



Variations in some biochemical parameters in a group of patients with primary brain tumours: a review of four studies

Variación en parámetros bioquímicos en un grupo de pacientes con tumor cerebral primario: revisión de cuatro estudios

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ABSTRACT

Introduction: It has been well established and evidence-based fact that serum levels of proteins, cholesterol, trace elements, and pseudouridines may suffer changes during a neoplastic disease process. This report encompassed four prospective studies, original in Iraq to our knowledge, had explored the serum total proteins (TP), pseudouridines levels, total serum cholesterol (TSC), and serum trace elements (TE), in groups of patients harboring primary brain tumours (PBT) compared to healthy persons. **Patients and Methods:****Study number 1:** A group of 107 patients, from both sexes, aged 2-75 years, harboring PBT were admitted to and operated upon via formal craniotomy by staff neurosurgeons at The Teaching Hospital at Kadhimiyah (TTHK) and Neurosurgical Hospital (NH); their sera were tested for serum total proteins (TP); the latter biochemical parameters were compared with those of 40 healthy persons. Study number II: the same patients and healthy controls were tested for pseudouridine measurement. Study number III: Another group of 30 patients with PBT were studied for TSC levels and were compared with 30 healthy volunteers. Study number IV: A third group of 26 patients with PBT, from both sexes, their sera were tested and measured for TE; the measurements were compared to 1630 volunteers from both sexes and of different age groups. The sera and brain tumor tissue samples were analysed and examined by appropriate methods at relevant laboratories of the TTHK, NH, The Medical Research Centre (MRC) of The College of Medicine, Al-Nahrain University and the Iraqi Atomic Energy Committee (IAEC). **Results and Discussion:** The serum TP and PBT study: Results are shown in table 1. The serum pseudouridines and PBT study: Mean levels of pseudouridine in serum of PBT patients, were significantly higher ($p < 0.01$) than its levels in the (normal) controls, table 2. The TSC and PBT study:

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1. Healthy persons from both sexes: age range, in years, 15 – 75, mean 40.5, SD \pm 19.8; TSC range 142 – 230 mg / dl, mean 185.6 mg / dl, SD \pm 24.9, (3.7 – 5.9 mmol / l, mean 4.8 mmol / l, SD \pm 0.6), table 3.
2. Thirty persons from both sexes having peripheral tumors, with no clinical evidence of brain tumors: age range, in years, 15 – 75, mean 54.3 \pm 12.8; TSC range 90 – 220 mg / dl, 143 \pm 36.3 (2.3 – 5.7 mmol / l, mean 3.7 \pm 0.9), table 4.
3. Thirty patients from both sexes with primary and secondary brain tumors, age range, in years, 15 - 75, mean 41.3 \pm 20.9; TSC range 140 – 284 mg / dl, 217.6 \pm 41.2 (3.6 – 7.3 mmol / l, 5.6 \pm 1.1), table 4.

Study number IV: Serum mean values (and S.D.) of all measured TEs were as follow: Se 0.045 \pm 0.011, Zn 0.320 \pm 0.095, Cu 0.607 \pm 0.154, Fe 0.880 \pm 0.456, Mg 13.625 \pm 3.994, Co 0.020 \pm 0.036, Ni 0.016 \pm 0.030, Mn 0.016 \pm 0.009, Cd 0.050 (one sample), and Cr 0.015 \pm 0.005 micrograms per milliliter (mcg / ml). All mean concentrations were consistently lower in the patients than healthy volunteers; both the Student's (t) and probability (p value) tests were performed; for Se, Zn, Cu, Mg, Co, Ni, Mn, and Cr the p value was $<$ 0.01 showing statistically significant results; however, for Fe, though the mean concentration was also lower in the brain tumor group, there was no statistical significance, $p >$ 0.05. Due to technical difficulties and very low concentration of Cd, it was not measured in healthy volunteers; however, it was measured in only one patient's serum sample; this has been discarded from the study, table 5.

Conclusions: Levels of serum TP, TC, and pseudouridine are higher in patients with PBT than in healthy people; however, those of serum TE are lower in the PBT group than healthy persons; the results of this report are in keeping with those of other researchers. The biochemical parameters can be an additional laboratory monitor in the investigation of PBT patients; however, both the specificity and sensitivity need to be ascertained. To our knowledge, this was the first study to be performed in Iraq in the setting of PBT.

INTRODUCTION

The serum TP and PBT study

Cancerous transformation entails major biochemical changes including modifications of the energy metabolism of the cell, e.g. utilization of glucose and other substrates, protein synthesis, and expression of receptors and antigens. Tumour growth also leads to heterogeneity in blood flow owing to focal necrosis, angiogenesis and metabolic demands, as well as disruption of transport mechanisms of substrates across cell membranes and other physiological boundaries such as the blood-brain barrier. All these biochemical, histological and anatomical changes can be assessed with emission tomography, X-ray computed tomography, magnetic resonance imaging and magnetic resonance spectroscopy; moreover, whereas anatomical imaging is aimed at the diagnosis of brain tumours, biochemical imaging is better suited for tissue characterization; the latter are used the diagnosis of primary brain tumours, as well as in follow-up ⁽¹⁾. Studying details of metabolic events in various human cancers has attracted the interest of many researchers, to the extent of using stereotactic microdialysis ⁽²⁾.

Study number II (serum pseudouridines and PBT)

Pseudouridine is a modified nucleoside derived from the degradation of some species of Ribonucleic acid (RNA), primarily transfer RNA, the level of which is elevated in biological fluids of tumor bearing subjects. This study was the first to be done in Iraq to measure Pseudouridine in serum of primary brain tumor patients.

Study number III (TSC and PBT)

Total serum cholesterol (TSC) level has been found to vary in many physiological as well as pathological processes. Though low TSC has been described in various malignancies especially those with rapidly proliferating cells, however, in brain tumour setting, the TSC value, in many patients has been found to be higher than normal.

Study number IV (The serum TE and PBT)

Micronutrients are a highly diverse array of dietary components necessary to sustain health. The physiologic roles of micronutrients are as varied as their composition; some are used in enzymes as either co-factor or prosthetic groups, others are used as biochemical substrates or hormones, and in some instances, the functions are not well defined. Under normal circumstances, the average daily dietary intake for each micronutrient required to sustain normal physiologic operations is measured in milligrams or smaller quantities. This quantification distinguishes micronutrient from macronutrients, the latter category encompassing carbohydrates, fats, and proteins, as well as the macrominerals calcium, magnesium, and phosphorus ⁽³⁾.

Fifteen trace elements have been identified as essential for health in animal studies: iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), selenium (Se), iodine, fluorine, manganese (Mn), molybdenum, cobalt (Co), nickel (Ni), tin, silicon, vanadium, and arsenic. Nevertheless, only for the first 10 of these elements is there compelling evidence of essentialness in humans. Some authorities consider Zn, Cu, Mn, and Cr, in additions to few other cations and anions, as typical nutrient contents of peripheral or central parenteral nutritional solution ⁽⁴⁾.

MATERIALS AND METHOD

All of the four studies were case series studies, conducted between November 2000 and October 2001 at both TTHK and NH in Baghdad. PBT patients were evaluated by full medical history to exclude any existing systemic disease that might have targeted the biochemical parameters to be diagnosed, particularly diabetes mellitus, liver disease, renal disease and chronic drug intake; otherwise the patient would have been excluded from the study. Patients were op-

erated upon by local staff neurosurgeons; tumor tissue samples were subjected for histological diagnosis by specialist neuropathologists. In the serum TP and PBT study, there were 107 patients suffering from PBT enrolled in the study; age ranged 2-75 years (35 ± 19). Regarding gender, there were 56 (52.33%) male and 51 (47.66%) female patients. Although 89% of the patients were under the age of 60 years, however, the most affected age group was 31-40 years (17.75%). Forty age - and sex - matched normal subjects were used as controls for serum measurements. In the TSC and PBT study, it was done between November 2000 and April 2001. Although thirty patients with PBT were compared with thirty healthy persons, however the thirty patients with peripheral tumours were mentioned in the current report for the sake of comparison. The ages ranged from 15 – 75 years. A serum sample was collected after 6-hour-fasting and the TSC was estimated by the enzymatic procedure. The mean, standard deviation (SD), and relevant p value have been determined and tabulated.

The results: The serum TE and PBT study: This was a pilot study concerning ten TEs among 26 patients, 17 females and 9 males, age range 2-75 years [36 ± 20.312], harboring benign or malignant brain tumours being operated upon at TTHK and NH during February till July 2001 inclusive. Estimation of levels of selenium (Se), zinc (Zn), copper (Cu), iron (Fe), magnesium (Mg), cobalt (Co), nickel (Ni), manganese (Mn), Cadmium (Cd), and chromium (Cr) was performed at the laboratories of The Iraqi Atomic Energy Committee (IAEC) in samples of serum by flame / flameless atomic absorption spectrophotometry. The results were compared with those of 1630 healthy Iraqi volunteers from both sexes. The serum pseudouridines and PBT study: The same groups of patients and healthy persons in the serum TP-PBT study were involved in this study. The samples were processed at the laboratories of TTHK, NH, MRC, and the IAEC.

RESULTS

Table 1. The mean values, and standard deviation (SD), of TP found in the serum of the PBT patients and the control groups.

Serum	Patients Mean±SD (g/dl)	Healthy Subjects Mean±SD (g/dl)	P value
TP	9.802 ± 0.802 (All PBT patients)	6.350 ± 0.307	<0.01
Albumin	2.219 ± 0.530 (All PBT patients)	3.908 ± 0.724	<0.01

Table 2. Mean concentration of Pseudouridine in serum of PBT patients and normal subjects.

Fluid	Patients Mean±SD (n mol/ml)	Control subjects Mean±SD (n mol/ml)	P value
Serum	3.9023±0.69	2.19±0.38	<0.01

Table 3. TSC level in healthy controls.

Mean age in years ± SD	40.5 ±19.8
Mean TSC in mg / dl (mmol / l) ± SD	185.6 ± 24.9 (4.8 ± 0.6)

DISCUSSION

Study number I

A highly significant increase in TP levels in serum of PBT patients was noticed when compared to those normal subjects (Table 1). The result shows a statistical significance, p value < 0.01.

This increase could be explained on the basis that the whole body of cancer patients is engaged in protein synthesis of various forms like: C-reactive proteins, tumor markers, enzymes, and immunoglobulins and other proteinous material. Our results are in agreement with Fiandra et al ⁽⁵⁾ who found that patients with neoplasm had higher values of total protein

(P<0.01). In addition, patients with oral squamous cell carcinoma had also markedly increased total protein concentrations ^(6,7). Nagashima and Schreiber ⁽⁸⁾ grouped several plasma proteins as acute phase reactant (APR), which significantly rise during inflammation and neoplasm. Wicher ⁽⁹⁾ on the other hand, found that the increase in synthesis of APR is accompanied by a decrease in the synthesis of prealbumin, albumin and transferrin, which are so called negative APR. Inflammatory tissue lesions generally induce changes in the concentrations of various serum proteins. The APRs may be increased, albumin may be decreased and the immunoglobulin production may be enhanced.

Table 4. TSC in brain tumour group (primary and secondary). BS=brain secondaries.

Histological type	Age in years	TSC mg per dl (mmol / l)	Histological type	Age in years	TSC mg per dl (mmol / l)
Anaplastic astrocytoma	42	260 (6.7)	Cerebellar astrocytoma	40	185 (4.8)
	26	225 (5.8)		15	284 (7.3)
	29	275 (7.1)		31	204 (5.3)
	56	255 (6.6)		16	140 (3.6)
	40	285 (7.3)		16	175 (4.5)
	59	160 (4.1)		40	185 (4.8)
	70	195 (5)		16	195 (5)
	16	168 (4.3)	Medulloblastoma	16	180 (4.7)
Gliomas of corpus callosum	24	245 (6.3)		46	190 (4.9)
	60	275 (7.1)	Meningioma	75	235 (6.1)
	47	247 (6.4)	Craniopharyngioma	52	275 (7.1)
Cerebello-pontine angle schwannoma	19	235 (6.1)	B S of bronchogenic carcinoma	75	225 (5.8)
	55	260 (6.7)	B S of nasopharyngeal carcinoma	34	185 (4.8)
	70	205 (5.3)	B S of breast carcinoma	73	210 (5.4)
Choroids plexus papilloma	15	200 (5.2)	B S of gastric carcinoma	65	170 (4.4)
Total: Mean age in years \pm SD				41.3 \pm 20.9	
Total: Mean TSC in mg / dl (mmol / l) \pm SD				217.6 \pm 41.2 (5.6 \pm 1.1)	

Table 5. TEs measurements in sera of healthy volunteers and patients with PBT (mcg/ml).

TE	Serum values in healthy volunteers	Serum values in brain tumours patients	Student test (t)	Probability test (p value)
Se	0.085 – 0.190 (0.099)	0.010-0.066 (0.045 \pm 0.011)	- 24.050	< 0.01
Zn	0.550 – 1.500 (0.980)	0.140-0.560 (0.320 \pm 0.095)	- 33.984	< .01
Cu	0.600 – 1.850 (1.410)	0.360-0.930 (0.607 \pm 0.154)	- 25.545	< 0.01
Fe	0.700 – 1.200 (1.010)	0.180-1.800 (0.880 \pm 0.456)	- 1.397	> 0.05
Mg	15.000 – 22.000 (16.000)	5.500-20.000 (13.625 \pm 3.994)	- 2.913	< 0.01
Co	0.020 – 0.060 (0.050)	0.007-0.190 (0.020 \pm 0.036)	- 4.083	< 0.01
Ni	0.015 – 0.056(0.040)	0.005-0.160 (0.016 \pm 0.030)	- 3.919	< 0.01
Mn	0.020 – 0.040 (0.030)	0.010-0.050 (0.016 \pm 0.009)	- 6.025	< 0.01
Cr	0.020 – 0.075 (0.055)	0.008-0.030 (0.014 \pm 0.005)	- 39.192	< 0.01

The increase in total protein concentrations, in this study, indicated that synthesis of APR proteins had exceeded the synthesis of negative APR, this imbalance leads to a marked increase in TP levels. Reduced serum albumin concentration is a common finding in patients with neoplastic diseases. Hypoalbuminaemia can appear early in the course of the disease, and indeed it may occur despite normal nutrition and even without effusion, protein loss or evident clinical signs of liver damage ⁽¹⁰⁾.

The presence of hypoalbuminaemia has been widely confirmed in the malignant lymphomas ⁽¹⁰⁾, prostatic cancer ⁽¹¹⁾, melanoma ⁽¹²⁾, colorectal cancer ⁽¹³⁾ and leukemia ⁽¹⁴⁾. It is well established that the acute phase reaction, usually involved interleukins, tumor necrosis factor (TNF), and C-reactive proteins causing a reduction in the concentration of albumin, is associated with the risk and development of cancer ⁽¹⁵⁾.

However, it has been shown that TNF may increase the permeability of the microvasculature, thus allowing an increased trans-capillary passage of albumin ⁽¹⁶⁾ and hence a lowering of the serum albumin concentrations.

The present study, like other researchers' findings, demonstrated a significant decrease in albumin concentration in serum of patients with PBT (2.21 ± 0.53 g/dl) in comparison to normal group (3.90 ± 0.72 g/dl) with statistical significance ($p < 0.01$) as seen in table 1. Also, in this study, TP have been found to be more in tissues of malignant PBT than in the benign ones with a statistical significance, $p < 0.01$. Using spectrophotometry to estimate the activity of glutathione peroxidase, glutathione reductase and superoxide dismutase in the brain tumours, the statistical analysis of their study revealed significant increase of enzymes within the brain tumours in comparison to the healthy brain tissue. Therefore, we think, this may also contribute to the increase in local tumour tissue protein ⁽¹⁷⁾.

Saadoun *et al* ^(18,19) have found that aquaporin 1 and 4, conserved water channel proteins, are present with increased expression in microvascular endothelium and reactive astrocytes of astrocytomas and metastatic adenocarcinomas. Therefore, it is also

our opinion that these may also contribute to the increased proteins in brain tumours ^(18,19). Vascular endothelial growth factor (VEGF), a key regulatory protein in neoangiogenesis, is strongly expressed in a variety of PBT, particularly malignant gliomas. Their findings indicated that immunoreactive VEGF was produced at the tumour site and abundantly released into the cyst fluid of primary and metastatic brain tumours. Interestingly, this abundant local release was not reflected in serum VEGF levels, even in the case of very high VEGF concentrations in tumour cysts. Thus, VEGF may be biologically relevant for the formation of tumour cysts in brain tumours and correlates with local disease progression ⁽²⁰⁾.

Studying the measurements of many biochemical items, including proteins, as far as the possible role of blood-brain barrier disruption in cyst formation in craniopharyngioma, researchers have proved the hypothesis of blood-brain barrier impairment in such patients and that the pathogenesis of craniopharyngioma cyst appears to be much more akin to those described for cysts accompanying other brain tumours than it was believed earlier ⁽²¹⁾.

Study number II

In this study, a rapid, efficient and precise method for analysis of pseudouridine in serum of patients and healthy individuals was employed to evaluate the usefulness of serum and salivary pseudouridine as a diagnostic and prognostic biochemical marker for PBT patients. Numerous studies ⁽²²⁾ have documented the occurrence of increased levels of modified nucleosides in the biological fluids of cancer patients. Of these nucleosides, pseudouridine is the most frequently and most significantly elevated. Pseudouridine is produced as a result of degradation of tRNA, since it's not metabolized, nor incorporated in tRNA formation, consequently, elevated level of pseudouridine have been considered to reflect the rate of tRNA turnover ⁽²²⁾, so that an increase in pseudouridine concentration could be possibly useful as a mean of determining tumor response to therapy and a valuable marker for monitoring the course of patient during treatment. It has been found that the level of pseudouridine excretion drops down to normal

after commencement of chemotherapy and remains normal as long as the patient is in remission, this was found in Burkitt's lymphoma and T-acute lymphoma leukemia (23). This study has shown that pseudouridine was found to be significantly elevated in serum of PBT patients. The use of nucleosides (particularly pseudouridine) as tumors marker, primarily for monitoring the progression of tumors and their response to treatment, has been proposed; however, the biological basis of the phenomenon has not been clarified, nor has the specificity or prediction value for cancer diagnosis been studied extensively (23,25).

Study number III

Reduced blood cholesterol levels were reported in patients with a variety of malignant peripheral tumors (26,29). This fact is likely related to increased cholesterol demand by proliferating tumor cells (4), or to nutritional status (28), though others think that the abnormality is a common feature of both hematological and solid tumors and is not entirely explained by poor nutrition (30). The question arises whether this 'tumor-associated hypocholesterolaemia occurs also in patients with brain tumors, and, if it does not, whether its absence can be related to the location of the tumors. Grieb *et al* (26) have compared fasting serum total cholesterol levels among three groups of patients: 52 patients with gliomas, 56 patients with symptomatic metastatic brain tumors, and 50 patients harboring malignant tumors of peripheral location but showing no clinical signs of brain metastases. Patients in the last group, despite being on an average more age-advanced, had lower total serum cholesterol levels than either the patients with gliomas, or the patients with brain metastases. No difference in the cholesterol levels was found between the two latter groups, and a majority of these patients had borderline or elevated cholesterol levels. This apparent absence of 'tumor-associated hypocholesterolaemia in brain tumor patients may be related to either brain tumors' ability to synthesize cholesterol *de novo* and their reduced dependence on peripheral cholesterol supply, the existence of brain tumor-blood barrier, effect of medications used to counteract brain edema and seizures, or a combination of these factors (28).

In her study, Al-Azzawi (31) has found that the mean value of TSC in 314 Iraqi healthy males and females, 172.4 ± 29.5 mg per dl (28). Comparing these values to that shown in Table 3, the healthy control, in this study, have a marginally higher level than those in Al-Azzawi's study (31). This is probably due to a lower number of patients, higher age inclusion, and that this study was performed in colder weather (autumn, winter, and early spring seasons). Patients with brain tumours have higher overall mean (and few absolute values) of TSC than both the healthy controls and those harboring peripheral tumours (32,34). This shows clearly the absence of tumour-associated hypocholesterolaemia in brain tumor patients. This finding is consistent with Grieb *et al*'s study (26).

Many explanations can be introduced but need more sophisticated methods for confirmation; it may be attributed to brain tumour cells' ability to synthesize cholesterol *de novo* and their reduced dependence on peripheral cholesterol supply, also the existence of brain tumour-blood-brain barrier can contribute to this reduced dependence. However, interestingly, researchers have found an increased concentration of cholesterol esters up to 100 times (0.1-10 $\mu\text{mol/g}$) in both tumour-tissue and surrounding areas compared with control material ($< 0.1 \mu\text{mol/g}$); the analyses also demonstrated that cholesterol esters in tumour tissue emanated mainly from serum [25]. The cholesterol concentrations were significantly lower in tumour tissue compared with surrounding areas as expected. These results indicate that tumour cell proliferation utilizes serum derived cholesterol esters presumably carried by LDL particles (29).

We, also, agree with Laurence *et al* that we should take into account the drugs used in brain tumour patients that counteract brain oedema and seizures like diuretics used to reduce intracranial pressure (35). Some studies have shown an increased synthesis of cholesterol by tumour cells in leukaemic patients but these cells were cholesterol deficient; these findings raise the possibility that proliferating cells in malignant blood diseases display abnormal cholesterol metabolism and are depressed as regards the negative feedback regulation normally exerted by LDL-receptors which normally inhibit endogenous

synthesis of cholesterol. But what might happen in brain tumour is that the increased rate of endogenous synthesis of cholesterol by tumour cells with an increase in the LDL-receptor negative feedback mechanism which might be due to the presence of brain tumour-blood-brain barrier.

Changes in the content and composition of lipids in brain tumours of different degree of malignancy are still the subject of numerous scientific studies. It is known that in developing brain tumours structural and functional changes of its cells, take place, in which lipids play a crucial role. Statistical analysis showed significant decrease of phospholipids (elements stabilizing cell membranes) in tumours in comparison to their adjacent areas (Wilcoxon's test: $p < 0.05$). At the same time in tumours an increase of level of plasmalogens took place: phosphatidylcholine, phosphatidylethanolamine typical elements of malignant tumours, responsible for cross cell membrane transportation processes. Changes in the level of lipids, and phospholipids in particular, in glioblastoma in comparison to adjacent areas can indicate the pathological processes in cells of these tumours ⁽³⁶⁾.

Regarding the synthesis and regulation of neurosteroids in human brain, researchers have examined the ability of human brain cells to synthesize steroids from a radiolabeled precursor and the mRNA and protein expression of key components of peripheral steroidogenic machinery. Oligodendrocytes are the source of pregnenolone in human brain. Human astrocytes do not synthesize radiolabeled pregnenolone, nor do human neurons. These results indicate that human brain makes steroids in a cell-specific manner and suggest that dehydroepiandrosterone synthesis can be regulated by intracellular free radicals ⁽³⁷⁾.

An important feature of malignant transformation of tumours is the loss of cholesterol feedback inhibition mechanism (cholesterol-feedback lesion) that regulates mevalonate pathway recognized to play a crucial role in cellular growth, death and differentiation. Recently, it was shown that Receptor-C(k)-dependent signaling regulates genes involved in main-

taining cellular cholesterol homeostasis through a transcription factor sterol response element binding protein (SREBP) having affinity for sterol regulatory element (SRE) present in the promoter region of these genes. The present study revealed that CNS tumours exhibit overexpression of Receptor-C (k) gene product which was accompanied by their inability to express SREBP gene product and this phenomenon has the inherent capacity to initiate the cholesterol feedback lesion in these tumours. Based upon these and our earlier studies, we propose for the first time that this loss of cholesterol feedback control may be responsible for the initiation of these tumours ⁽³⁸⁾.

Study number IV

Associations between diet and central nervous system tumours (CNS) in humans remain hypothetical ⁽³⁹⁾. It is believed that the consumption, and endogenous production of N-nitroso compounds and their precursors might increase brain tumour risk ⁽⁴⁰⁾. On the other hand, the consumption of orange juice and vitamin supplements (which contain antioxidant substances such as ascorbic acid which inhibit endogenous nitrosation activity) has been associated with reduced risk of childhood CNS tumours ⁽⁴¹⁻⁴²⁾. Most dietary epidemiologic studies of CNS tumours, however, have used poor measures of intake and have been too small to detect significant risks. Burch et al suggested a protective effect of fruit but not vegetables ⁽⁴³⁾. Preston-Martin et al suggested that citrus fruits were protective of meningioma but the odd ratio was not statistically significant ⁽⁴⁴⁾. A prospective study of Seventh-Day Adventists showed discrepant and non-significant associations with dietary items ⁽⁴⁵⁾. A case-control study in Germany detected an increased glioma risk associated with the consumption of ham, processed pork and fried bacon, but no association with endogenous N-nitrosation, or with the intake of vitamin C, or fruit and vegetables ⁽⁴⁶⁾. In an Australian study, no increase in risk of glioma or meningioma was found with the regular consumption of food rich in N-nitroso precursors, nor were any decrease in risk associated with the regular use of foods and supplements containing endogenous nitrosation inhibitors ⁽⁴⁷⁾. Similarly, the results of a

case-control study of maternal diet during pregnancy and risk of astrocytoma, the most common childhood brain tumour, was conducted by the Children's Cancer Group, provided a limited support for the nitrosamine hypothesis and concluded that a future research should investigate the effect of dietary components not assessed in this study ⁽⁴⁸⁾.

The biochemical functions of TEs have not been as well characterized as those for the vitamins, but most of their functions appear to be as components of prosthetic groups or as cofactors for a number of enzymes. Determination of essential TEs status is problematic except for iron, selenium, and iodine. The vanishingly low concentrations of these elements in body fluids and tissues, the fact that blood levels frequently do not correlate well with levels in the target tissues, and the fact that functional tests cannot be devised until biochemical functions are better understood preclude an accurate and convenient laboratory method of assessment for most of the TEs ⁽⁴⁹⁾. However, estimation of TEs in serum, CSF, and brain tumour tissue in patients harboring brain tumours and experimental animal brain tumour models, whether by flame / flameless atomic absorption spectrophotometry or instrumental neutron activation analysis techniques have attracted many researchers ^(46, 51).

During 1984 –1990, El-Yazigi et al ^(49, 52) conducted four different studies estimating values of 20 TEs in CSF of control persons and patients with cerebral (both benign and malignant), non-cerebral tumours (e.g., leukaemia), and some neurological disorders. They found that most of the TEs were lower in patients with neoplasia, especially malignant tumours, than in the control group. However, silver and lead values were higher in the malignant brain tumour group, concentrations of arsenic in CSF of patients with non-brain malignant tumors were significantly (p less than 0.05) higher than in the controls, the ratio for mean CSF concentration of arsenic in patients with non-brain tumors/control patients was 2.9, and that differences in the concentrations of Fe, lithium, or molybdenum among the various groups were nonsignificant ^(53, 54). However, although we have measured the concentration of TEs in the CSF of

our patients, we have no values for the CSF among healthy volunteers or appropriate control group for comparison. Moreover, not all TEs in the El-Yasigi et al's study were assayed by us.

This study has shown that Se mean level, like others, is lower in the brain tumour patients than in the healthy volunteers (Table 5) ^(55, 56). This finding is in accord with that of other researchers ⁽⁵³⁾. Some of the latter could demonstrate the inhibitory effect of Se on rat glioma and activity of glutathione peroxidase after development of tumors was significantly higher in the high Se group at 18 and 30 days. In some studies, Se has exhibited an antiproliferative effect on human glioma cell lines (and induced the typical ladder pattern of DNA fragmentation commonly found in apoptosis), which were prevented by catalase. These findings demonstrate that selenium may induce, by apoptosis, cell death of human glioma cell lines, which are resulting from free radical oxygen forming ^(55, 56). However, others have even tried giving Se supplements to the diet of patients with brain tumours but without convincing useful result. Similar to Se, Zn has been found to be lower in the brain tumour group than the healthy people (Table 5). Zn has been found to selectively inhibit, *in vitro*, human glioblastoma cell multiplication but at the same concentrations do not inhibit astrocyte multiplication ^(57, 62).

Sciaudone et al ⁽⁶³⁾ have demonstrated that chelation of Zn amplifies induction of growth hormone mRNA levels in cultured rat pituitary tumour cells ⁽⁶³⁾. Circulating Zn levels have been found to be low in patients with pituitary tumours such as prolactinomas ⁽⁶⁴⁾. However, other researchers indicated that orally administered Zn to rat with experimental brain tumour had a cocarcinogenic (promotion) effect of Zn in neurooncogenesis of the rat ^(65, 66). Copper ion has been coupled with tumour angiogenesis both in human and animal model brain tumours; they found that Cu ions promote angiogenic phenomena and that Cu depletion could reduce brain tumour growth by angiosuppression ⁽⁶⁷⁾.

Microvascular proliferation, a hallmark of malignant brain tumors, represents an attractive target of anti-

cancer research, especially because of the quiescent nonproliferative endothelium of the normal brain. Cerebral neoplasms sequester copper, a trace metal that modulates angiogenesis. Using a rabbit brain tumor model, normocupremic animals developed large vascularized VX2 carcinomas. By contrast, small, circumscribed, relatively avascular tumors were found in the brains of rabbits copper-depleted by diet and penicillamine treatment. Metabolic and pharmacologic withdrawal of copper suppresses intracerebral tumor angiogenesis; angiosuppression is a novel biologic response modifier for the in situ control of tumor growth in the brain (68).

There was a very interesting finding by Kaiser et al: The copper content of astrocytomas and glioblastomas was investigated by the cuproin-method; the peritumoral tissue of glioblastomas contained more copper than the tumour tissue itself. In astrocytomas, on the other hand, more copper was detected in the central parts of the tumours (69). Brem *et al* and Yoshida *et al* (70,72) therefore, suggested manipulating pharmacological and metabolic cellular microenvironment represent a novel useful therapeutic approach in the treatment of brain cancer.

Many studies have investigated not only the levels of TEs, but also the role(s) of their dependent enzymes (73). Rao et al and others (74) have found a significant decrease in red blood cells (RBC) glutathione reductase (GRx) and superoxide dismutase (SOD) activity in most types of brain tumor cases. Patients with acoustic neurinoma showed a significant reduction in selenium-dependent glutathione peroxidase (Se-GPx) activity. A decrease in catalase activity was seen in most of the brain tumor patients but remained statistically insignificant when compared to controls. A significant increase in plasma ceruloplasmin concentration was observed in patients with glioma (75). These enzymes were also studied in 27 post-treatment cases. GRx activity returned to normal levels in these patients. RBC SOD and plasma ceruloplasmin levels showed a tendency to return to normal. Hence, a marked decrease in the antioxidant enzymes may have a role in the genesis of considerable oxidative stress in patients with brain tumors (69,70).

Some investigators have linked the levels of these TEs-dependent enzymes with the prognosis of some types of brain tumours. For example, Kurisaka et al using a monoclonal antibody against human copper- and zinc-SOD, have exhibited abundant SOD in tumors from patients with poor outcomes and little SOD in patients with good outcomes. Their results had suggested, they stated, that resistance to adjuvant therapy depends on the amount of SOD in tumor tissues, and since the effects of adjuvant therapies for medulloblastomas depend on the production of free oxygen radicals, so if tumor cells contained a free radical scavenger such as SOD, the effects of adjuvant therapy might be reduced. Measurement of SOD in tumor tissues they concluded, might be useful as a prognostic indicator for medulloblastoma (76).

CONCLUSIONS

A highly significant increase in TP levels in serum of PBT patients was noticed when compared to those normal subjects. The result shows a statistical significance, p value <0.01 . This study has also shown clearly the absence of tumour-associated hypocholesterolaemia in patients harboring brain tumour; on the contrary, there is elevation of TSC in the majority of patients although some are younger due, probably, to an increased synthesis of cholesterol by tumour cells themselves and/or to the drugs in the treatment of seizures and raised intracranial pressure as well as by other means as mentioned above. Therefore, we may conclude, although can be too early, that when the clinician is confronted with brain tumours in clinical practice and gets confused whether the intracranial lesion is a primary or metastatic tumour, an estimation of TSC level may help in dissolving the dilemma. Also, we recommend that more future work is needed to correlate this relation between TSC and brain tumour in the context of prognosis and the response to treatment, and in case any concomitant dietary modifications or pharmacological therapy to correct such biochemical abnormality will have any consequences on the outcome.

TEs can be used as biochemical parameters in the investigation of various disease processes including brain tumours, though the artiological association

between the latter and TEs need to be convincingly conclusive at a universal scale. They should be estimated in the common food stuff consumed by the Iraqis in order to establish the approximate intake to help directing health authorities to educate people regarding their daily allowances, intake and/or if any supplements are needed to be given to certain age groups such as growing children and pregnant mothers. Also, the normal values of TEs in various biological fluids of healthy people should be assayed and established. Future studies should be performed on a larger group of tumour victims an expanded to include the role of other TEs in this context. Though we have discussed only few TEs in the context of brain neoplasia, we think that investigations should be continued in this field to include many other TEs and their dependent enzyme system(s) in the aetiology, prognosis, and/or therapeutic approaches to brain neoplasia.

Many levels of pseudouridine in serum of PBT patients, were significantly higher ($p < 0.01$) than its levels in normal and they can be an extra tool in the investigation of PBT patients; particularly, the analysis by HPLC technique is a sensitive, accurate and specific one. However, both the specificity and sensitivity need to be ascertained. We think that in order to decide upon the “Specificity and Sensitivity” of the findings in relation to brain tumours, as well as in case there has been any deleterious effect on the body health, in any, we need to repeat the study upon a large group of patients with brain tumours compared to control “healthy” group of persons, randomly selected from the community, from both sexes, and of different age groups stretching over significant time period, i.e. for many years. However, the latter time period duration may have certain limitation, especially so in the case of patients with highly malignant PBT, e.g. glioblastoma multiforme who have relatively short survival after establishing the histological diagnostic nature of the disease, despite the modality of treatment adopted. However, we need:

1. The intention to do this.
2. Researcher (s).
3. Patients harboring PBT.

4. Other logistic support: laboratory services, materials and well trained personnel. And,
5. Finance.

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