Evaluation of levels Ceruloplasmin, Copper, Zinc, Calcium and Non-Ceruloplasmin_bound Copper in Lung and Liver cancer Patients.

تقدير مستوى السيرولوبلازمين والنحاس والزنك والكالسيوم السيرولوبلازمين الغير مرتبط بالنحاس في مصل مرضى سرطان الكبد والرئة .

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Abstract

The present study was conducted to investigate the relationship between Serum levels of Zinc(Zn), Copper(Cu), calcium(Ca) Ceruloplasmin(Cp),and Non–Ceruloplasmin-Bound Copper (NCC) in lung and liver cancer patient, by used serum of forty male,(40 – 65) years aged were divided into three groups, which included liver cancer patients group composed of ten patients, lung cancer patients group composed of ten patients and twenty healthy or control groups. The results of this study showed significant increase in levels of Ceruloplasmin , copper, calcium and Non-ceruloplasmin _ bound copper in lung cancer and liver cancer groups if compare to control group. The level of Zinc (Zn) was significantly ($P<0.05$) decreased in patients lung and liver cancer groups if compare to control group.

Key words: Lung Cancer, Liver Cancer, Trace Element, Ceruloplasmin, NCC

الخلاصـــــــــــة

أجريت هذه الدراسة لتقييم العلاقة بين معدل السيرولوبلازمين والعناصر (النحاس والزنك والكالسيوم) السيرولوبلازمين الغير مرتبط بالنحاس لمرضى سرطان الكبد والرئة باستعمال أربعون عينة مصل رجال أعمارهم تتراوح بين 40-65 سنة وقسمت العينات إلى ثلاثة مجاميع تشمل مجموعة مرضى سرطان الكبد وتتألف من عشرة مرضى ومجموعة مرضى سرطان الرئة وتتألف من عشرة مرضى إضافة إلى عشرون عينة من مصل رجل سليم كمجموعة سيطرة . اظهرت النتائج وجود ارتفاع معنوي ($P\leq0.05$) في معدل السيرولوبلازمين والعناصر (الكالسيوم والنحاس) السيرولوبلازمين الغير مرتبط بالنحاس في مجاميع المرضى (سرطان الرئة والكبد) ، وانخفاضا معنويا عند ($P\leq0.05$) في تركيز الزنك لمرضى سرطان الرئة والكبد جميع هذه المستويات تمت بالمقارنة مع مجموعة الأصحاء .

Introduction

Cancer is one of the leading causes of death in the world. Among the many known malignant tumors of the liver and lung. Hepatocellular carcinoma (HCC) has been almost diagnosed in an advanced phase with an association with serious cirrhosis occurring in 70–80% of the cases [(1)]. Hepatocellular carcinoma (HCC) has become one of the top three causes of death in world [(2)]. HCC is estimated to cause between 250,000 and 1 million deaths annually [(3)]. To improve the prognosis of HCC, it is important to diagnose its presence as early as possible. Prescreening for HCC in an early stage is widely believed to be an effective form of prevention of HCC [(4)]. According to the World Health Organization WHO guidelines, Lung cancer generally appears after the age of 45 and its incidence increases by age. Together with smoking which is the most important risk factor (92% in men, 78% in women) conditions of labour, nourishment, age, gender, socioeconomic condition and genetic susceptibility have a big role on the etiology of lung cancer [(5)]. Despite well-defined

risk factor associations, particularly tobacco consumption, gender, occupation, diet, exposure to radon, and air pollution, Interactions between risk factors, and in particular with cigarette smoking, may increase lung cancer risk significantly [6]. Hereditary factors and genetic susceptibility to lung cancer currently remain ill-defined. Recent molecular epidemiologic studies have used lung cancer as a model to investigate gene environment interactions, and individual susceptibility to tobacco carcinogen metabolism, populations, and individual susceptibility to malignancy appears to be modified by the genotype of the enzyme. Polymorphisms of CYP1A1, CYP2E1, and CYP2D6 have been implicated in the development of lung cancer [7]. Lung cancer is histologically divided into 5 subtypes; squamous cell carcinoma, adenocarcinoma, small cell carcinoma, nonsmall cell carcinoma and adenosquamous carcinoma [8]. Tumors can be divided into two main groups, benign or malignant. Benign tumors are rarely life threatening, grow within a well-defined capsule which limits their size and maintain the characteristics of the cell of origin and are thus usually well differentiated. Malignant tumors invade surrounding tissues and spread to different areas of the body to generate further growths or metastases [9]. It is this process which is often the most life threatening. Different clones within a tumor will have differing abilities to metastasize, a property which is genetically determined, the process of metastasis is likely to involve several different steps and only a few clones within a tumor will have all of these properties [10]. The biochemical and nutritional roles of copper and zinc are widely recognized in the human body, and copper and zinc are constituents of many metalloproteins and metalloenzymes in normal metabolism [11]. Trace element contents and their ratios are frequently reported to be good biomarkers for diagnosis of various cancers.[2 ,12]Copper is an essential cofactor for many enzymes, including cytochromes, but it is toxic in its unbound form. The vast majority of serum copper is transported bound to ceruloplasmin; the rest is bound to albumin, transcuprein, and copper–amino acid complexes. Copper deposition occurs in hepatic parenchymal cells, the brain, the periphery of the iris, and the kidney. The age of onset and form of presentation of Wilson disease are very variable. Initially, copper accumulates in the liver, and accordingly, hepatic presentations are common [13]. Essential elements copper (Cu) and zinc (Zn) have a role in many biochemical reactions as a micro-source [14]. Zn stimulates gene transcriptions and cell multiplication.. While Cu and Zn are necessary for activation of RNA and DNA polymerase enzymes, they also have a role as co-factors of antioxidant enzymes. In addition, Zn is necessary for the optimum performance of the immune system [15, 16]. While Cu serum concentration levels are found to be increased in leukemia [17]. Lymphomas, sarcomas, bronchiogenic carcinomas, melanomas and gynecological cancers [18,19]. Ceruloplasmin levels are found to be increased in breast, cervix and endometrium cancers and oral leukoplakia [20,12]. Ceruloplasmin is a copper-carrying glycoprotein. It uses ferric oxidase activity to prevent the occurrence of toxic Fe products. In addition, it controls membrane lipid oxidation [19]. As an acute phase reactant, ceruloplasmin was found to be increased in gastrointestinal (GIS) cancers In fact, it is claimed to be a prognostic and diagnostic factor in various malignancies [21]. Calcium is the fifth most common element and the most prevalent cation in the body, it is play roles in such basic physiologic processes and regulation of exocrine and endocrine glands [22]. The concentration of calcium increased in malignancy (hypocalcaemia) in breast cancer, lung cancer, liver cancer, bone metastases, leukemia and other [23, 2]. The aim for this study, serum trace element (Zn, Cu, calcium) and ceruloplasmin levels were measured in patients operated for liver and lung cancer. The relations with NCC between each factor were studied to determine the importance of these parameters in malignant illnesses

Material & Methods

1- **Experimental design** : forty male healthy and patients ages approximately between 40 – 65 years, (Range = 52.5) divided to three groups (control group composed of from twenty men, lung cancer group composed of ten men and liver cancer group composed of ten men) were admission in hospital of atomic therapy and radiation , Baghdad , Iraq .

2- **Parameters of Study** :

I. Determination of free copper, zinc and calcium in blood Serum were determined by utilizing atomic absorption spectrophotometer method. Two sets of standard solutions in the ranges (0.1 to 1 µg/ml) for zinc copper and (0.05 to 0.8 µg/ml) for calcium was prepared. The serum were precipitated by using equal volume of 1.2N (TCA) after centrifugation, several dilution of supernatant were used (1: 100) for ca and (1: 10) for Zn and cu measurements by the Atomic Absorption Spectrophotometer according to. Method kurz (1972) [24] .

II. Determination of total serum Ceruloplasmin (Cp) : Cp was measured by used method according to the coupled method of Oliver (1995) .this method depended to oxadated Para phenylenediamine (PP) by used soluble 50mg of PP in 5ml distal water which contain 1ml Glacial acetic acid , soluble 8.15gm from sodium acetate trihydrate in 30ml water and mixed with PP and equal the size into 50ml distal water , waited 3 hours and keeped in cooled frozen . Procedure : 1ml from standard solution in taste tube and added 0.1 ml serum incubatered in 37C for 15 mint , added 5ml (inhibitor solution), mixing in 25 C and reader to 525nm . and used factor 0.68 [25] .

III. Non-Ceruloplasmin bound copper (Non-Cp-Cu) can be calculated as follows Non-Cp-Cu (µmol/l) = total serum Cu (µmol/l) − 0.047 × serum Cp (mg/litre) [26].

IV. Copper / Ceruloplasmin ratio can be calculated as follows: Cu/Cp ratio = Copper × 0.132⁄Cp where Cu is in µmol⁄l and Cp is in g⁄l [27] .

Statistical analysis of data

Other data were analyzed by SPSS11.0 software and reported as mean ± standard deviation using one-way ANOVA. Student's t-test was used for comparison between groups. P values of 0.05 or less are considered statistically significant[28] .

Results

The result of the present study showed significantly increased ($P \leq 0.05$) in levels of copper, Ceruloplasmin, copper⁄Ceruloplasmin ratio and Non Ceruloplasmin _ bound copper in lung , liver patients Table (1). and the table (2) acted, the results of this study showed significant increase in levels of calcium, and copper and copper zinc ratio and showed , decreased ($P \leq 0.05$) in level of zinc in lung , liver cancer groups . These result of patients groups compared with control groups.

Table 1: Level of Ceruloplasmin & Copper, Cu/Cp ratio and NCC in Lung and Liver cancer Patient, M±SD .

Parameter / Group	Cu µg/dl	Cp mg/dl	Cu / Cp ratio	NCC µmol/l
Control	90±18.2	32.1±6.33	4.631±1.23	1.82±0.2334
Lung Cancer	168±16.1*	56.5± 8.5*	6.233±2.41*	2.13±0.858*
Liver Cancer	185.8±14.23*	62.1± 8.66*	6.186±2.41*	2.218±0.8788*

*Significant deference in probability P≤0.05, Cu (Copper) , CP(Ceruloplasmin) , NCC(Non-Ceruloplasmin bounded Copper

Table 2: Level of Zinc, Calcium, Copper and Cu/Zn ratio in Lung and Liver Cancer Patient, M±SD.

Parameter / Group	Zn µg /dl	Ca mg/dl	Cu µg/dl	Cu/Zn ratio
Control	85±9.2	4.9 ±1.5	90±18.2	1.83±0.778
Lung Cancer	72±7.32*	5.82±1.32*	168±16.1 *	2.13±0.895*
Liver Cancer	75±8.66*	5.82±1.4*	185.8±14.23*	2.218±0.996*

* Significant deference in probability P≤0.05 , Zn (Zinc) , Ca (Calcium)

Discussion

The present investigation indicated that the levels of Ceruloplasmin, trace element (copper, zinc and calcium) in lung and liver cancer patient. Copper is an essential cofactor for many enzymes, including cytochromes, but it is toxic in its unbound form. The vast majority of serum copper is transported bound to ceruloplasmin [26]. The rest is bound to albumin, transcuprein, and copper amino acid complexes [29]. Ceruloplasmin is an enzyme which has a role as an oxidant or antioxidant depending on the existence of Fe ions and similar material levels in the micro base. It also stops lipid peroxidation by direct oxidation of cations. As a result, membrane lipid oxidation is controlled [30]. Recently, the antioxidant defense system that affects the occurrence and advancement of neoplastic illnesses has been under research. This defense system consists of Co-factors which are element enzymes and low weighted molecular compounds [10]. Ceruloplasmin makes up 95% of the total Cu contained in these isotopic elements. Cu makes up the main compound of the metalloproteins responsible for oxidation-reduction reactions [11]. Zn is essential for the development of, for example, nucleic acid and protein synthesis. In addition, Zn ensures the stability of the fullness of the'd' orbital, and this makes oxidation-reduction impossible in any environment containing Zn [31]. In our study, we found that in patients with tow types of tumors studied (lung, liver cancer) the baseline serum levels of a series of trace element were significantly different when compared with a healthy control group's. Ceruloplasmin is a copper binding protein, which increases in several carcinomas [32]. in present study high increased in ceruloplasmin levels, copper and decreased in concentrations of zinc and the increased concentrations of copper in serum do not seem to result from a shift of zinc into or release of copper out of the malignant tumor tissue.

Secondary in the liver might be contributory to the high levels of ceruloplasmin. The liver is the main regulator of copper metabolism. In the hepatocyte, copper is distributed among the various copper metalloenzymes and also incorporated into the plasma protein ceruloplasmin, where it re-enters the circulation [33]. Ceruloplasmin bound copper accounts for over 90% of plasma copper. In the bile, specific isoforms of ceruloplasmin have been identified that appear to be involved in copper excretion The measurement of serum ceruloplasmin and copper concentrations should be available bound _ copper. The factor multiplying the serum ceruloplasmin concentrations is derived from the molecular weight of ceruloplasmin and the number of copper atoms (six) bound to it [27]. Normally, Non-ceruloplasmin bound copper is less than 10% of the total copper. Interpretation of the values should bear in mind the imprecision of measurement of ceruloplasmin if the serum concentration is below 60 mg/litre. Note that in acute liver failure concentrations can be increased to 30–50 μmol/litre total serum copper, with greatly increased calculated Non-ceruloplasmin copper (70–80%)[26].In our study increased in copper concentration and increase in NCC this result may be used in diagnostic factor and also the Ceruloplasmin has been found in some studies to be a diagnostic factor, the value of serum ceruloplasmin and NCC with liver and lung cancer patients were found above the level of the control group, these result, it was very important to detected of changed which take place during cell physiology in patient Although some publications defend the possibility that ceruloplasmin may increase due to the fact that it is an acute phase protein, there is no definite conclusion on this subject. Some studies support the possibility of an increase of oxidative stress reduction in the nature of the cancer related to a Ceruloplasmin prognosis [34]. Our data seem to be in agreement with the heterogeneity in the relationship between ceruloplasmin and total copper concentrations, Accordingly, any factor n used to describe the relationship for ceruloplasmin- bound copper in individual patients will have an associated confidence interval. This has important implications for the calculation of NCC and its use in routine clinical practice [26]. the decrease in Zn levels was found to be significant .In some publications, an increase in serum Cu and a decrease in Zn in breast cancers and gynecological cancers were decided to be prognostic and diagnostic factors, While the decrease in Zn levels in diseases with benign characteristics appears to be less than in normal cancer cases with malignancy, Zn levels drop more [23]. In our research, the decrease in Zn levels seems to correlate with the strength of the cancer in the patient group. The levels of Zn, Cu and ceruloplasmin were compared with the Cu/Zn ratio. As a result, instead of analyzing the effects of Cu, Zn and ceruloplasmin individually, it was found to be important to evaluate them in relation to the Cu/Zn ratio in the monitoring of the trace elements in cancer patients [21]. In this study, there was some indication that relatively high levels of calcium in liver and lung were positively associated with risk of subsequent cancer. Although it is possible that this was a chance finding, it might suggest that accumulates relatively high concentrations of calcium is predisposed to progress to cancer because an adequate supply of calcium is necessary to sustain the proliferation this tissue [2].

Conclusion:

In conclusion, serum Cu, Zn, calcium, ceruloplasmin, Cu / Zn ratio and NCC levels show changes in liver and lung cancers, but further research is needed to show the importance and significance of these parameters and their relation with other contributing neoplastic factors.

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