Copper and zinc levels in sera and tissue of patients with lymphoma and the effect of storage solution and the time on tissue content of Cu and Zn .

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Abstract

Copper and zinc concentration were measured in 14 patients with malignant lymphoma and 18 with benign tumors,higher levels of copper (154.2 ±36.7), (20.2 ±4.2)in sera and tissues respectively in patients having lymphoma when compared with those of benign tumors .estimation of zinc metals showed no significant decrease in sera of lymphoma patients but higher concentration (34.45±11.73) in tissues of them when compared with respect of benign tumors .

The storing effect on copper and zinc concentration in tissue samples which were stored in both storage solution (normal saline and formalin) were also studied .It was found that the copper concentration levels could be measured in tissue which was stored in normal saline up to fife days in benign tissues and up to three days in malignant tissues , and up to four days in benign tissues and up to seven days in malignant tissues for zinc determination .Using formalin as a storage solution , copper could be measured in tissue which was stored in formalin up to fife days in benign tissues and up to eight days in malignant tissues , and up to fife days in benign tissues and up to seven days in malignant tissues for zinc determination.

الخلاصة

تم قياس تركيز كل من النحاس والخارصين عند 14 مريض مصاب بالأورام اللمفاوية الخبيثة و 18 مريض من المصابين بالأورام الحميدة ،وجد زيادة ملحوظة في تركيز النحاس (154.2±36.7) و(20.2±4.2)في الأمصال والأنسجة على التوالي لدى المصابين بسرطان الغدد اللمفاوية عند مقارنتها مع المصابين بالأورام الحميدة أما عند تقدير الخارصين فتبين انه ليس هناك انخفاض معنوي في تركيز الخارصين في أمصال المصابين بالأورام السرطانية ولكن وجد زيادة ملحوظة (34.45±11.73) في أنسجة هؤلاء المرضى عند مقارنتها مع أنسجة الأورام الحميدة .

كما تم دراسة تأثير فترة حفظ النسيج على تركيز العنصرين ،اذ وجد أنه لا يمكن الاحتفاظ بالأنسجة المحفوظة بالمحلول الطبي لأكثر من خمسة أيام للأنسجة الحميدة و ثلاثة أيام للأنسجة الخبيثة عند قياس عنصر النحاس ولأكثر من أربعة أيام عند قياس عنصر الخارصين في حالة الأورام الحميدة وسبعة أيام في حالة الأورام السرطانية . أما عند حفظ العينات في الفورمالين فيجب مراعاة عدم الحفظ أكثر من خمسة ايام في الأنسجة الحميدة وثمانية ايام في الأنسجة الخبيثة عند قياس عنصر النحاس وخمسة أيام عند قياس عنصر الخارصين في حالة الأورام الحميدة وسبعة أيام في حالة الأورام الخبيثة.

Introduction

The trace metals copper (Cu)and zinc (Zn) play an essential role in biochemical processes in creature cells ,for example the copper as a cofactor for many enzymes that include Cu superoxide dismutase ,cytochrome oxidase ,ceruloplasmin and lysyl [1] .Consequently, Cu have a critical events for a wide variety of biological processes as transport in the cell surface and the delivery of Cu to intercellular compartments .The poisoning in copper is less and rare and to be exposed to 10 mg/day for many weeks leads to poisoning[2] .High serum copper levels were found in plasma of patients in different type of cancers[3] . Zinc metal found in active sites of multi enzymes such as phosphatase,polymerase ,alcohol dehydrogenase and alkaline DNA,RNA super oxide dismutase [4,5] as well as the zinc have specific role to devour free radicals that product from excess oxygen (O_2) and peroxide(H_2O_2) that damage the cell membrane of creature[6].The previous studies found strong correlation between zinc metal and immunity system diseases and cancer diseases, that noticed decrease in zinc levels in plasma of patients with breast cancer [7,8]. Elevated serum copper and decrease serum zinc have been detected in patients with sarcoma[9,10] ,lung cancer[11,12]and carcinoma of digestive system[12,13].

Materials and methods

Perkin-Elmer atomic absorption spectrophotometer used the determination of Cu and Zn in this study which consisted of patients that were treated from malignant and benign lymphoma. The benign (n=18) and malignant (n=14) lymphoma patients underwent lymph node biopsy and the diagnosis was validated histological from the lymph node biopsy specimen. The patients underwent an operative biopsy under general anesthesia and lymph nodes tissues were obtained. Tissue specimens were weighted, then immersed in normal saline once and in formalin once more and stored immediately at -4ºC for many days to assay the effect of storage solution and time on it. Prior to each experiment , the frozen tissues were homogenized in solution containing 2.5 mL HNO_3 and 0.5 mL $HCLO_4$ for an hour then digested on a heat block at first 10ºC and then 20 ºC .After digestion the solution mixed with 1٪ V/V HNO_3 then this mixture was used for determination of Cu and Zn .The blood samples were draw from same patients after operative to compare the concentration of each metal in tissue and serum of patients.

Result and discussion

Serum and tissue levels of copper and zinc were measured in 14 patients with malignant lymphoma were selected for the study for the comparison of the result of 18 normal control subjects who had been operated for benign diseases .The results were analyzed using students *t* test .It was found there were significant ($p<0.05$) elevations of serum copper and non significant decrease of serum zinc in the cancer patients when compared with the pathological controls (table 1). In addition the result of evaluation of copper and zinc concentration in tissues revealed significantly elevated ($p<0.05$)for both metals copper and zinc in malignant tissues when compared with those of benign tissues(table 2).

Several possible explanation for copper increase in serum or tissue of various cancers that considered according to Samuni *et,al* [14],superoxide radicals or other reducing agents ,such as ascorbate reduce the copper complexes to the cuprous state .In turn these complexes

react with hydrogen Peroxide to form hydroxyl radicals that damage proteins , RNA and most importantly DNA. Some authors reported elevated serum copper levels in various cancers such as cervical carcinoma [14, 15],breast cancer [17],Hodgkin's disease ,carcinomas of the large bowel and stomach [10,14,16] .Atlintas *et,al* [18] have stated that tissue copper content showed non significant difference between early and advanced clinical stage of cervical carcinoma. Sonmez *et,al* [19]have found an increase in levels of serum copper concentration that associated with marked elevation in tissue and serum sialyltransferase activities of breast cancer patients ,the elevations was correlated with the grade (I ,II ,III)of the disease. Fisher *et,al* [9] have showed that copper levels were elevated in cancerous tissue of sarcoma when compared with the normal tissue .Seuret *et ,al* [20]have demonstrated that tissue copper content showed no significant differences between early advanced clinical stage of the patients with the endometrial neoplasia.

In this study ;the behavior of the trace metal zinc was tested in both serum and tissue of benign and malignant tumors .We found no significant decrease in levels of serum zinc in lymphoma patients when compared with benign subjects .

On the other hand ;we found significant increase ($p<0.05$) of this metal in tissues of malignant tumors when compared with benign tumors .Several studies have reported rises in zinc concentration while other reported lower in zinc concentration ,for example the zinc concentration was significantly increased in malignant breast tissue as compared to its counter part [16,21] Whereas Daniesen *et,al* [22] have stated that tissue zinc content showed vastly lowered in kidney tissue .Thus cancers for each tissue concentrates different metal , Karicioglu *et,al* [23] assume that during the neoplastic growth of renal carcinoma the tubule cells lose their anatomical access to the glomerular filtrated consequently prohibit the intake of zinc by renal absorption. These data unequivocally show that the alterations occurring in the zinc concentration of cancerous tissues follow a different pattern in each particular organ. frust [24] improved that metals can be carcinogenic if their local cellular concentration becomes high so that they can compete with the normal essential metals for the available binding site in the lattice ,and thus aid in the synthesis of abnormal polymeric nucleic acid. The general trend towards slightly decreased zinc concentrations in serum of malignant tumors,as demonstrated in the current study supports the experimental results obtained by Brown *et,al* [25] they suggest that zinc deficiencies associated with the etiology of cancer. Many investigators have reported rises in copper while the zinc level was low in both sera and tissues of patients with colon and stomach cancer [13].In addition sueret *et,al* [20] have demonstrated that the elevation of serum copper and decrease of zinc levels was compatable with the burden the tumor. Our results are in agreement with these results.

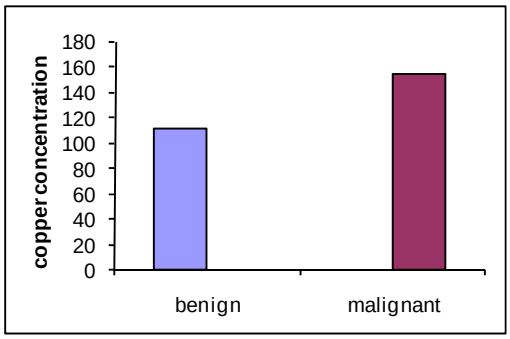
The influences of storage solution and storage period were examined to achieve the most suitable conditions for the estimation the two metals copper and zinc ; there for we evaluated the copper metal in tissue storage in normal salin ,we found significant decrease in levels of copper after five days of storage in benign tissue and after three days of storage in malignant tissue when compared with original value (fresh sample).Whereas in formalin as a storage solution ,we found the decrease start from the fifth

day of storage in benign tissue and from the eighth day of storage in malignant tissue show figure(1). Some investigators have suggested that decrease in copper levels in tissue due to adsorption of this metal between tissue and storage solution (8, 26, 27), there for we noticed increase copper levels in storage solution while decrease in tissue and the change was constant in tissue and storage solution.

In the present study we noticed significant decrease in zinc concentration that due to storage in formalin and the change was more clearly in malignant tumors tissue than benign tumors tissue because of the zinc concentration in tissue of cancer tumors was higher than it's in tissue of benign tumors. So we can determine zinc metal in normal saline up to four days in benign tissues and up to seven days in malignant tissue ;but when using formalin as a storage solution ,we could measure the zinc concentration up to five days in benign tissue and up to seven days in malignant tissue show figure (3) . We believed that the cause of the decrease was due to the osmosis pressure that leads to burst the cell membrane and go out the contents of cell to storage solution ;as well as we show the increase of zinc concentration in storage solution figure (4) and decrease its in tissue. our ideas are in agreement with those reported by others (8,26,27).

Table (1): Levels of copper and zinc in serum of benign and malignant lymphoma tumors.(without storage)

metal	Range (µg / dl)	Mean ± SD	P value
Cu Benign tumors Malignant tumors	 85-145 100-220	 112.3±25.1 154.2±36.7	P<0.005
Zn Benign tumors Malignant tumors	 14.2-31.4 13-29	 20.83±4.41 21.5±4.74	N.S



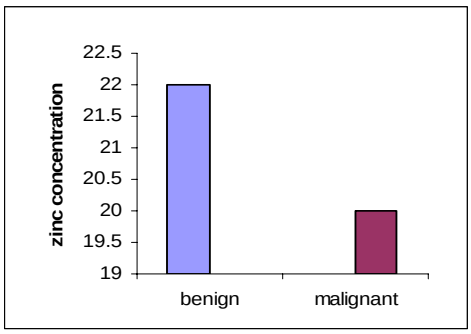
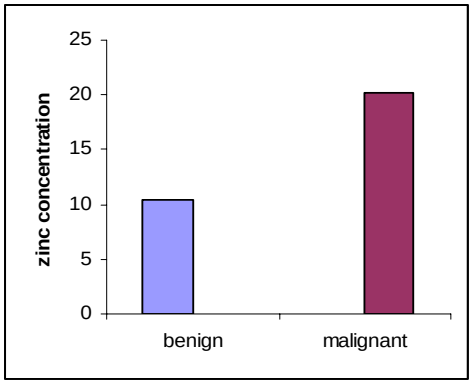
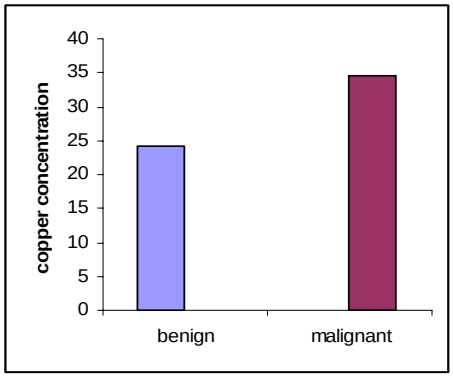
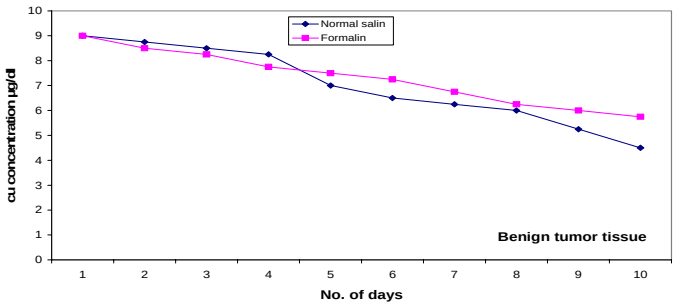


Table (2): Levels of copper and zinc in tissue of benign and malignant lymphoma tumors. (without storage)

Metal	Range(µg /dl)	Mean ± SD	P value
Cu Benign tumors Malignant tumors	 6.2-14 10. 5-31.5	 10.4±2.2 20.2±4.20	 P<0.05
Zn Benign tumors Malignant tumors	 12.3-36.8 18.3-58.5	 24.22±6.92 34.45±11.73	 P<0.05







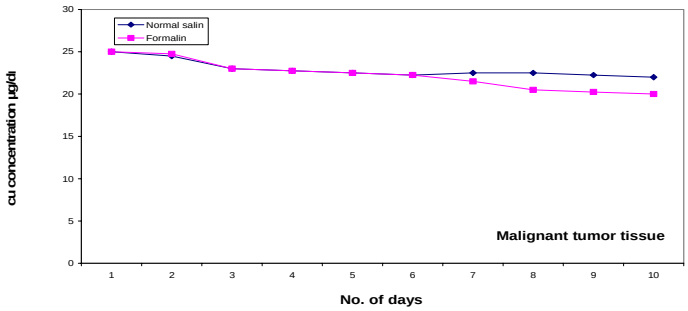


Figure (1): Decrease of copper concentration in storage tissue in normal salin and formalin.

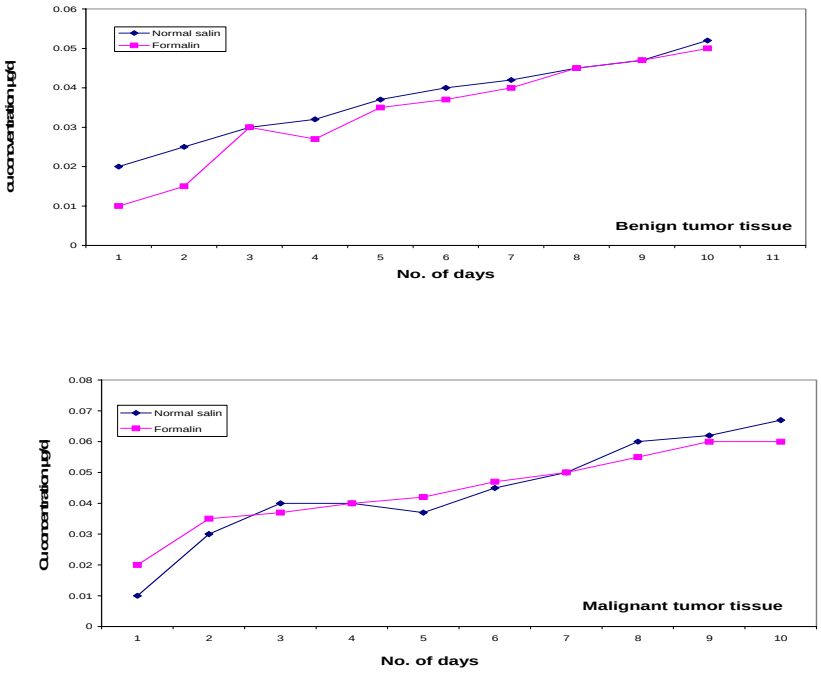


Figure (2): Increase of copper concentration in normal salin and formalin.

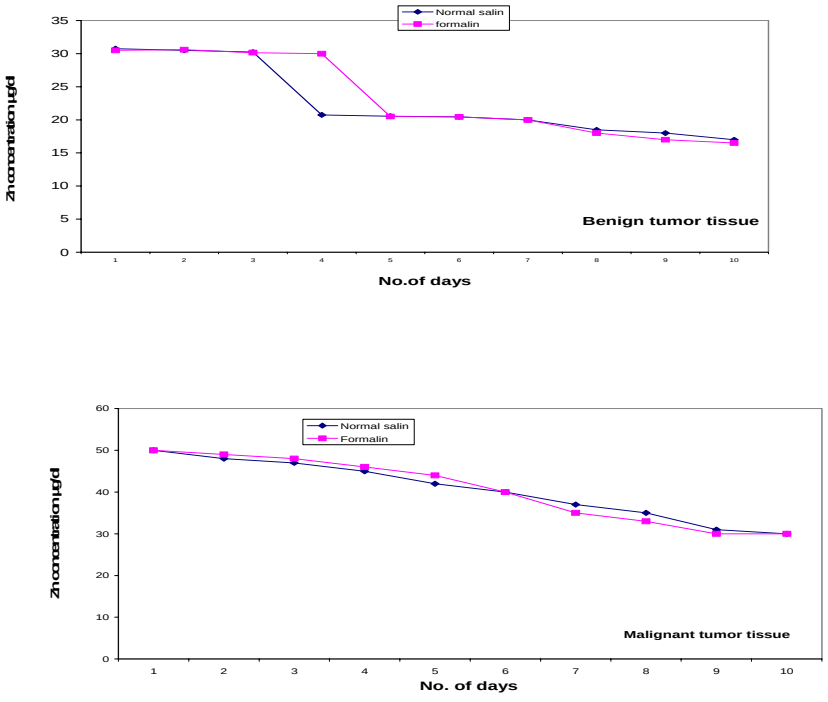


Figure (3): Decrease of zinc concentration in storage tissue in normal salin and formalin.

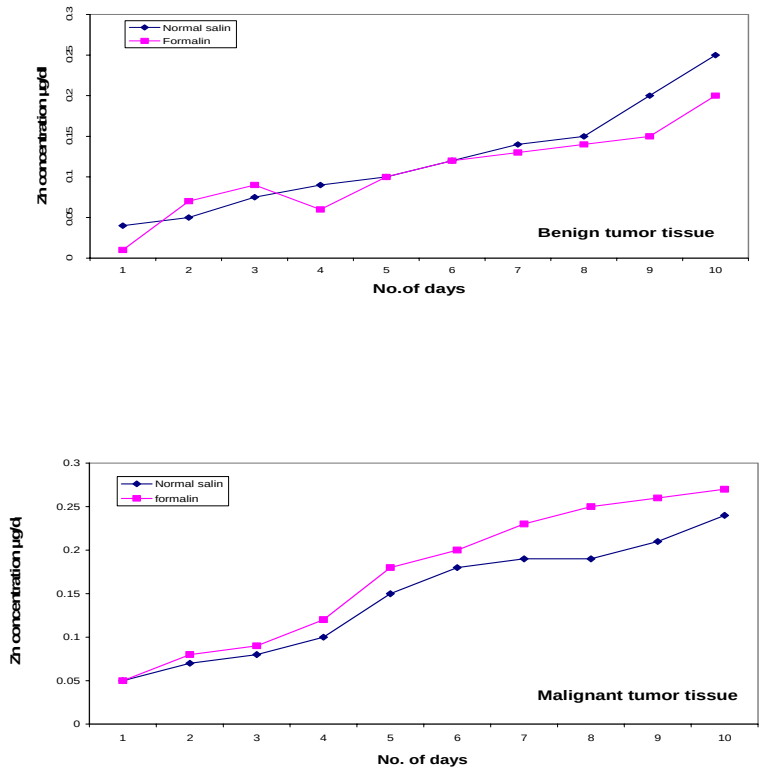


Figure (4): Increase of zinc concentration in normal saline and formalin.

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