THE RELATIONSHIP BETWEEN TRACE ELEMENTS IN PATIENTS WITH DIFFERENT TYPES OF BRAIN TUMORS (BENIGN AND MALIGNANT)[+]

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Abstract

Copper, zinc and iron trace elements were measured in tissue extracts of (37) patients with brain tumors (benign 19 and malignant 18) using flameless atomic absorption technique. Astrocytoma, Glioma and Meningioma are subgroups of brain tumors, which were investigated in this study. The results indicate a highly significant increase ($p<0.001$) in these trace elements with malignant brain tumors compar with that of benign brain tumors, the same results were found in subgroup astrocytoma and glioma, while insignificant increase ($p>0.05$) in subgroup meningioma of malignant patients.

المستخلص:

تم قياس تركيز العناصر الضئيلة النحاس ، الزنك والحديد في خلاصة انسجة (٣٧) مريضاً بورم الدماغ(١٩ حميد ، ١٨ خبيثاً) باستخدام تقنية الامتصاص الذري أللالهبي ، وتم التحري عن الفروقات الموجودة بين تركيز هذه العناصر أعلاه في المستخلص النسيجي لتلك الاورام(الحميدة والخبيثة) في المجاميع الفرعية لأورام الدماغ وهي astrocytoma , glioma , meningioma .أشارت النتائج الى ما يلي :

إن تركيز هذه العناصر قد ارتفعت بصورة معنوية عالية ($P<0.001$) في خلاصة أنسجة المصابين بالأورام الخبيثة مقارنة مع المصابين بألاورام الحميدة . وظهرت في النتيجة نفسها نتائج المجاميع الفرعية astrocytoma , glioma .بينما لم يظهر أي اختلاف معنوي في تركيز هذه العناصر ($p<0.001$) في المجموعة الفرعية من نوع meningioma .

Introduction

Trace elements are micro nutrients (micrograms to milligrams) that are essential for certain biochemical processes and metabolic functions, including oxidative metabolism regulation, immunologic response, and cell-differentiation ,these regulations have been reported to be disturbed during the malignant process[1].

Among these trace elements zinc that is the second most abundant transition element in the body, over (300) enzymes require zinc for their function. Zinc plays as three roles in enzyme containing zinc, catalytic, coactive (or cocatalytic) and structural role2].Zinc is transported into the adult brain,probably as required components for neural function.The zinc concentration in the brain increase with

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growth after birth and is maintained constant in the adult brain[3,4],Approximately 90% of the total brain zinc is zinc metallo proteins in neurons and glial cell[5],and 10% of the total brain zinc probably ionic zinc,exists in the synaptic vesicles,and may serve as an endogenous neuro modulator in synaptic neurotransmission[2,6].Zinc can be neurotoxic in the brain when its amounts increase in the extracellular fluid[2].Whole blood and serum zinc levels in human patients with such neoplasm are generally subnormal[7].

Copper is another essential element in biological systems ،including the central nervous system (CNS)[8,9],it is an essential trace element for several enzymes and is important,for a variety of processes in homeostasis such as electron transport,aminoacid metabolism , connective tissue biosynthesis,pigment formation,and neuro transmitted hormone production[10].

In plasma, copper is present in two forms. Approximately 90% of the total plasma copper is firmly bound to the-α 2 globulin Ceruloplasmin, and 10% of plasma copper is loosely bound to albumin[11]. In human brain, the copper distribution varies from region to region[12].

Brain tissue contains a relatively high concentration of copper[13]..Copper is toxic to humans only when it is ingested as the copper ion[14].

Iron is considered one of essential metals for almost all living organisms due to its presence in a large number of enzymes and proteins[15].Hemoglobin is the most abundant heme proteins and accounts for 62% of the body iron.While myoglobin accounts for about 8% of the body iron,it transpos and stores oxygen to be used during muscle contraction[15].

Iron levels in the brain may be closely related to disease severity and reflect the free radicals damage that is considered part of the pathology[16,17].

Materials and Methods:

All chemicals are of highly analytical grade.

a-Patients :

Thirty-one brain tumor patients who were attended to teaching hospital of Iraqi medical college,over a period of eight months from Sebtember 2003 to May.

Nineteen tissue samples of different types and stages of benign brain tumors and eighteen tissue samples of different types and stages of malignant brain tumors were used, their mean age was (42.3 ± 22.1) ranging from (2-82) Years.

Samples	N	Mean age ±S.D
Benign	19	41.52±22.80
Malignant	18	43.11±21.48
Total	37	42.3± 22.1

b-Methods:

1. **Preparing of tissue homogenates[18]**

A weight of (0.1) gm of each tumor tissue that was obtained from the benign and malignant of patients with brain tumor was washed thoroughly with normal

saline, then homogenized by homogenizer using veronal buffer in a ratio of tissue weight to buffer volume of (1.5). The homogenate was sonicated, the supernatant (S1) was separated from the plette by centrifugation at (10.000 xg) for (30) minutes in a cooling centrifuge then the obtained supernatant was used for trace elements measurement.

2.Determination of trace elements:

Tissue trace elements contents (Zn, Cu, Fe) were evaluated using flameless atomic absorption technique, zinc was estimated at a wavelength of 213.9nm,copper at 324.8nm and Iron at 248.3nm.

3.Statistics

The Student's *t*-test was performed: differences between two groups were considered significant if $p<0.05$.

Results:

Alteration in tissue extract Copper, Zinc and iron levels in the cancer patients was observed, in comparison with that of the benign group. Table (1); illustrates the mean levels of these trace elements in both groups.

Table(1): Mean concentration for tissue Zn,Cu,Fe in total benign and malignant human brain tumors.

	Benign groups(n=19) Mean±S.D	**Malignant groups (n=18) Mean±S.D**	**P-value**
Cu µmol/L	0.078±0.029	0.115±0.028	P<0.001
Zn µmol/L	0.297±0.128	0.520±0.218	P<0.001
Fe µmol/L	1.177±0.477	2.502±1.162	P<0.001

P<0.001=High significant

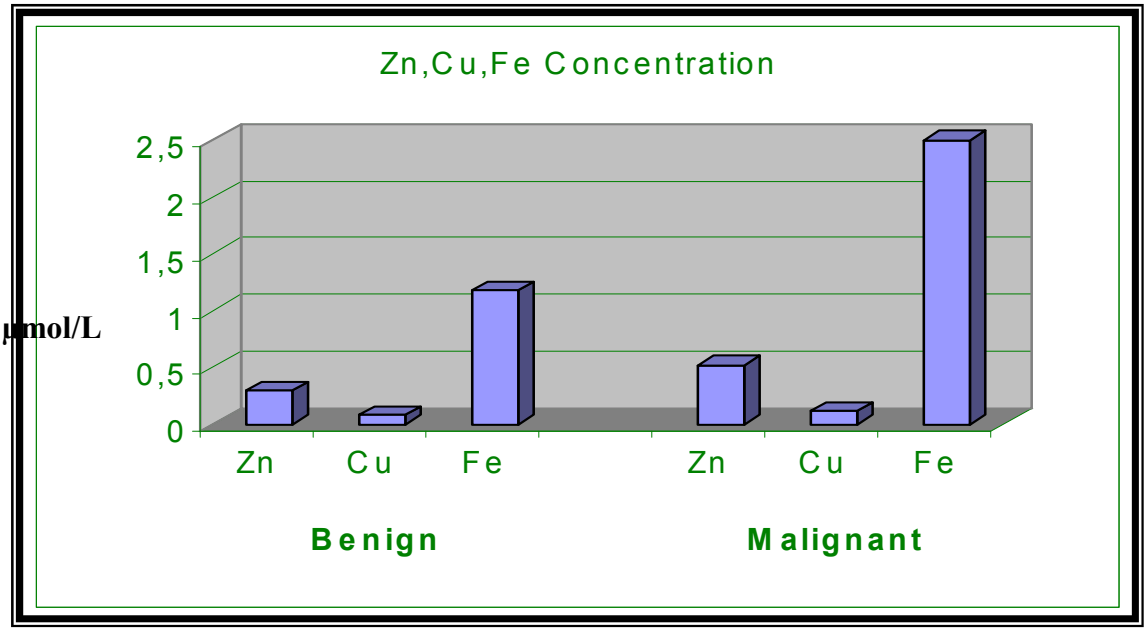


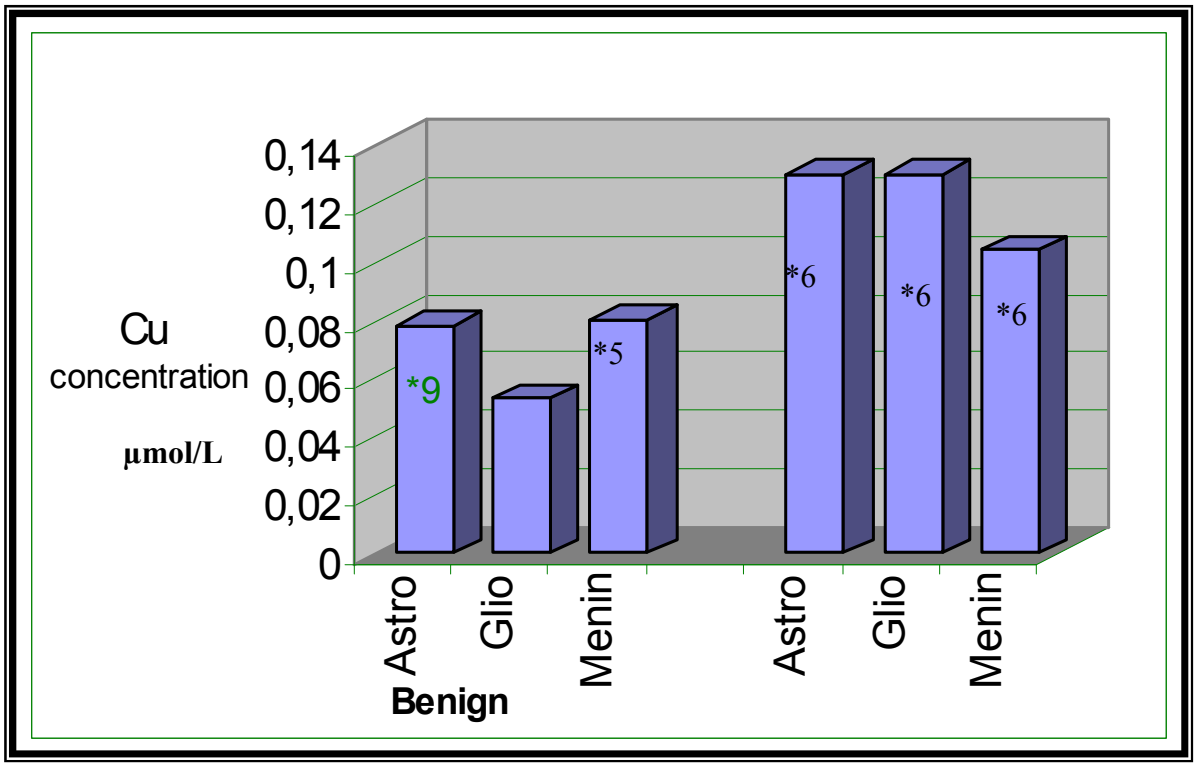
Figure (1): Values of Zn ,Cu, Fe Concentration in tissues homogenates of benign and malignant human brain tumors.

Table (2): Mean concentration of Cu,Zn,Fe in subgroups astrocytoma, glioma and meningioma tissues homogenates of benign and malignant human brain tumors.

Sample size	Benign group n=(19) Mean ±S.D			Malignant group n=(18) Mean ±S.D		
	Astrocytoma	***Glioma***	***Meningioma***	***Astrocytoma***	***Glioma***	***Meningioma***
Copper µmol/L	0.077±0.009	0.057±0.009	0.087±0.023	0.035±0.013	0.058±0.009	0.097±0.043
Zinc µmol/L	0.247±0.1	0.386±0.03	0.307±0.08	0.179±0.156	0.273±0.067	0.296±0.115
Iron	0.354±0.62	0.158±0.16	0.319±0.22	0.218±1.355	0.215±0.752	0.347±0.37

Copper , zinc and iron (Benign and Malignant) $p<0.001$ (high significant) in astrocytoma and glioma subgroups.

Copper , zinc and iron (Benign and Malignant) $p>0.05$ (not significant) in meningioma subgroup.



Figure(2) :Ratio of Cu concentration in astrocytoma,glioma and meningioma tissues homogenates of benign and malignant human brain tumors.
(Astro=Astrocytoma,Glio=Glioma,Menin=Meningioma) *=n=number of patients.

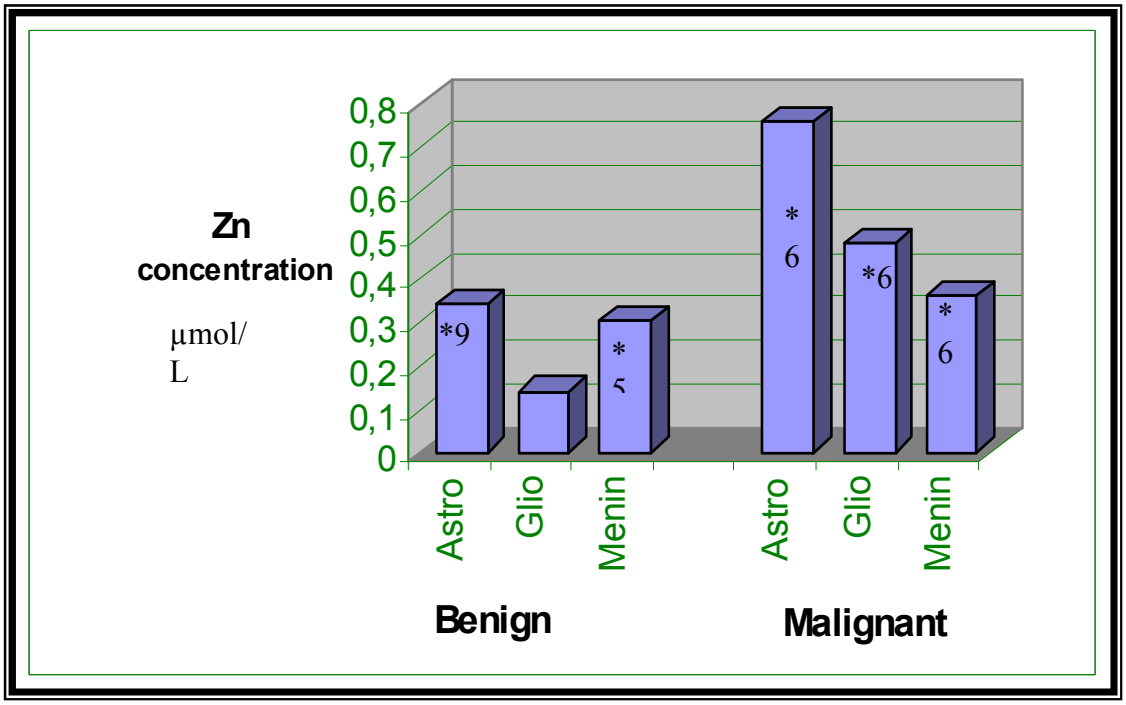
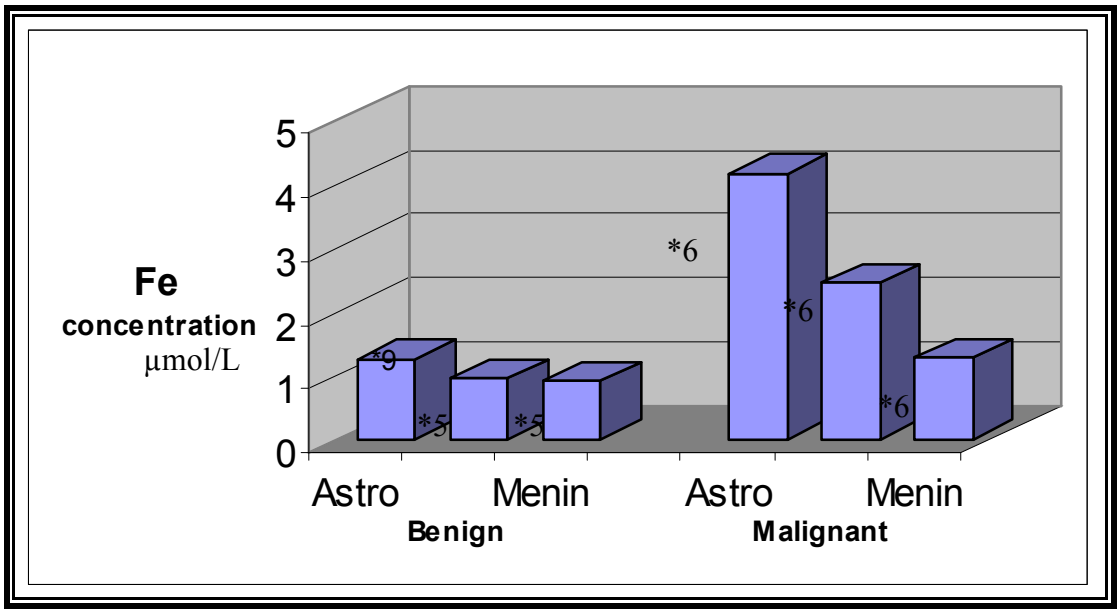
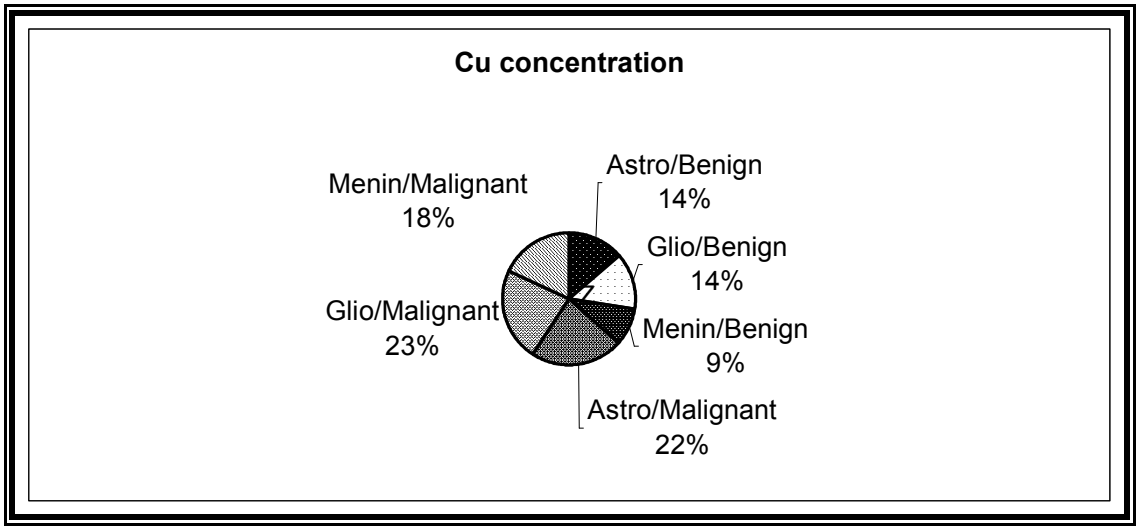
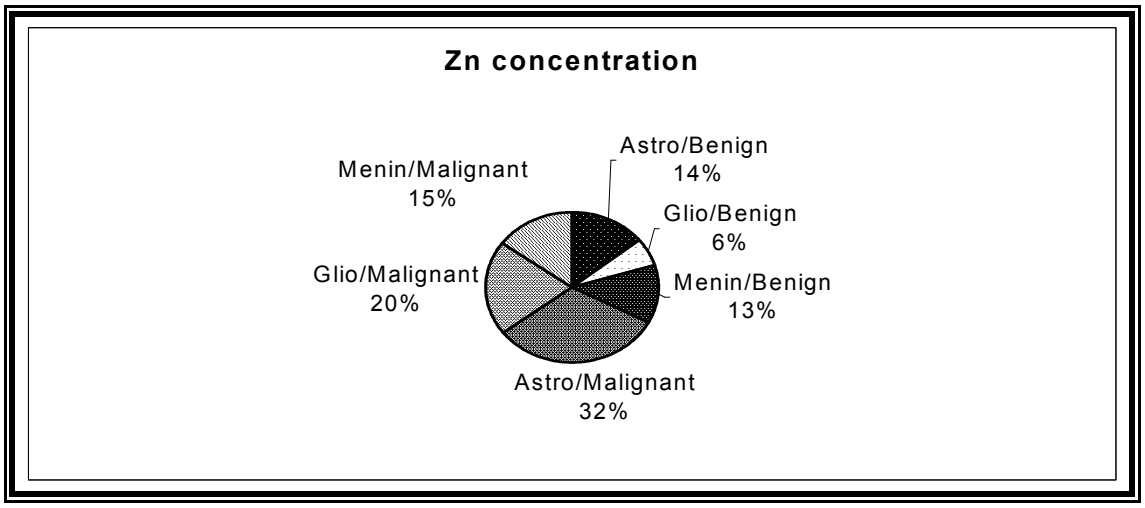


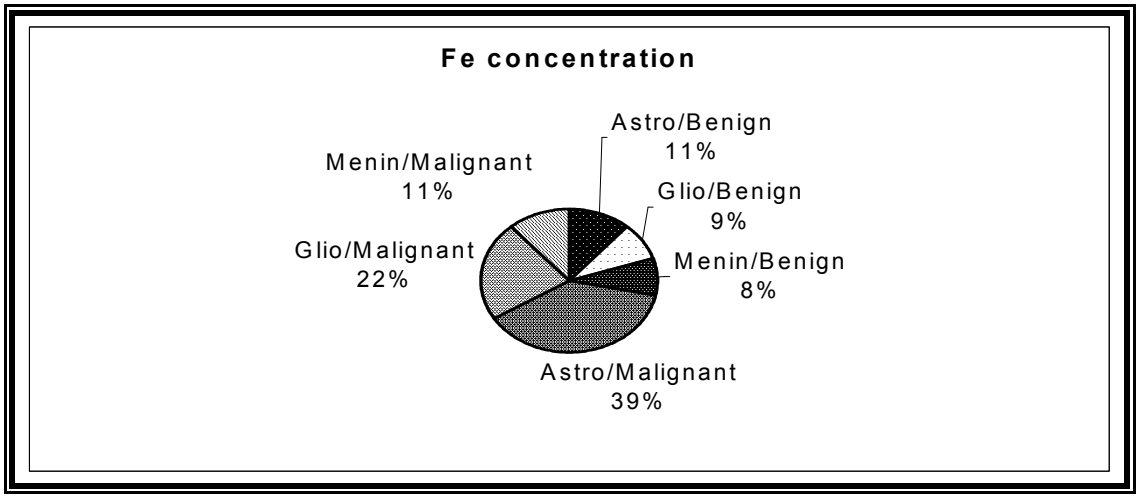
Figure (3) : Ratio of Zn concentration in astrocytoma,glioma and meningioma tissues homogenates of benign and malignant human brain tumors.
(Astro=Astrocytoma,Glio=Glioma,Menin=Meningioma)
*=n=number of



Figure(4) : Ratio of Fe concentration in astrocytoma,glioma and meningioma tissues homogenates of benign and malignant human brain tumors.
(Astro=Astrocytoma,Glio=Glioma,Menin=Meningiom







Figure(5): Pie graph showing distribution percentage of Cu,Zn and Fe concentrationin astrocytoma ,glioma and meningioma tissues homogenates of benign and malignant human brain tumors.

Trace elements such as Cu and Zn have important chemical and biological properties. Recent study by Siegel et al [19] report that trace nutrients in the diet have a vital role in maintaining normal brain function ,tissue Cu and Zn concentrations have been correlated with prognosis in selected malignancies. In addition, depletion of trace metals has suppressive effects on tumor growth[3].

The results above give that copper levels in the malignant group were significantly higher than the benign group ($p<0.001$) as shown in Figure (1), the increase in copper level observed has been correlated with the result obtained by Yoshida D et al[20]. That metastatic carcinomas and malignant gliomas revealed significantly higher tissue copper concentrations than control tissues and meningioma. Malignant gliomas demonstrated significantly higher tissue copper/zinc ratios, that due to the copper,an important angiogenic factors,is accumulated within the malignant tissues of metastatic carcinoma and malignant glioma but not meningioma .

In human brain, the copper distribution varies from region to region as shown in Figure (2) ,and the locusceruleus has the highest concentration of copper[21].It has been suggested that copper plays an important role in complex processes of neurotransmission,function of nerve cells and in several enzymatic processes that accompany the synthesis,storage and release of neurotransmitters[22,23].

Copper deficiency during development causes impairment of mitochondria and myelination[22,24,25],as well as lower catecholamines and depression of tyrosine hydrolase activity[26].Neuronal copper deficiency should be more fully investigated as possible etiological factor in the more common neurodegenerative disease,such as Al Zheimer`s disease,Menkes disease in man and amyotrophic lateral sclerosis[23].

Copper concentration was increased in proportion to ceruloplasmin[27,28],that due to Cp , the major plasma copper transport protein is synthesized in several tissues including the brain[29].

Zinc levels show a highly significant increase in malignant group than the benign group ($p<0.001$) as shown in figure(1) ,this result agrees with previous result done by Yoshida D et al[20] .Because zinc is necessary for DNA replication and transcription and protein synthesis, this metal powerfully influences cell division and differentiation[30,31].Thus, dietary zinc deprivation retards the growth of humans and animals [32,33].Severe Zn^{+2} deficiency during the period of rapid brain growth has effects similar to that seen with protein calorie malnuishment,including altered regulation of emotion;food motivation;lethargy(reduced activity and responsireness),and deficits in learning,attention and memory.In addition to the many deficits produced by Zn^{+2} deficiency in the brain ,the severe effect of Zn^{+2} deficiency on other tissues leads to additional peripheral mechanisms that alter brain function[34].

As shown in figure (3) a highly significant increase in subgroups (astrocytoma and glioma)and not significant increase in meningioma subgroup of malignant brain tumor when compared to that of their benign subgroups,due to zinc and ions,which are contained in nerve terminals,are extruded into the extracellular space during neuronal activity .Excessive levels of zinc may be released during intense neuronal activation and tissue damage.

High Zn^{+2} concentrations enhance and prolong the firing rate of neurons,significantly depress rapi-pulse potentiation,block the action of N-methyl-D-

asparatate(NMDA) on cortical neurons,enhance quisqualate receptor-mediated injury and inhibit the Ca+2-dependent release of transmitter by inhibiting the entry of Ca+2 into nerve terminates[35].

Iron level shows a highly significant increase in malignant group than the benign group ($p<0.001$) as shown in Figure (1).

Brain cancer like other cancers,arises as a result of gene defects that cause changes in control of cell metabolism[39].Therefore ;any factor which can damage the cell,s DNA, is potentially important for the development of cancer and its progression[40] .One category of substance which can induce mutations is metal ions,like Cu I / II and Fe II / III.These ions can take part in and catalyse oxidation / reduction reactions which produce hydroxyl radical (OH) from hydrogen peroxide.This radical is highly reactive and destructive to macromolecules and may lead to local damage to the molecules of life, if its production is uncontrolled[39] .Oxidation may also be due to other active oxygen species,like superoxide radicals and peroxide in the absence of sufficient SOD orcatalase activities respectively. The intra-cellular level of iron might reflect uncontrolled oxidation events,where superoxide ions can release iron from ferritin leading to the production of the active oxygen species such as hydroxyl radicals.This may underline the importance of the anti-oxidant copper that is present in ,for example, superoxide dismutase[40] .This explanation of iron role agrees with the suggestion of many workers who revealed a higher risk of cancer in individuals with larger iron stores than with small iron stores[39] .

Intestinal exposure to ingested iron may be a principal determinant of human colorectal cancer risk. Evidence exists associating iron with early phases of carinogenesis as well as somatic defenses against early cancer through hypoferremia[36].

As shown in Figure (4) there are a significant increase in tissue Fe concentration in (astrocytoma and glioma) subgroups and not significant increase in meningioma subgroup of malignant brain tumor when compared to that of their benign subgroups,because Iron and transferrin are required for DNA synthesis and cell division,cellular iron uptake is mediated by transferrin receptors.In order to investigate whether iron uptake in brain tumors is associated by with their histological grade [37] ,and serves as important factor in many metabolic reactions,including protein synthesis as cofactor of both heme and nonheme enzyme and important in the development of neuronal processes.

However free iron particularly Fe+2 is highly toxic (it is able to trigger cellular deletrons effects), including fenton reaction, and generate free radicals and lipid peroxidation[35].Oxidative stress resulting from increased iron levels in the brain and possibly also from defects in antioxidant defense mechanisms is widely believed to be associated with neuronal death[27,37].

Conclusion :

Out of our results we can conclude that there is a significant differences in levels of these trace elements (Cu,Zn,Fe) between malignant and benign brain tumor with also a differences in subgroup in the same tumors,this means that these elements are important in the growth of brain tumor with increasing demans in high grade brain tumor.

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