Biochemical Changes in Serum of Benign and Malignant Brain Tumors Patients

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ABSTRACT

The present investigation includes study and determination of some vitamins (C, A and E), trace elements [iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg) and selenium (Se)] and electrolytes [sodium (Na^+), potassium (K^+) and chloride Cl^-)] in serum of benign and malignant brain tumor patients compared with control group.

Blood samples (93) from brain tumors patients, and (65) blood samples from healthy subjects as control group are collected for the present study.

The results obtained show a significant decrease in the concentration of vitamins (C, A and E) and in the trace elements (Zn, Se). On the other hand a significant increase in the concentration of (Fe, Cu, Na^+ and Cl^-) were observed in the serum of benign and malignant brain tumor patients compared with the control group.

التغييرات الكيموحيوية في مصل مرضى أورام الدماغ الحميدة والخبيثة

الملخص

يتضمن البحث الحالي دراسة وقياس بعض الفيتامينات (A, C و E) والعناصر النادرة [الحديد (Fe)، النحاس (Cu)، الخارصين (Zn)، المغنيسيوم (Mg) والسيلينيوم (Se)] والالكتروليتات [الصوديوم (Na^+)، البوتاسيوم (K^+) والكلوريد (Cl-)] في مصل دم المرضى المصابين بأورام الدماغ الحميدة والخبيثة ومقارنتها مع مجموعة السيطرة.

جمعت في الدراسة (93) عينة دم لمرضى أورام الدماغ و (65) عينة دم لمجموعة سيطرة أصحاء. تشير النتائج إلى وجود نقصان معنوي في تراكيز الفيتامينات (C، A و E) وفي العناصر النادرة (Zn و Se). يلاحظ من جهة أخرى أن هناك زيادة معنوية في تراكيز (Fe، Cu، Na^+ و Cl^-) في مصل دم مرضى أورام الدماغ الحميدة والخبيثة مقارنة مع مجموعة السيطرة.

INTRODUCTION

Cancer is a major health problem, not just in industrialized nations, but also in developing nations around the world, that cannot be ignored. It is like other chronic disease not only caused by a single factor, but is by multifactorial events (like genetic, environmental factors, chemical agents, radiation, reactive oxygen species "ROS"….etc.)

(WHO, 2003). Therefore, there is an increasing evidence suggesting that certain antioxidants compounds act as preventive or protective factors (Kirk, 1999).

Environmental factors play important roles in cancer development. Because some individuals within the same environment develop cancer and others do not, (Harrison's, 2001).

Living close to certain industries is a recognizable cancer risk factor. Although it is difficult to determine cancer risk from outdoor pollution alone because investigators must have accurate control of smoking and radon. Radon is a natural radioactive gas derived from the radioactive decay of uranium that is ubiquitous in rock and soil (WHO, 2003). Also, ulraviolet of sunlight causes increase in risk of cancers (Harrison's, 2001). Exposure to ultraviolet (UV.) radiation occur from both natural and artificial sources. Another risk factor to human is the exposure to ionizing radiation includes emissions from X-ray, radioisotopes, and other radioactive sources (Kumar and Clark, 2002).

A brain tumor is a mass of abnormal cells that is growing in or around the brain. They are generally named after the type of cell they developed from, benign and malignant terms are used to describe these tumors (Thomas and Graham, 1980).

Free radicals are continuously produced during aerobic metabolism. These unstable species may cause oxidative damage to DNA, carbohydrates, proteins and lipids that are normally counteracted by protective antioxidants (Bishop et al., 2005). Oxidative defense is provided by a number of enzymes and vitamins, including the chain-breaking scavengers vitamin E, vitamin C and glutathione (Halliwell, 1997). In times of increased free radical production, individuals may become deficient in these antioxidants. Incomplete scavenging of reactive radicals lead to oxidation of cellular lipids, proteins, nucleic acids and glyco-conjugated which result in fragmentation and cross-linking. This may ultimately lead to cell death with widespread pathological consequences (Baynes, 1991). The imbalance between protective antioxidants (antioxidant defence) and increased free radicals production, leading to oxidative damage, is known as oxidative stress (Halliwell, 1997). Molecular oxygen is a major source of reactive species. Reactive oxygen species (ROS) are thought to be implicated in the pathogenesis of various human diseases. They are generated endogenously under physiological and pathological conditions but also upon exposure to exogenous challenges. The organism maintains defense system against ROS, including enzyme and low molecular weight antioxidants. These antioxidants act together as an integral system. (Stahl and Sies, 1997).

The aim of this study is to determine some antioxidants, like vitamins, trace elements in serum of brain tumor patients and comparing it with healthy control group.

Materials and Methods

Patients and Control Subjects

Patients were enrolled in the present study to the neurosurgery unit in Ibn-Sina Hospital in Nineveh Governorate. Samples of (93) patients diagnosed clinically and radiologically as having brain tumor were included in this study (47 males and 46 females) ranging in their age between (15-70) years, were collected during the period May 2004 to July 2005.

Depending on histopathological examination, (41) patients were diagnosed as having malignant brain tumor, while (52) patients were diagnosed as benign brain tumor.

Sixty-five age matched healthy individuals were included (45, males and 20 females) in this study as a control group.

Samples

Ten milliliters of venous blood was taken from each patient before the operation and left for (15) minutes at a room temperature for coagulation, serum then were separated by centrifugation at (3000 Xg) for (10) minutes. Serum was divided in aliquot and kept frozen at (-20 °C) for the biochemical tests (Tietz, 1999).

Methods

Vitamin C was determined photometrically with 2,4-dinitro phenylhydrazine (DNPH) (Colowick and Kaplan, 1979).

The chemical methods for determination of vitamin E are based on an oxidation reduction reaction. Following specific elution techniques, fractions were commonly subjected to the Emmerie-Engel procedure (Harold et al., 1976).

The optical density differences between irradiated and non-irradiated serum extracts can therefore be used to measure the vitamin A content (Wootton, 1974).

Iron was assayed using colorimetric method by kit of Syrbio (Ceriotle, 1980).

Copper and zinc were assayed using atomic absorption spectrophotometry technique (Tietz, 1999).

Magnesium was determined by colorimetric method using manufactured kit by Syrbio (Pesce and Kaplan, 1987).

Selenium was determined using the method based on (Cummins et al., 1965).

Sodium and potassium were determined using flame emission spectrometry (Bishop et al., 2005).

Chloride was determined by colorimetric method (Snell, 1981).

Results and Discussion

The results in table (1) showed that there was a significant decrease ($P<0.001$) in vitamin C concentration in serum of patients with benign and malignant brain tumors, compared with control group. The percent of decrement was about (49% and 52%) respectively. Neither significant differences had been found on the levels of vitamin C in serum of benign and malignant brain tumors, nor between males and females cases, table (1).

The decrease in vitamin C level in benign and malignant cases was higher than that in control which might be due to the free radical formed in the body fluids patients with cancer. The detoxified by antioxidant including vitamin C, that is the consumption of vitamin C will be higher which leads to the decrease of its concentration in the blood (Yochum et al., 2000). In addition of the fact that more ascorbic acid is used to regenerate vitamin E in membrane in these cases (Halliwell and Gutteridge, 1991).

Serum vitamin A level showed a significant decrease ($P<0.001$) in patients with benign and malignant brain tumors, in comparison with control groups. The percent decrement in total patients was about (62% and 58%) in benign and malignant tumors respectively, as shown in table (1). Within the patient groups there was no significantly change in the serum of benign and malignant. Also it was found that there was no significant effect between males and females with brain tumors groups.

Table 1: Vitamins concentration in blood serum of patients with benign and malignant brain tumors

Vitamins	Sex	Mean ± SE		
		Control	Benign tumors	Malignant tumors
Vitamin C (μmol/L)	Males	27.68 ± 1.29	14.68±1.09***	13.92±1.08***
	Females	30.09 ± 0.84	14.45±1.22***	13.62±1.30***
	Total	28.56 ± 0.88	14.55±0.82***	13.78±0.82***
Vitamin A (μmol/L)	Males	1.62 ± 0.045	0.46±0.046***	0.64±0.080***
	Females	1.57 ± 0.083	0.65±0.070***	0.73±0.13***
	Total	1.60 ± 0.040	0.61±0.059***	0.68±0.071***
Vitamin E (μmol/L)	Males	16.63 ± 0.97	7.82±1.43***	8.11±1.33***
	Females	15.83 ± 1.31	5.37±1.04***	6.41±1.40***
	Total	16.38 ± 0.79	6.68±0.92***	7.26±0.96***

*** Significant difference between control at ($p \leq 0.001$)

The decrease in vitamin A concentration is due to that retinol and it analogues act as inhibitors of superoxide radical production in polymorphonuclear leukocytes (Sthelin et al., 1991).

The increased risk of cancer in vitamin A deficiency is thought to be the result of a depletion in β-carotin. This compound is a very effective antioxidant and is suspected to reduce the risk of cancers known to be initiated by production of free radicals (Sthelin et al., 1991). Of particular interest is the potential benefit of increased β-carotene intake to reduce the risk of lung cancer (Rao et al., 2003).

Table (1) showed that there was a significant decrease ($P<0.001$) in vitamin E concentration in serum of benign and malignant groups in comparison with control. The percent decrement was about (59% and 56%) in benign and malignant tumors respectively. No significant differences had been shown on the vitamin E level in serum of benign and malignant brain tumor patients. Also, it was found that there was no significantly effect of sex on the vitamin E concentration in serum of benign and malignant tumor patients.

The low levels of vitamin E concentrations might facilitate oxidative damage in patients with brain tumor. The major site of vitamin E is to act as a natural antioxidant by scavenging free radicals and molecular oxygen (Maureen, 2004). It has other roles, unrelated to antioxidant activity, including the maintenance of cell membrane structure and effects on DNA synthesis and cell signaling (Kasai, 1997).

Furthermore, vitamin E plays a crucial role in the maintenance of the immune system (Halliwell, 1997). The immune function is linked to the release of O_2 radicals that participate in macrophages. Thus the immune system has been shown to be more sensitive than other systems to antioxidant deficiencies in the diet (Delafuente et al., 2000).

The results in table (2) revealed that it was significantly increased (P<0.001) in iron concentration of patients with malignant tumors. This increase represents a (40%) higher in the main value of patients when compared with control. Table (2) also showed that there was no significant increase in iron concentration of benign tumor when compared with control. Neither significantly difference had been found on the iron concentration in serum between benign and malignant brain tumor patients, nor between sex group.

Iron overload might be caused by an increase absorption of dietary iron, parenteral administration of iron or both. Whatever the source of excess iron, when the accumulation overwhelms the body's capacity for safe storage, tissue damage is the result. Excess iron, as with excess copper, can cause free radicals production and damage (Cox, 1995).

Table 2: Trace elements concentration in blood serum of patients with benign and malignant brain tumors

Elements	Sex	Mean ± SE		
		Control	Benign Tumors	Malignant Tumors
Fe µmol/L	Males	16.64 ± 3.37	19.69 ± 0.12	24.28 ± 0.19***
	Females	16.82 ± 3.89	18.96 ± 9.11	22.25 ± 0.15***
	Total	16.64 ± 2.61	19.33 ± 7.21	23.27 ± 0.12***
Cu µmol/L	Males	15.11 ± 4.13	19.50 ± 7.54**	19.40 ± 6.59**
	Females	15.83 ± 5.70	23.57 ± 7.12***	25.85 ± 6.00***
	Total	15.33 ± 4.63	21.63 ± 7.52***	22.52 ± 7.02***
Zn µmol/L	Males	19.82 ± 6.20	12.91 ± 2.81***	14.32 ± 3.22***
	Females	20.40 ± 5.74	11.43 ± 4.25***	13.32 ± 2.89***
	Total	20.00 ± 6.02	12.13 ± 3.67***	13.84 ± 3.06***
Mg mmol/L	Males	0.83 ± 0.02	0.91 ± 0.05	0.85 ± 0.05
	Females	0.81 ± 0.05	0.76 ± 0.03	0.81 ± 0.05
	Total	0.83 ± 0.02	0.83 ± 0.03	0.83 ± 0.04
Se µmol/L	Males	0.92 ± 0.03	0.58 ± 0.09***	0.39 ± 0.04***
	Females	0.96 ± 0.05	0.57 ± 0.06***	0.37 ± 0.08***
	Total	0.93 ± 0.02	0.57 ± 0.05***	0.38 ± 0.04***

*** Significant difference between control at (p≤0.001)
** Significant difference between control at (p≤0.01)

Statistical analysis showed a significant difference (P<0.001) in copper concentration between benign and malignant patients when compared with control. No significant differences noticed between sex group, table (2).

Copper plays important role as a component of enzymes or proteins involved in redox reactions, such as ceruloplasmin, superoxide dismutase (SOD), dopamine β-hydroxylase, ascorbate oxidase, tyrosinase and cytochrome C oxidase (Cox, 1995). Many studies attributed the redox activity of copper to its electronic configuration of d orbital which helped it to scavenge the free radical as Cu-Zn-SOD reaction. On the other hand,

copper has an oxidant property by generating free radical such as $^{\bullet}OH$, $O_2^{\bullet-}$ (Prohaska, 1988).

Other workers had suggested a carcinogenic role for copper. They stated that trace levels of copper dramatically enhanced the biologic damage caused by superoxide radicals. These radicals reduce copper complexes to the coprous state. Then, the reduced complexes react with hydrogen peroxide to form hydroxyl radicals that damage proteins, RNA and DNA initiating a malignant process (Margalioth et al., 1983).

In the present research, the results in table (2) showed that there was a significant decrease ($P<0.001$) in zinc concentration, in serum of benign and malignant cases in comparison with control. The percent decrement was about (39% and 31%) in benign and malignant tumor respectively. It has also been found that there was neither significant differences between benign and malignant tumors groups, nor sex groups.

The reduction in concentration of zinc in biomembranes underlies some of the disorders associated with zinc deficiency with a loss of zinc from the membrane resulting in an increase susceptibility to oxidative damage, structural strains and alterations in specific receptor sites and transport systems (Margalioth et al., 1983).

Zinc might exert its antioxidant effect by decreasing the susceptibility of essential sulfhydryl group of proteins to oxidation and by competing with pro-oxidant metals, such as iron and copper for biological binding sites (Aldor et al., 1982).

Table (2) showed the results of magnesium concentration which indicated that there was no significantly difference of magnesium concentration in benign and malignant brain tumors when compared with control group.

The level of selenium determined in serum of patients with brain tumors. It was found a significant decrease ($P<0.001$), when compared with control group. The percent decrement was about (39% and 59%) respectively. No significant differences had been found on the selenium level in serum of benign and malignant tumor patients, also no significant differences between males and females with brain tumors were found, as shown in table (2).

There are a number of hypotheses that have been postulated to account for the experimental data that selenium reduced cancer. They are: (1) selenium's enzyme, (2) selenium's enhancement of immunity, (3) selenium's effect on the metabolism of carcinogens, (4) selenium's interactions that affect protein synthesis and cycle of cell division, and (5) the formation of anti-cancer selenium metabolites (Garry and Larry, 2003).

Other studies showed low selenium concentration and attributed their finding to the acting of selenium as antioxidant by binding with vitamin E and as a constituent of GSH peroxidase to scavenge free radical to detoxify tissue peroxidation (Al-Niami et al., 2001).

Sodium, potassium and chloride were determined in serum of patients with benign and malignant brain tumors. Table (3) shows that there was a significant increase ($P<0.001$) in serum sodium concentration in all patients with benign and malignant tumors when compared with control group.

Potassium concentration obtained a non-significant difference between patients and control, table (3). On the other hand a significant increase ($P<0.05$) of chloride concentration was observed on serum of malignant brain tumor patients when compared with control group, table (3). While the concentration of chloride in benign tumor gave a

non-significant increase when compared with control. The results also showed that there was no significant difference in the concentration of electrolytes between benign and malignant tumor patients and no significant differences within the sex of patients with brain tumors.

The patients with different types of cancer are usually presented with prolonged vomiting due to the increase in the intracranial pressure. The vomiting will lead to dehydration and hypernatremia (increased serum sodium concentration) results from excess loss of water relative to sodium loss, decreased water intake or retention or increased sodium and chloride intake due to an excessive intravenous infusion (Laker, 1996).

These symptoms are commonly attributed either to the cancer treatment or the cancer itself. Other causes include tumor lysis syndrome when cancer cells are killed by therapy, they may spill their inner (intracellular) contents, which accumulate in the body faster than can be eliminated. These excess intracellular contents cause the metabolic and electrolyte disturbances that result in tumor lysis syndrome (Harry et al., 1996).

Table 3: The electrolytes concentration in blood serum of patient with benign and malignant brain tumors

Electrolytes	Sex	Mean ± SE		
		Control	Benign tumors	Malignant tumors
Na^+ (mmol/L)	Males	135.48±0.92	143.18±2.25***	152.05±3.08***
	Females	135.50±2.03	143.06±2.76***	147.09±2.93***
	Total	135.49±0.88	143.13±1.85***	149.13±2.19***
K^+ (mmol/L)	Males	4.02±0.057	4.02±0.084	4.12±0.14
	Females	3.09±0.10	4.00±0.10	3.95±0.081
	Total	3.98±0.051	4.01±0.066	4.10±0.087
Cl^- (mmol/L)	Males	99.98±2.44	100.69±7.22	115.18±5.72*
	Females	87.87±2.93	91.38±6.13	96.74±4.42*
	Total	96.25±2.03	98.62±4.10	103.18±4.15*

*** Significant difference between control at ($p \leq 0.001$)

* Significant difference between control at ($p \leq 0.05$)

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