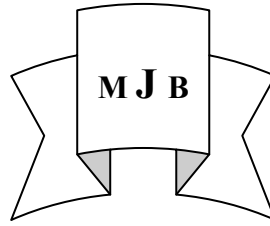# Serum Ceruloplasmin, Copper, Zinc and Copper Zinc Ratio Levels in Different Types of Malignancy

Jamal A Abdul-Barry[*]   Lamia M Al- Naama   Zenib.A Al- Manaseer

Hashim S Al- Khayat

Dept. of Biochemistry, College of Medicine, University of Basrah, Basrah, Iraq.
Basrah General Hospetal, Basrah, Iraq.
* Corresponding other.
Email: aldohan57@yahoo.com

M J B

Abstract

This study aimed to know the levels of serum ceruloplasmin (Cp), copper (Cu), and zinc (Zn) and Cu/Zn ratio concentrations in different type of malignancy and to clarify the relation ships of those parameters when used as a markers for detection the   severity of malignancy.

Study design: A prospective case-control study, conducted through a period of 8 months, from October 2000 to May 2001, during which 184 patients admitted to the Surgical Ward of Basrah General and Teaching Hospitals.There were 100 patients with different types of malignancy including 43 patients with gastro intestinal tract cancer (GIT), 18 females having breast cancer, 18 patients with urinary system malignancy and the reminder 21 patients having other types of malignancy. The other 84 patients suffered from surgical ailments other than malignancy serve as a control group.

Venous blood sample was collected from each patient for measurement Cp, Cu, Zn, and Cu\Zn ratio by using standard methods.

Results: Serum (Cp) level was significantly elevated (P‹0.05) in all types of malignang with respect to age (less than 45 years and between 45-65 years olds), sex, smoking and nutritional habit when compared with non malignant patients.

Serum Cu concentrations not-significantly elevated (p>0.05) in all types of malignant patients in comparison with non malignant subjects. There was significant increase in its levels in specific type of malignant and within specific limits including age mainly less than 45 years, smoking and nutritional habit, particularly among those consuming fruits and vegetables 3-5\week.

Serum Zn levels showed significant decrease (p<0.05) in malignant patients according to age, sex, smoking and nutritional habits mainly in patients characterized by less frequent intake of fruits and vegetables less than 3 times per week.

Serum Cu\Zn ratio was highly significantly elevated (p<0.05) in all types of malignancy with respect to age, sex, smoking and nutritional habit particularly those consuming fruits and vegetables frequency >3 times and 3-5 times per week.

Conclusion: On the basis of this study, it can be concluded that, substantial differences in those parameters studied in malignant and-non malignant patients and in different types of malignancy were important and useful for the measurement of the severity of malignancy.

مستويات السريلوبلازمين ،النحاس ،الزنك ،ونسبه النحاس إلى الزنك بمصل الدم في أنواع مختلفة من مرضى السرطان

الخلاصة

الهدف: صممت هذه الدراسة للتعرف على مدى تراكيز السيروبلازمين النحاس ،الزنك ،ونسبه النحاس إلى الزنك عند أنواع مختلفة من السرطانات من جهة ولتوضيح العلاقة بين هذه العوامل عند استخدامهم كمَعلمات لتعين شدة السرطان من جهة أخرى .

نمط الدراسة: هذه دراسة مستقبلية امتدت على نحو ثمانية اشهر من الأول من أكتوبر سنة ٢٠٠٠ إلى شهر مايس من سنة ٢٠٠١ .تم خلالها اخذ ١٨٤ مريضا ممن كانوا يراجعون قسم الجراحة في مستشفى البصرة العام و مستشفى التعليمي في البصرة .مئه من هؤلاء

المرضى كانوا يعانون من أنواع مختلفة من السرطانات منهم ٤٣مريضا مصابا بسرطان الجهاز الهضمي ،١٨ مريضـة مصـابة بسرطان الثدي ،١٨ مريضا مصابا بسرطان الجهاز البولي و ٢١مريضا مصابا بأنواع مختلفة من السرطانات . المتبقي منهم ٨٤ مريضا كانوا يعانون أمراض مختلفة غير السرطان عدوا" كمجموعة ضابطة .

جمعت عينـات مـن دم جميـع المرضى ليتعين مستويات السـريلوبلازمين ،النحاس ،الزنك ،ونسبه النحـاس إلى الزنك وذلك باستخدام الطرق القياسية.

النتائج : كان هنالك ارتفاعا معنوي يعتد به 0.05 <P بمستوىCp عند مصل دم المرضى لجميع أنواع السرطانات نسبة إلى الفئات العمرية وخصوصا المرضى الذين تقل أعمارهم عن ٤٥ سنه أو الذين تتراوح أعمارهم بين ٤٥ – ٦٥ سنة وكذلك عند دراسـة تأثير نوع الجنس أو حالة التدخين أو طريقة الغذاء أو عند مقارنة النتائج مع المجموعة الضابطة .

في حين أظهر مستوى النحاس ارتفاعا غير معنويا p>0.05 في جميع مرضى السرطانات من جهة أو عند مقارنة مستواه مع مرضى المجموعة الضابطة من جهة أخرى ، وكان الارتفاع المعنوي بمستوى النحاس محدد ويمثل نوع معين من أنواع السرطان ولفئة عمرية واحده وهي اقل من ٤٥ سنة أو لحالة التدخين أو طريقة الغذاء خصوصا عند الأشخاص الذين يتناولون الفواكه بمعدل أقل من ٣ مرات أسبوعيا.

واظهر مستوى الزنك انخفاضا معنويا P<0.05 عند مرضى السرطان استادا لتأثيرات العمر الجنس التدخين أو العادات الغذائية خصوصا عند المرضى الذين يتناولون الخضروات والفواكه بمعدل أقل من٣ مرات أسبوعيا .

كمـا وارتفعت نسبة Cu/Zn بشكل عالي المعنويـة p <0.01 عند جميع مرضى السرطانات نسبة لتأثير العمر ونوعية الجنس وحالة التدخين أو طريقة الغذاء في تناول الفواكه والخضروات خصوصا عند تناول اقل من ٣ مرات أسبوعيا.

الاستنتاج: واستادا لقواعد الدراسة الحالية ،يمكننا استخلاص حقيقة هامه وهي أن هنالك تغيرات واختلافات بمستويات هذه العوامل عند المقارنة بين مرضى السرطانات من جهة والمرضى غير المصابين بالسرطان من جهة أخرى أو عند المقارنة بين الأنواع المختلفة من السرطانات ، هذه الحقائق المهمة يمكن استخدامها فيما بعد كخير دليل ومعّلم لمتابعة شدة وفعالية السرطان.

Introduction

Neoplasm is an abnormal mass of tissue, the growth of which exceeds and un-coordinated with that of normal tissue. Two basic components of all tumors were associated with proliferating neoplastic cells that constitute their parenchyma or supportive stroma made up of connective tissue and blood[1].

Radiant energy, viral and chemical carcinogen is are types of agents leading to genetic damage and induced neoplastic transformation of the cells[2]. Radiant energy whether in the form of ultra violet rays of sun light or as ionizing radiation can transform cells and induce neoplasm. The carcinogenicity of radiation was attributed to its formation of pyrimidine dimmers in DNA and if such defects are not repaired before mitosis, they may lead to mutation and some instances to cancer[1].

While, the viral carcinogens are either integrated into the host's genome which then leads to transformation of the host cell into a cancer cell and production of tumor [3,4] or RNA virus, which is subsequently inserted on a host chromosome leading to transformation of host cell into a cancer[3]. On the other hand, the chemical carcinogens are a wide verity of chemicals, the great majority of which are organic compounds, their action is either directly and it is called direct carcinogens or pro-carcinogens that required prior metabolism to become carcinogenic[5].

It has been shown that, there was an association between a diet as a possible contributor to cancer, obesity, stressed, tobacco, cultural habit, life style as well as occupation and radiation with the incidence of cancer[6-8]. Epidemiological studies have consistently reported a reduction in risk for several cancer sites in relation to high consumption of vegetables and fruits. These studies suggested that some micro nutrients

particularly vitamin C, vitamin E and B carotene, selenium, magnesium and zinc could reduce the risk of oral cavity, lung ,esophagus and stomach cancer because they act as antioxidants[9,10].

While the dietary fiber was more specifically related to reduce the risk of colorectal cancer by diluting the fecal carcinogens and reducing the colonic transient time, so it would limit the exposure of mucous membrane to potential carcinogen[10].

The mechanisms by which dietary compounds can affect cancer development include either alteration of carcinogen metabolism as antioxidant compounds, or by the enhancement of differentiation and growth inhibition or as immunological modulation as vitamin A, C and minerals as zinc, copper, selenium and Iron[12, 13].

The copper was suggested as anti oxidant element in super oxide dismutase enzyme, which is alysosomal type containing copper and zinc responsible for removal of super oxide anion in which copper acts as catalyses function while zinc plays a structural[14].

On the other hand ceruloplasmin acts by the prevention the of oxidation process in serum by preventing the formation of free radical intermediates that might be generated by none enzymatic oxidation of complexes and to prevent the oxidation of ferrous ion to ferric , that occurs usually during the normal metabolic process[15].

Therefore, the present study was undertaken to estimate the serum levels of copper and its related protein ceruloplasmin, zinc and Cu /Zn ratio in different type of malignancy and compared with non malignant patients, and then use the ratio in estimating the extent and prognosis of malignant tumor, and also used as a dividing line between the benign and malignant case.

Materials and Methods

A prospected case control study, conducted through a period of 8 months between October 2000 till may 2001, a total of 184 patients were admitted to the Surgical Ward of al-Basrah General and Teaching Hospital in Basrah city where. There ages range from 22 – 84 years old. They were 100 patients with different types of malignancy and 84 patients were suffered from surgical ailments other than malignancy serve as a control group.

The malignant patients (42 males and 58 females) were allocated in to 4 different groups according to the type of malignancy. Group A, 43 patients (21 males and 22 females) having gastrointestinal tract cancer (GIT). Group B, 18 patients with breast cancer. Group C, 18 patients were urinary system malignancy (13 males And 5 females). Group D, including other types of malignancy as cancer of larynx (2 males and 3 Females), cancer of thyroid (2 males and 3 females) and secondary tumors (7 males and 4 females).

The eighty four non – malignant patients in there ages and type of sex (40 males and 44 females) participoted in this study as control group admitted to the surgical word for hernia, frssure, fistula and piles.

From each patient full information was obtained by using questionnaire that include name, agea, occupation, as well as the question about the medical history for any previous and recent illness and their type of treatment, family history for any malignant condition, social history for smoking and drinking habits and dietary question about the type and frequency per week including fruits mainly citrus fruit, and vegetables

intakes also vitamins supplementations , mainly C and E including dose and duration was performed.

Physical and radiological examination was done including ultra sound and X-rays especially for those suspected cases with malignancy, biopsy was taken for histo patholological examinations and classification of patients according to their type of malignancy by consultant of surgery Dr. Hashim. Alkhayat Head of the Department of the Ward of Surgery in Basrah General Hospital.

Laboratory investigation for hemoglobin, fasting blood sugar, blood urea, general urine examination and estimation of sedimentation rate were obtained from their case sheet.

Blood samples were collected from each patient (malignant and non-malignant after an average fast of 10 hours and serum was separated and tested for copper and its related protein ceruloplasmin, zinc and Cu/Zn ratio using direct method with acetylene and air flame atomic absorbance spectrophotometer after simple dilution for (zinc and copper analysis)[16] the procedure was carried out in The Petrochemical Company in Basrah and enzymatic assay for ceruloplasim activity was accomplished according to the procedure of Rice and Menmorial [17].

Statistical analysis was expressed as mean ± SD, and the t-test and chi-square test were used to determine the relative importance of various variables. The comparison between groups was performed with one way analysis of variance ANOVA, and P value at < 0.05 was regarded as significant and 0.001 as highly significant.

Results

The basic clinical characteristics of all patients participated in this perspective study regarding age, sex, cigarette smoking ,family history, type of malignancy and nutritional habits are presented in Table 1.

a- Effect of age:
Table 2 shows the mean ± SD of serum ceruloplasmin in different types of malignancy and at different age groups. There was highly significant increase p <0.01 in serum level of Cp for malignant patients with GIT, breast cancer and urinary system at the age group <45 and 45-65 years old ,and non significant differences(p>0.05) in older age group i.e. above 65 years. The same observation was found for the level of serum copper Table 3.

However the mean± SD for serum zinc level was highly significantly decreased(p<0.05) in other types of malignancy at age < 45 years ,in GIT cancer with age ranging form 45-65 years respectively. Aso there was highly significant decrease (p<0.01) in serum Zn level for patients with breast cancer having age above 65 years Table 4.

The effect of age on Cu/Zn ratio among different types of malignancy and as compared with non malignant patients was as discussed for (Cp and Cu levels) presented Table 5.

B-Type of sex:
The mean ±SD of serum Cp, Cu, Zn and Cu/Zn ratio levels according to the sex was presented in Table 6. Highly significantly increase in serum Cp (p< 0.01) was found for male patients having GIT and urinary system. Non-significant alteration (p> 0.05) was observed in serum Cu in different type of malignancy and among different sex groups. There was highly significant decrease (p< 0.01) in serum Zn for female patients presenting with GIT cancer.

Serum Cu/Zn ratio level was significantly elevated (p< 0.05) for both sexes for patients with GIT and

for female presented with urinary system of malignancy.

C-Smoking habit:

Table 7 describes the mean ± SD of serum Cp, Cu, Zn and Cu/Zn ratio levels in malignant and non malignant patients related to their smoking habit. There was highly significant increase (p< 0.01) in serum Cp for smoker patients with urinary system malignancy and in non smoker patients presented with breast cancer. While the only significant elevation in serum Cu was found for smoker patients having GIT and other type of malignancy.

Non significant alteration was found for serum Zn levels among different type of malignancy, but the Cu/Zn ratio levels was significantly elevated (p< 0.05) for both smoker and non smoker patients spially in GIT cancer patients.

d- Family history:

The results in Table 8 illustrate the mean ± SD of serum Cp, Cu, Zn and Cu/Zn ratio levels among the family history for all patients. High significant increase (p< 0.01) in serum Cp level was found in those patients having negative family history in GIT and urinary system malignancy comparing with the control patients. However serum Cu level showed non significant alteration (p> 0.05) in all types of malignancy and also in those having both +ve or -ve family history . Serum Zn level was significantly decrease (p < 0.05) for GIT patients having –ve family history when compared with non malignant patients. However, serum Cu/Zn ratio levels were significantly elevated in GIT patients having –ve family history as compared with non-malignant control group.

E-Nutritional habits (fruits and vegetables intake :)

E.1: Efect of fruits:

The effect of frequent intake of fruits and vegetables per week on serum levels of Cp ,Cu, Zn and Cu/ Zn

ratio in different types of malignancy and in non-malignant patients were presented in Table 9. The levels of serum Cp, Cu and Cu/ Zn ratio were significantly elevated (p < 0.05) in patients having breast cancer and urinary system malignancy with frequency of 3-5 and > 5/week.

Serum Zn level was shows significant decreases (p < 0.05) among those consumed fruit and vegetables < 3/week mainly in patients suffering from GIT and urinary system malignancy.

E.2: Efect of vegetables:

Table 10 shows the levels of Cp, Cu, Zn and Cu/ Zn ratio among frequent intake of vegetables in different types of malignancy and in non-malignant patients. There was significantly elevation (P< 0.05) in the concentrations of Cp, Cu, Zn and Cu/ Zn ratio in patients suffering from breast cancer and urinary system malignancy specifically those consuming vegetables in frequent 3-5 and > 5/week.

Serum Zn level showed significant decreases (p < 0.05) in GIT and urinary system malignancy when those patients consumed < 3times per week.

Note: Any differences in numbers of patients during estimating the parameters, are due to in adequate volume of serum due to the large previous parameters that measured.

Discussion

In the present study there was significant increase in serum level of Cp in all types of malignancy, a fact confirmed by other investigators [18,19]. This significant elevation in serum level was demonstrated with regard to age, sex, smoking and nutritional habit. The possible cause responsible for this elevation was

related to increase hepatic synthesis of the protein in consequence to cytokine and leukocyte endogenous mediator stimulants that are released during an acute phase response to tissue damage since tumor growth is a dynamic process associated with damaging to the surrounding normal tissue, there fore, there will be aggregation of leukocytes and phagocytes around damaged tissue which are responsible for the releasing of these stimuli[20,21]. in spite of high frequency of fruit and vegetable intake which leads to decrease in the serum level of Cp as result to inhibitory effect of vitamin C against intestinal copper observation[22].

The results of serum copper in this study were confirmed with other workers. The possible cause of this observation could be related to the raising serum cupric-enzyme (ceruloplasmin, which represents about 95% of hepatic serum copper) as result to the increased hepatic synthesis of this protein[22]. With regard to the influence of nutritional habit on serum Cu concentration, studies had shown that as the amount of citrus fruit intake increased there would be reduction in serum copper concentration; this is due to decrease intestinal copper absorption as a result to inhibitory effect of vitamin C[22].

The significant decrease in serum zinc level in all patients with cancer, specifically the GIT cancer was almost as the same finding that was observer by other studies[23-25]. This observation was found in respect to age, sex,smoking and nutritional habit. The patterns of changes in serum zinc concentration was typical of an acute phase response to inter leukin 1 and leukocyte endogenous mediator stimuli , that responsible for enhancing the hepatic metallothionin synthesis which required for zinc sequestration from circulation[26]. In addition, there

will be increases in the uptake of zinc by cancer cells due to its metabolic requirements[27].

Concerning the nutritional habit, there was significant decrease in serum zinc level in those patients consuming fruit < 3/ week .Since vitamin C enhances intestinal zinc absolution, therefore there was a direct
 relationship between the amount of vitamin C intake and intestinal zinc absorption[22]. At the same time, high intake of vegetables will inhibit intestinal zinc absorption due to the effect cellulose that enhances the rate of passage of diet through the digestive tract and consequently reduction in serum zinc level was mainly in these consuming vegetables 3-5 /week[28].

The Cu\Zn ratio represents the distance between two populations benign conditions and malignant tumors. This ratio was significantly increased in all types of malignancy specifically in GIT and breast cancer. The same findings were mentioned in other studies[29,30]. This elevation in Cu/Zn was ratio mainly due to increase in serum copper concencentration and a contributory effect of decrease serum zinc concentration. In addition, certain studies revealed that Cu/Zn ratio is useful measure in estimating the extent and prognosis of malignant tumor because it is correlated with tumor size and activity[31,32].

Therefor a high Cu/Zn ratio indicates poor prognosis and vice versa.

References

1.Cotran RS., Kumar V., Collin T. Pathologic basis of disease.6th ed., Philadelphia:
W.B . Saunders Company., 1999.
2. Murray RK. Cancer, Carcinogens and growth factors. In: Murray RK., Granner DK., Mayes PA eds. Harper's Biochemistry 24 th ed., California:

Appleton and Lange Company, 1996; 757-777.

3. Talaro K., Talaro A. Foundation in Microbiology. 2nd ed., Chicago: Wm. C. Brown publishers,1996.

4. Sedjo RL., Inserra P., Abrahamsen M., et al. Human papilloma virus persistence and nutrient involved in the methylation pathway cohort of young women. Cancer Epid. Biomarkers Prev. 2002; 11: 353-359.

5.Miller JA., Miller EC. Perspectives on the metabolism of chemical carcinogens. In: Emmelot P. and Kriek E.,eds. Environmental Carcinogenesis, Occurrence Risk Evaluation and Mechanisms Lsevier/North –Holland Biomedical Press. 1979; 25-45.

6.World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition and the prevention of cancer: a global perspective. 1st ed. Washington: the American Institute for Cancer Research, NW. 1997 .

7. Higginson J. Environmental Carcinogenesis: A global perspective. In: Emmelot P. and Kriek E.,eds Environmental Carcinogenesis, Occurrence, Risk Evaluation and Mechanisms. Lsevier/North –Holland Biomedical Press. 1979; 9-22 .

8. Doll R., Peto R. The causes of cancer. JNCI .1981; 66(6):1191-1308.

9. Malone WF. B. Studies evaluating antioxidants and β-Carotene as chemo preventives .AM. Clin. Nutr. 1991 ;53:305S-313S.

10. Trichopoulos D., Petridou E., Lip Worth L., et al. Epidemiology of cancer. In: Devita V.T., Hellman S .and Rosenberg SA., eds . cancer principles and practice of oncology .5th ed., Philadelphia: Lippincott-Raven publishers, 1997;231-251.

11. Meydani M., Martin A., Gong J., et al. β-Carotene supplementations increases antioxidant capacity of plasma in older women. Nutrion 1994; 24: 2397-2403.

12. Goodman D. Vitamin and Retinoid in health and disease. New England Journal of medicine. 1984; 310 (16) 1023-1030.

13. Spom MB and Roberts AB. Role of Retinoid in differentiation and Carcinogenesis

14.Hassan HM., Fridovich I. Super oxide Dismutases: Detoxication of a free radical.

15. Gutteridge JMC. Antioxidant properties of ceruloplasmin towards Iron and Copper dependent oxygen radical formation. FEBS 1983; 157 (1):37-40.

16. Tylor A. Clinical application of flame techniques in Haswell S J . Atomic absorption spectrophotometry. Theory, design ,and application. Amsterdam,
Elsevier science publisher. 1991; 351-379.

17. Rice EW., Menmorial WHS. Ceruloplasmin Assay in serum :standardization of
ceruloplasmin activity in terms of international enzyme units. In: Selgson D;eds. Standard methods of clinical chemistry. New York. Academic press. 1963 ; 39-46.

18.Linder MC., Moor JR., Wright K. Ceruloplasmin assay in diagnosis and treatmentof human lung, breast and GIT cancer. J. Natt.Cancer Inst. 1981; 67 (2): 263-275.

19. Onizuka K., Migita S., Yamada H et al. Serum protein fraction in patients with Laryngeal cancer undergoing radiation therapy. Possibility as a prognostic factor.
Fukuoka. Igaku .Zasshi. 1999 ;90 (2):46-58.

20. Dinarello CA. Interleukin 1 and the pathogenesis of the acute phase response. The New England Journal of Medicine 1984; 311(22):1413-1418.

21. Daffado AA., Young SP. Coordinated regulation of

ceruloplasmin and metallothionin mRNA by Interleukin 1 and copper in $HepG_2$ cells. FEBS. Lett. 1999; 457(2):214-218

22.Andersn L., Dibble MV., Turkki PR., et al. Nutrition in health and disease. 17[th]ed. Philadelphia: J. B. Lippincott Company. 1982.

23. Li W., Lu W., Zhu M., et al. Determination of copper, zinc, iron and calcium in wheat and maize and three nitrogen compounds in high and low areas of esophageal cancer. Wei. Sheng. Yen .Chin . 1998; 27 (1):69-71.

24. Faber M., Coudray C., Hida H., et al., Lipid per oxidation products and vitamin and trace elements status in patients with cancer before and after chemotherapy including a drimaycin , A pre Lime nary study. Biol. Trace . Chem. . Res. 1995; 47 (1-3):117-123.

25.Issel BF., Macfadyen BV., Gum ET., et al. Serum zinc levels in lung cancer patients. Cancer .1981;47: 1845- 1848.

26.Fisher GL., Spitler LE., Macneill KL., et al. Serum copper and zinc levels in melanoma patients. Cancer . 1981; 47 1838-1844.

27. AtukoralaS., Basu TK., Dickerson JWT., et al. Vitamin A, zinc and lung cancer. BRJ of Cancer. 1979; 40 (6): 927-931.

28. Underwood EJ. Trace elements in human and animal nutrition. 4th ed. New York: Academic Press, Inc.1977.

29. Inustuka S., Araki S. Plasma copper and zinc levels in patients with malignant tumors of digestive organs. Cancer Aug . 1078; 42: 626-631.

30. Brandes JM., Light man A., Dru gan A., et al. The diagnostic value of serum Cu/Zn ratio in gynecological tumors. Acta Obstet Gynecol Scand. 1983; 62: 225-229.

31. Ferriono D., Buccheri G., Camilla T. Serum copper and zinc content in non-small cell lung cancer abnormalities and clinical correlates'. Monaldi. Arch. Chest. Dis. 1999;54(3):204-208.

32. Poo J, Romero, RR., Robles JA. Diagnostic value of Cu/Zn ratio in digestive cancer . Arch. Med. Res. 1997; 28(2);259-263.

Table 1 Clinical characteristics of malignant and non- malignant subjects.

Variable		Malignant N %	Non – malignant N %
Age (years)	<45	26 26% 53	41 48%
	45-65	53%	35 41%
	>65	21 21%	8 9%
Sex	Female	59 59%	44 52% 40
	Male	41 41%	47%
Smoking	+ ve	36 36%	16 19%
	- ve	64 64%	68 80.9%
Family history	+ve	18 18%	5 5.9%
	- ve	82 82%	79 94%
Fruit intake / week	< 3	51 51%	21 25%
	3-5	28 28%	29 34.5%
	> 5	21 21%	34 40%
Vegetable intake / week	< 3	28 28%	11 13%
	3-5	38 38%	11 13%
	> 5	34 34%	62 73%
Type of malignancy	GIT	18 18%	- -
	Breast	43 43%	- -
	Urinary system	18 18%	- -
	Others	21 21%	- -

Table 2 Serum level of cerloplasmin according to the age in malignant and non-malignant patients.

Age	Non-malignant	Type of malignancy				
		GIT	Breast	Urinary system	Other type	Total
<45	51.17±3 (37)	70.6±17.3** (8)	70.6±26.9** (8)	78±24** (3)	57.5±4.6 (4)	71.79±3.7** (23)
45-46	50.9±3 (31)	64.15±18.5** (19)	63.6±10.3** (8)	73.8±22** (6)	60.9±11.7 (11)	63.55±2.6** (44)
>65	72.8 ± 10.2 (16)	57.28±24 (7)	70±0 (1)	64.8±17.5 (7)	69.7±24.4 (4)	64.3±4.5 (19)
Total	52.1±17 (84)	64.2±19* (34)	67.3±19* (17)	70.6±19.8** (16)	62±14.9 (19)	65.5±18.5*** (86)

Values are expressed as mean ± SD
Types m'alignant patients vs. non –m'alignant
P*<0.05,   p**<0.01
All values were expressed as I.U

Table 3 Serum level of copper in malignant and non –malignant patients in different age groups.

Age	Non-malignant	Type of Malignancy				
		GIT	Breast	Urinary system	Other type	Total
<45	81.6±4 (37)	92.3±17.8* (9)	89±18.4* (9)	100±12.7* (2)	72.7±12.5 (4)	96.29±5.28 (24)
45-46	92.37±4.6 (31)	92.6±24.5 (23)	102.5±32 (6)	80±12.39 (8)	91.6±31 (13)	90.96±3.41 (50)
>65	93.59±10.9 (16)	79.8±13.16 (6)	127±0 (1)	89.5±24.6 (7)	92.25±28.1 (4)	83.9±6.59 (18)
Total	83.7±24.8 (84)	90.5±21.7 (38)	96.4±25.1 (16)	86.4±18.7 (17)	88±27.9 (21)	90.25±23.2 (92)

Values are expressed as mean ± SD
Types m'alignant patients vs. non –m'alignant
P*<0.05,   p**<0.01
All values were expressed as I.U

Table 4 Serum zinc level according to the age in malignant and non –m'alignant patients.

Age	Non-malignant	Type of Malignancy				
		GIT	Breast	Urinary system	Other type	Total
<45	95.8±3.7 (37)	98.6±32.5 (9)	90.5±19.3 (9)	89.5±20.5 (2)	71±7.78** (4)	90.58±4.9 (24)
45-46	98±4.39 (31)	78.6±24.7* (23)	86.5±30.7 (6)	88.3±27.9 (8)	91.7±18.1 (13)	84.7±3.2** (50)
>65	100.6±10.3 (16)	72.2±24.3** (6)	59±0* (1)	84.5±22.4 (7)	108±6.6 (4)	84±6.231* (18)
Total	97.9±21.1 (84)	82.3±27.6* (38)	87±23.9 (16)	86.9±23.6 (17)	90.9±18.8 (21)	85.9±24.3* (92)

Values are expressed as mean ± SD
Types m'alignant patients vs. non –m'alignant
P*<0.05,   p**<0.01
All values were expressed as I.U

Table 5 Serum Cu/Zn level in malignant and non –malignant Patients in different age groups.

| Age | Non-malignant | Type of malignancy | | | | |
		GIT	Breast	Urinary system	Other type	Total
<45	0.91±0.07 (37)	1±0.5 (9)	1±0.2 (9)	1.16±0.4 (2)	1.01±0.06 (4)	1.13±0.09** (24)
45-46	0.996±0.08 (31)	1.3±0.59* (23)	1.3±0.6* (6)	0.98±0.317 (8)	1.02±0.4 (13)	1.173±0.06* (50)
>65	0.96±0.2 (16)	1.28±0.71 (6)	2.15±0 (1)	1.15±0.25 (7)	0.85±0.26 (4)	1.14±0.12 (18)
Total	0.9±0.35 (84)	1.25±0.59* (38)	1.18±0.48 (16)	1.07±0.4 (17)	0.99±0.34 (21)	1.15±0.49* (92)

Values are expressed as mean ± SD
Types m'alignant patients vs. non –m'alignant
P*‹0.05,   p**‹0.01
All values were expressed as I.

Table 6 Serum level of cerloplasmin, copper zinc and Cu/Zn ratio according to the sex in malignant and non –malignant patients.

| Sex | parameters | Non-malignant | Type of Malignancy | | | | |
			GIT	Breast	Urinary system	Other type	Total
Female	Cp	58.7±16.2 (33)	61.2±17 (19)	67.3±19.4 (17)	69±27.6 (5)	61.15±17.5 (13)	63.5±19 (54)
	Cu	88.14±21.7 (35)	87.47±17.3 (21	96.4±25.16 (16)	102.5±25.3 (4)	80±23 (13)	89.4±22.3 (54)
	Zn	100.7±18.4 (35)	73.8±24.3*** (21)	87±23.9 (16)	75.5±23.3 (4)	89.8±17.9 (13)	83.6±22.7 ** (54)
	Cu/Zn	0.92±0.3 (35)	1.27±0.6* (21)	1.18±0.48 (16)	1.44±0.48* (4)	0.9±0.3 (13)	1.2± 0.52* (54)
Male	Cp	46.4±15.9 (34)	69.3±20.7** (15)	-	71±16.8** (11)	64±8 (6)	69±17.4** (32)
	Cu	82.4±21.4 (39)	94±26 (17)	-	81±13.9 (13)	101±31.5 (8)	91.34±24.7 (38)
	Zn	90.27±20.7 (39)	88±27 21	-	90±23 (13)	92±21 (8)	89.3±26.4 (38)
	Cu/Zn	0.97±0.41 (39)	1.27±0.56* (17)	-	0.96±0.3 (13)	1.1±0.39 (8)	1.1±0.44 (38)

Values are expressed as mean ± SD
Types m'alignant patients vs. non –m'alignant
P*<0.05,   p**<0.01
All values were expressed as I.U

Table7 Serum level of cerloplasmin, copper zinc and Cu/Zn ratio according to the smoking habit in malignant and non-malignant patients.

Smoking habit	parameters	Non-malignant	Type of malignancy				
			GIT	Breast	Urinary system	Other type	Total
+ve	Cp	52.25±15.2 (12)	74±16.47 (11)	63±0 (1)	79.8±21.4** (8)	65.8±18.9 (10)	72.7±18.6* (30)
	Cu	82±29.4 (14)	97.5±29.4* (12)	46±0 (1)	82.2±15.2 (9)	95.6±22.6* (10)	91±25** (32)
	Zn	103±27 (14)	92.5±27.7 (12)	85±0 (1)	92±19.7 (9)	97.4±17.6 (10)	93.5±21.7 (32)
	Cu/Zn	0.84±0.4 (14)	1.2±0.58* (12)	0.54±0 (1)	0.94±0.32 (9)	0.98±0.2 (10)	1.1±0.42* (32)
-ve	Cp	52.1±17.6 (55)	59.6±19 (23)	67.5±20* (16)	61.5±13.7 (8)	58.6±10 (9)	62±17.7* (56)
	Cu	85.6±22 (60)	87.3±17 (26)	99.8±22 (15)	91±22 (8)	81.4±31.5 (11)	91.3±27 (60)
	Zn	96.8±19.6 (60)	77.6±27** (26)	87±25 (15)	81.5±27.7 (8)	85±18.6 (11)	81.9±23.9** (60)
	Cu/Zn	0.92±0.33 (60)	1.28±0.6* (26)	1.23±0.46 (15)	1.2±0.46 (8)	0.99±0.43 (11)	1.23±0.55* (60)

Values are expressed as mean ± SD

Types m'alignant patients vs. non –m'alignant

P*‹0.05, p**‹0.01

All values were expressed as I.U

Table8 Serum level of cerloplasmin, copper zinc and Cu/Zn ratio according to the family history in malignant and non –malignant patients.

Family history	parameters	Non-malignant	Type of malignancy				Total
			GIT	Breast	Urinary system	Other type	
+ve	Cp	47.8±10.6 (5)	44±13.07 (3)	62.3±10.9 (8)	-	57.5±15.5 (4)	57.8±13 (15)
	Cu	76.6±28.5 (4)	89±25 (2)	98±26 (7)	87±0 (1)	80.7±26.6 (4)	92.5±26.2 (14)
	Zn	105±9.8 (4)	84.5±32 (2)	91.7±29 (7)	75±0 (1)	95±21.3 (4)	88.4±20 (14)
	Cu/Zn	0.732±0.2 (4)	1.2±0.73 (2)	1.12±0.4 (7)	1.2±0 (1)	0.86±0.3 (4)	1.1±0.4 (14)
-ve	Cp	52.5±17.5 (62)	66±18.9** (31)	71.6±4.6* (9)	70.6±19.8** (16)	63.2±15.2 (15)	67.2±20*** (71)
	Cu	85.5±23 (70)	90.6±22 (36)	95±26 (9)	86.3±19.3 (16)	89.8±28.7 (17)	92±26.2 (78)
	Zn	97.5±21.7 (70)	82.2±27.8* (36)	83.4±20 (9)	87.6±24 (16)	89.9±18.7 (17)	84.5±24.3* (78)
	Cu/Zn	0.92±0.35 (70)	1.25±0.59* (36)	1.23±0.56 (9)	1.07±0.4 (16)	1.02±0.35 (17)	1.2±0.54* (78)

Values are expressed as mean ± SD

Types m'alignant patients vs. non –m'alignant

P*‹0.05,   p**‹0.01

All values were expressed as I.U

Table 9 Serum level of cerloplasmin, copper zinc and Cu/Zn ratio according to the frequency 0f fruits intake in malignant and non –m'alignant patients

Fruits take/ week	Para- meters	Non- malignant	Types of Malignancy				
			GIT	Breast	Urinary	Other type	Total
<3	Cp	58±18.6 (16)	64±21 (19)	62±16.4 (7)	62±12 (9)	65.2±11 (6)	63±17 (41)
	Cu	84±26 (20)	97±23 (22)	87±13 (7)	85±22 (10)	92±35	92±24 (47)
	Zn	102±14 (20)	78±29* (22)	85±21 (7)	79±26* (10)	93±18 (8)	80±26** (47)
	Cu/Zn	0.8±0.3 (20)	1.5±0.6* (22)	1.1±0.5 (7)	1.2±0.5 (10)	1±0.45 (8)	1.3±0.56* (47)
3-5	Cp	50±15.5 (25)	61±19.5 (11)	65±11 (8)	74±31* (3)	66±3.5 (3)	64±17* (25)
	Cu	85±23 (26)	81±16 (13)	109±24* (7)	93±16 (3)	92±7 (3)	91±21 (26)
	Zn	95±17 (26)	9124 (13)	85±28 (7)	97±19 (3)	89±17	90±23
	Cu/Zn	240.9±0.3 (26)	0.96±0.4 (13)	1.4±0.5 (7)	1±0.4 (3)	1±0.2 (3)	1.1±0.4 (26)
>5	Cp	50±17.3 (26)	69±11 (4)	94±44* (2)	85±20* (4)	65±18* (10)	73±21* (20)
	Cu	85±22 (28)	89±23 (3)	86±56 (2)	85±13 (4)	84±27 (10)	85±25 (19)
	Zn	97±28 (28)	99±1.5 (3)	101±0.3 (2)	100±9 (4)	93±21 (10)	95±17 (19)
	Cu/Zn	0.9±0.4 (28)	0.9±0.2 (3)	0.8±0.4 (2)	0.8±0.14 (4)	0.95±0.3 (10)	0.9±0.3 (19)

Values are expressed as mean ± SD

Types m'alignant patients vs. non –m'alignant

P*‹0.05,   p**‹0.01

All values were expressed as I.U

Table 10 Serum level of cerloplasmin, copper zinc and Cu/Zn ratio according to the frequency of vegetables intake in malignant and non-malignant patients.

Vegetables / take week	Para-meters	Non-malignant	Types of Malignancy				
			GIT	Breast	Urinary	Other type	Total
<3	Cp	54±21 10	71±17 7	61±9 7	60±9 3	54±11 4	63±15 21
	Cu	91±30 10	105±29 11	102±20 6	74±12 4	83±11 5	87±28 26
	Zn	105±14.5 10	85±32 11	101±25 6	75±33 4	83±11 5	87±28 26
	Cu/Zn	0.87±0.3 10	1.4±0.6 * 11	1.1±0.3 6	1.1±0.4 4	1.2±0.5 4 5	1.2±0.5 26
3-5	Cp	47±16 7	63±21 17	68±13 7	66±21 7	60±5 2	65±19 33
	Cu	84±23 10	90±13 18	97±25 7	99±19 7	79±16 2	93±17 34
	Zn	100±15 10	77±27* 18	71±16* 7	89±22 7	69±4.2 2	78±24* 34
	Cu/Zn	0.8±0.2 10	1.3±0.6 18	1.5±0.6 * 7	1.2±0.5 7	1.1±0.2 2	1.3±0.5 * 34
>5	Cp	53±17 50	61±1.9 10	80±39 3	81±39* 6	65±17* 13	68±21* 22
	Cu	82±21 54	73±10 9	85±40 3	80±13 6	88±25 14	82±21 32
	Zn	96±23	90±22 9	98±18 3	93±20 6	97±19 14	94±19 32
	Cu/Zn	0.9±0.4 54	0.9±0.4 9	0.8±0.3 3	0.9±0.2 4 6	0.9±0.2 6 14	0.9±0.3 32

Values are expressed as mean ± SD
Types m'alignant patients vs. non –m'alignant
P*‹0.05, p**‹0.01
All values were expressed as I.U