically with poor prognosis [30]. Dario Rahelic et al. [31] explained that the decreased level of zinc may be due to low ingestion, protein reluctance, increased loss in gastroenterological system, diarrhoea, intestinal malabsorption and increased urinary losses. Naciye Semnur Buyukasik et al. [32] found lower levels of serum iron in cirrhosis of liver patients when compared to that of chronic hepatitis patients.

4. CONCLUSION

Our results showed that there are differences in trace elements, especially copper and ceruloplasmin levels in liver cirrhosis and ovarian cancer patients in both serum and ascitic fluid samples. The cutoff value of copper and ceruloplasmin in serum samples are 134.5 µg/dl and 43.5 mg/dl, while in ascitic fluid were 46.5 µg/dl and 21.0 mg/dl respectively. As the diagnostic efficiency of copper and ceruloplasmin in serum and ascitic fluid samples are 97% to 99% with highest sensitivity and specificity, analysis of trace elements with ceruloplasmin in depth with their ratios may be helpful in differentiating the cirrhotic and neoplastic illness.

CONSENT

An informed consent was taken from the patient and patient's attendant; the patient's history was recorded in the specific proforma approved by the Ethics, Scientific Committee.

ETHICAL APPROVAL

This study was approved by the ethics scientific committee of Osmania Medical College and Basavatarakam Indo-American Cancer Hospital & Research Institute, Hyderabad.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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