

Primary Tumours and Few Vitamins

Abdul Wahab R. Hamad¹, Walid W. H. Al-Rawi², Nuha Auwaed Mashaly Al-Kenany³,
Raad K. Muslih³

¹ Department of Chemistry and Biochemistry and Medical Research Center, College of Medicine, Al-Nahrain University.

² Teaching Hospital at Kadhimiyah, College of Medicine, Al-Nahrain University

³ Department of Chemistry, Al-Mustansiriyah University

Abstract

The requirements and / or the availability of various nutritional factors for the living cell(s) can be quite variable under both physiological and pathological situations. Vitamins are established components of the spectrum of necessary constituents of food for the living tissues and many of them have proved a protective role in cancer etiology.

Design and aims of study: This is a prospective study to investigate the levels of few vitamins in sera, saliva, cerebrospinal fluid (CSF), and tumour tissues in the context of primary brain tumours (PBT), both benign and malignant, among a group of Iraqi patients.

Setting: The Teaching Hospital at Kadhimiyah (THK) and Neurological Hospital (NH) in Baghdad.

Patients and methods: This study had been conducted between November 2000 and October 2001 at both THK and NH in Baghdad. Out of the 107 patients suffering from PBT with an age range 2-75 years (mean 35, the SD = 19), 56 were males (52.3%), and 51 were females (47.6%). The most affected age group was 31-40 years (17.75%), 89% of the patients were under the age of 60 years (table 1).

There were 77 gliomas (both benign and malignant) and 32 meningiomas (benign).

A group of 50 patients with congenital hydrocephalus were involved in the study for CSF sampling. Forty age- and sex-matched normal subjects were used as controls in serum and saliva measurements. High performance liquid chromatography (HPLC) was used in the study.

Results: All 3 vitamins, A, C, and E, have shown lower values in malignant tumours patients (in serum, saliva, CSF, and tumour tissues) compared to those with benign tumours and controls in the biological fluids and tissues that were assayed with statistical significance ($p < 0.1$).

Conclusions and recommendations: These changes are quite significant, the findings should be considered for further research to determine both the specificity and sensitivity of low vitamins levels in the possible aetiological association with PBT.

Introduction

Vitamins have proved and remained essential dietary components of the balanced diet that is needed for a healthy growth of the living organism. They have varieties of mechanisms of actions in the living tissues. Vitamin deficiency syndromes are well established and evidence-based clinical entities. Vitamins are needed in many physiological and pathological situations to meet the requirements of the living cells. Moreover, there are certain situations where the daily requirements become more than during ordinary times, such as that occurs during pregnancy. The figures that represent the daily allowances for the vitamin intake have been well established in the literature.

Classical and new therapies in anaplastic astrocytomas and glioblastomas do not yield sufficient results. Agents able to redifferentiate neoplastic cells *in vitro* are known. Patients with glioblastomas and anaplastic astrocytomas were enrolled in a phase II trial involving surgery or biopsy, radiotherapy (64 Gy), chemotherapy. Alfacalcidol, a vitamin D analogue able to bind to nuclear receptors regulating mitotic activity, has been used in the treatment of malignant gliomas as an *in vitro* agent of redifferentiation; it is safe and

seems able to induce in some patients, in synergy with classical surgery-radiotherapy-chemotherapy treatments, a particular progressive and durable regression of the tumor. The responders might represent about 20% of malignant gliomas⁽¹⁾.

A protective effect among glioma patients relating to frequency of use of vitamin C and other vitamin supplements was reported⁽²⁾.

Similarly, studies have been demonstrated that vitamin C (ascorbic acid) exhibit a protective role in certain types of cancer (in glia tumor cells); Vitamin C inhibited DNA adduct formation and arylamine N-acetyltransferase activity and gene expression in rat glial tumor cells⁽³⁾.

The aim of the study is to investigate the levels of few vitamins, A, C, and E, in the context of PBT (benign astrocytoma, malignant, or anaplastic, astrocytomas, and meningiomas) among a group of Iraqi patients (107 persons), in their sera, saliva, brain tumour tissues, and CSF and to compare the CSF figures with that of a cohort of 50 hydrocephalic patients and sera of another cohort of 40 healthy volunteers.

Patients and methods

This study had been conducted between November 2000 and October 2001 at both THK and NH in Baghdad. Patients were evaluated by full medical history to exclude any existing systemic disease that may affect the parameters to be diagnosed, particularly diabetes, liver disease, renal disease and chronic drug intake, other wise the patient was excluded from the study.

Out of the 107 patients suffering from PBT with an age range 2-75 years (mean 35, the SD + 19), 56 were males (52.39%), and 51 were females (47.6%). The most affected age group was 31-40 years (17.75%), 89% of the patients were under the age of 60 years.

A group of 50 patients with congenital hydrocephalus were involved in the study for CSF sampling. Forty age- and sex matched normal subjects were used as controls in serum and saliva measurements.

Duration of the disease

The duration of the disease ranged from <1- >9 years. The majority of the patients (57.94%) presented within less than 1 year from onset of symptoms.

Chemical and Reagents

All Chemical and standard solutions used in this work, were the highest analytical grade obtained from commercial source, and used without further purification. All volumetric glassware were cleaned in a solution of 5 N HCl, for at least 24 hrs, then washed repeatedly in deionized water prior to use.

Vitamins (A and E) standard were obtained from Supelco Park, Bellefont USA.

Sample collection and preparation

Serum

A bout 5 ml's venous blood was drawn aseptically into sterile test tube with silicon coated, by utilizing disposable needle and plastic syringes. The blood was allowed to clot (10 minutes), centrifuged at 4000 rpm for 15 minutes. Serum sample were immediately transferred into four tube and frozen at (-20°C) for subsequent analysis; haemolyzed samples were discarded.

One milliliter of venous blood, after clotting, was centrifuged at 600 rpm for 10 min, serum was diluted with 0.2 ml (0.2 M) sodium potassium phosphate buffer (pH8.4) and centrifuged for 20 min, at 1000 rpm thoroughly to remove protein. The filtrate was kept frozen at (-20°C) until analyzed. (20-100 ml) aliquot of the filtrate was used for HPLC analysis.

Assays were done at the laboratories of The Medical Research Centre (MRC), College of Medicine, Al-Nahrain University and The Iraqi Atomic Energy Commission (IAEC).

Saliva

Un-stimulated whole saliva was collected after the patient have rinsed his mouth several times with deionized water, then the accumulated saliva in the floor of the mouth was drawn by a plastic disposable pipette, collection time was always between 8-9 a.m.

The collected saliva was cold centrifuged at 2500 rpm for 10 minutes at 5°C; this was done within one hour after collection to eliminate debris and cellular matter. The centrifuged supernatants were divided into 5 equal parts.

All sample were stored frozen at (-20°C) in polyethylene tubes till assayed.

Tumor tissue

Tumor tissue was taken from the lesion on the day of surgery, which immediately transferred, for mincing and homogenization. An equal volume of Triton X-100 buffer (sodium - phosphate buffer) is added to the minced tissue, and then cold centrifugation was performed at 4000 rpm for 30 minutes at 4°C.

The centrifugal supernatant was aspirated and divided into 5 equal parts. All samples were stored frozen at (-20°C) in polyethylene tube until assayed.

CSF

The 107 patient included in this study had fasted for 8 to 12 hrs, before surgery. A CSF specimen (3 to 4 ml) was collected in a plastic specimen container from each patient at the time of operation through a ventricular catheter.

CSF samples were collected via a ventricular catheter that was used in treatment. The CSF specimens were collected in plastic containers, promptly frozen, and stored at (-20 °C) until analysis.

Assays were done within one week to one month of collection at the laboratories of NH and IAEC.

Vitamins (A, C and E)

Chromatographic conditions used were established by Abid et al¹⁰. The HPLC system used was LC 6A liquid chromatography, equipped with UV-visible detector model SPD-6AV operating at 210 nm for water soluble vitamin (vitamin C) and 295 nm for fat soluble vitamins (A and E).

A Rheodyne 7125 valve injector with 100 µl injection loop was used. SCL-6A system controller controlled the solvent delivery system. The resulting retention time and peak area were displayed and processed on chromatopak C-18 ODS (250 × 4.6 mm I.d.), 5µm particle size, and propylamine column (250 × 4.6 mm I.d.), 5µm particle size, were used throughout this work. The column temperature was maintained at 40 °C using column's oven model CTO-6A. Acetonitril,

methanol, sodium octane sulfonate, hexane and ethyl acetate were used as mobile phase.

The buffer sodium dihydrogen phosphate were prepared in deionized water and adjusted to pH 2.1 with sodium hydroxide. Flushing the elements with stream of helium for 10 minutes degassed all elements.

Vitamin C (ascorbic acid), 0.5mg was dried at 80 °C for 2 hours then cooled and stored over phosphorous pentoxide for 24 hrs. The weight required to prepare 50 ppm of each vitamin were dissolved in 200 ml deionized water containing 60% methanol. Diluted hydrochloric acid (0.1N) was added through the volume to 500 ml with deionized water. Standards of vitamins (A and E) were prepared as mentioned above.

Results

The current investigation provides data of the levels of vitamins A, C, and E in serum, saliva, tumor tissue and CSF of primary brain tumor patients and normal subjects. These are well shown in tables 1, 2, 3, and 4 respectively.

Table (1): Mean concentration of vitamins in serum of PBT patients and normal subjects.

Vitamin	All Patients'	Normal	P value
	sera mean + SD (mg/ml)	serum mean ± SD (mg/ml)	
A	0.4436±0.2119	2.1±0.751	<0.01
C	3.085±1.422	5.66±0.691	<0.01
E	3.2166±1.363	4.32±0.465	<0.01

Mean concentrations of same vitamins in saliva of primary brain tumor patients and normal subject are shown in table 2.

Mean concentrations of vitamins A, C, and E in malignant and benign tissue of primary brain tumor patients are shown in table 3.

Table 4 shows the mean concentration of the vitamins in CSF of PBT patients (both malignant and benign tumors)

Table (2): Mean concentration of vitamins in saliva of PBT patients and normal subjects.

Vitamin	All Patients'	Normal	P value
	saliva mean ± SD (mg/ml)	saliva mean ± SD (mg/ml)	
A	0.055±0.002	0.227±0.014	<0.01
C	0.584±0.226	1.602±0.751	<0.01
E	0.396±0.142	0.543±0.3	<0.01

Table (3): Mean concentration of vitamins in malignant and benign tissues of PBT patients.

Vitamin	Malignant PBT	Benign PBT	P value
	tissue mean ± SD (mg/ml)	tissue mean ± SD (mg/ml)	
A	0.667±0.191	0.747±0.17	<0.01
C	1.742±0.674	3.340±1.195	<0.01
E	1.585±0.445	3.081±0.725	<0.01

Table (4): Mean concentration of vitamins in CSF of PBT patients malignant and benign.

Vitamin	CSF of patients with malignant PBT	CSF of patients with benign PBT	P value
	mean ± SD (mg/ml)	mean ± SD (mg/ml)	
A	0.03±0.007	0.05±0.05	<0.01
C	0.52±0.035	1.32±0.38	<0.01
E	1.57±0.175	2.85±1.06	<0.01

Vitamin A

Serum vitamin A levels of PBT patients (0.44 ± 0.75 mg/ml) are lower than their levels in normal serum (2.1 ± 0.75 mg/ml), statistical analysis showed that this decrease is significant ($p < 0.01$) (Table 1).

A highly significant decrease in salivary vitamin A levels of PBT patients was observed in comparison to that of normal subjects as noticed in table 2.

A significant decrease in vitamin A concentration was observed when malignant tumor tissue was compared with benign tissue (Table 3).

Vitamin A levels were significantly lower in CSF of malignant PBT patients when compared with that of benign ones. The difference was significant ($p < 0.01$) as demonstrated in table 4.

Vitamin C

Serum vitamin C concentrations of PBT patients are lower than their levels in normal subject (Table 1).

Table 2 shows a significant decrease in vitamin C level in saliva of PBT patients compared to that of normal subjects.

Vitamin C levels were significantly lower in malignant tissue (1.74 ± 0.67 mg/ml) of PBT patients when compared with that of benign tissue (3.34 ± 1.195 mg/ml) as demonstrated in table 3. Table 4 demonstrates that there was a significant decrease in vitamin C level in CSF of patients harboring malignant PBT.

Vitamin E

Table 1 demonstrates that there was a significant decrease in vitamin E serum levels of patients, when compared to its level in normal subject.

A highly significant decrease in salivary vitamin E was noticed in PBT patients in comparison to that of normal subjects (Table 2).

A highly significant decrease ($p < 0.01$) of vitamin B levels in malignant tissue was observed in comparison to its level in benign tissue (0.58 ± 0.44 mg/ml respectively) (Table 3).

The decrease in malignant CSF content of vitamin E was highly significant in comparison to benign CSF as observed in table 4.

Discussion

A highly significant decrease in vitamins levels (A, C, E) was observed in serum, saliva, malignant tissue and malignant CSF of PBT, as shown in the relevant tables.

These findings are considered to be reasonable due to the inverse association between vitamins and cancer risks⁽⁵⁾. It is believed that these vitamins function as antioxidants and act as scavengers of free radicals, either independently or as part of large enzyme systems⁽⁶⁾, vitamins (A, C, and E) have been postulated to play a protective role against bladder cancer⁽⁷⁾. Vitamin E is concentrated in the lipid regions that are exposed to the highest partial pressure of oxygen, such as cells lining the outer surface of the lung and red blood cell membranes. Beta-carotene is located in the membranes and organelles that are exposed to the lowest partial pressure of oxygen, but its action may not be restricted to such location, as seen by its possible protection against lung cancer⁽⁸⁾. Vitamin C is located in the water-soluble component of the body, vitamin C is believed to be the first line of defence⁽⁹⁻¹⁰⁾ and appears to have a role in sparing or reconstituting the active forms of vitamin E and carotenoids.

The decrease in vitamin A (retinal) levels, are characteristic of acute phase responses to infection or trauma has been known for decades⁽¹¹⁻¹²⁾. Mechanisms that have been suggested are losses of holo-retinal binding protein (holo-RBP) in the urine⁽¹³⁻¹⁴⁾, decreased release of RBP from the liver⁽¹⁵⁾ and loss of holo-RBP into the extra cellular fluid due to increased vascular permeability⁽¹⁶⁾. However, all of these mechanisms for lowering retinal during acute phase responses postulate losses of retinal bound to RBP and ignore evidence that the molar decrease in retinal during infection or trauma frequently seen to be greater than the decreases of RBP⁽¹⁷⁻¹⁸⁾. Results from laboratory studies suggest that vitamin A may exhibit anti-carcinogenic activities that may reduce the risk of cancer, particularly, cancer of lung⁽¹⁹⁻²⁰⁾ and stomach⁽²¹⁾. It has also been associated with a decreased risk of

prostate cancer⁽²²⁾. However, reviews of studies investigating cancer outcome, indicate less consistent findings.

Conclusions and recommendations

These changes are quite significant, the findings should be considered in further research to determine both the specificity and sensitivity of low vitamins levels in the possible aetiological association with PBT.

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الخلاصة

خلفية البحث: تشكل البيتا كاروتين أحد مكونات لطيف الغذائي المهمة و A، B، C، E، H، K، L، M، N، O، P، Q، R، S، T، U، V، W، X، Y، Z، AA، AB، AC، AD، AE، AF، AG، AH، AI، AJ، AK، AL، AM، AN، AO، AP، AQ، AR، AS، AT، AU، AV، AW، AX، AY، AZ، BA، BB، BC، BD، BE، BF، BG، BH، BI، BJ، BK، BL، BM، BN، BO، BP، BQ، BR، BS، BT، BU، BV، BW، BX، BY، BZ، CA، CB، CC، CD، CE، CF، CG، CH، CI، CJ، CK، CL، CM، CN، CO، CP، CQ، CR، CS، CT، CU، CV، CW، CX، CY، CZ، DA، DB، DC، DD، DE، DF، DG، DH، DI، DJ، DK، DL، DM، DN، DO، DP، DQ، DR، DS، DT، DU، DV، DW، DX، DY، DZ، EA، EB، EC، ED، EE، EF، EG، EH، EI، EJ، EK، EL، EM، EN، EO، EP، EQ، ER، ES، ET، EU، EV، EW، EX، EY، EZ، FA، FB، FC، FD، FE، FF، FG، FH، FI، FJ، FK، FL، FM، FN، FO، FP، FQ، FR، FS، FT، FU، FV، FW، FX، FY، FZ، GA، GB، GC، GD، GE، GF، GG، GH، GI، GJ، GK، GL، GM، GN، GO، GP، GQ، GR، GS، GT، GU، GV، GW، GX، GY، GZ، HA، HB، HC، HD، HE، HF، HG، HH، HI، HJ، HK، HL، HM، HN، HO، HP، HQ، HR، HS، HT، HU، HV، HW، HX، HY، HZ، IA، IB، IC، ID، IE، IF، IG، IH، II، IJ، IK، IL، IM، IN، IO، IP، IQ، IR، IS، IT، IU، IV، IW، IX، IY، IZ، JA، JB، JC، JD، JE، JF، JG، JH، JI، JJ، JK، JL، JM، JN، JO، JP، JQ، JR، JS، JT، JU، JV، JW، JX، JY، JZ، KA، KB، KC، KD، KE، KF، KG، KH، KI، KJ، KK، KL، KM، KN، KO، KP، KQ، KR، KS، KT، KU، KV، KW، KX، KY، KZ، LA، LB، LC، LD، LE، LF، LG، LH، LI، LJ، LK، LL، LM، LN، LO، LP، LQ، LR، LS، LT، LU، LV، LW، LX، LY، LZ، MA، MB، MC، MD، ME، MF، MG، MH، MI، MJ، MK، ML، MM، MN، MO، MP، MQ، MR، MS، MT، MU، MV، MW، MX، MY، MZ، NA، NB، NC، ND، NE، NF، NG، NH، NI، NJ، NK، NL، NM، NN، NO، NP، NQ، NR، NS، NT، NU، NV، NW، NX، NY، NZ، OA، OB، OC، OD، OE، OF، OG، OH، OI، OJ، OK، OL، OM، ON، OO، OP، OQ، OR، OS، OT، OU， OV، OW، OX، OY، OZ، PA، PB، PC، PD، PE، PF، PG، PH، PI، PJ، PK، PL، PM، PN، PO، PP، PQ، PR، PS، PT، PU، PV، PW، PX، PY، PZ، QA، QB، QC، QD، QE، QF، QG، QH، QI، QJ، QK، QL، QM، QN، QO، QP، QQ، QR، QS، QT، QU، QV، QW، QX، QY، QZ، RA، RB، RC، RD، RE، RF، RG، RH، RI، RJ، RK، RL، RM، RN، RO، RP، RQ، RR، RS، RT، RU، RV، RW، RX، RY، RZ، SA، SB، SC، SD، SE، SF، SG، SH، SI، SJ، SK، SL، SM، SN، SO، SP، SQ، SR، SS، ST، SU، SV، SW، SX، SY، SZ، TA، TB، TC، TD، TE، TF، TG، TH، TI، TJ، TK، TL، TM، TN، TO، TP، TQ، TR، TS، TT، TU، TV، TW، TX، TY، TZ، UA، UB، UC، UD، UE， UF، UG، UH، UI، UJ، UK، UL، UM، UN، UO، UP، UQ، UR، US، UT، UY، UZ، VA، VB، VC، VD، VE، VF، VG، VH، VI، VJ، VK، VL، VM، VN، VO، VP، VQ، VR، VS، VT، VU، VV، VW، VX، VY، VZ، WA، WB، WC، WD، WE، WF، WG، WH، WI، WJ، WK، WL، WM، WN، WO، WP، WQ، WR، WS، WT، WU، WV، WW، WX، WY، WZ، XA، XB، XC، XD، XE، XF، XG، XH، XI، XJ، XK، XL، XM، XN، XO، XP، XQ، XR، XS، XT، XU، XV، XW، XX، XY، XZ، YA، YB، YC، YD، YE، YF، YG، YH، YI، YJ، YK، YL، YM، YN، YO، YP، YQ، YR، YS، YT، YU، YV، YW، YX، YY، YZ، ZA، ZB، ZC، ZD، ZE، ZF، ZG، ZH، ZI، ZJ، ZK، ZL، ZM، ZN، ZO، ZP، ZQ، ZR، ZS، ZT، ZU، ZV، ZW، ZX، ZY، ZZ.

أهداف الدراسة: قياس مستويات بعض الفيتامينات (A، C، and E) في مجموعة من المصابين بأورام الدماغ الأوتية و مقارنتها بمجموعة سيطرة (في معدل نمو و العال و نساء كندية متوكي و سيج الورم).

المرضى و طرق الدراسة: تم إجراء الدراسة بين 1998 و تشرين ثان 2000 م و تشرين أول 2001 م في مستشفى تعليمي في الكاتمية و مستشفى جراحة الجملة العصبية بيفند و 107 مريضاً بأورام الدماغ الأوتية تراوحت أعمارهم بين 2 - 75 سنة (تحت 35 سنة، الانحراف المعياري ± 19)، 56 نكراً (52.33%) و 51 أنثى (47.67%) . وفي الوقت الذي أظهرت فيه دراسة بالغ 89% من المرضى هم تحت عمر 65 سنة، إلا أن المجموعة الغالبة منهم تقع أعمارها بين 21 - 40 سنة (75.5%).

شملت الدراسة أيضاً مجموعة من 50 و من 40 مريضاً (مستشفى صرا و جنساً لمجموعة أورام الدماغ) كمجموعة سيطرة. التحليل الإحصائي العام للبيانات (32 يوم التحليل التجمي (حمى و غير صيفاً) 32 يوم التحليل الشهرية (صيفاً). تم استخدام جيز الأداء العادي للبيانات (أورام الدماغ).

النتائج: أظهرت النتائج انخفاضاً واضحاً في الفيتامينات (A، C، and E) التي شملتها الدراسة (في معدل النمو و العال و السائل الدماغي الشوكي و سيج الورم) لدى المرضى المصابين بأورام الدماغ الأوتية. بقيمة إحصائية عالية جداً بين المصابين و الأورام الخبيثة و مجموعة السيطرة.

الاستنتاجات و التوصيات: تتغير مستويات الفيتامينات عن المعتاد الطبيعي في مرضى الأورام و توصي بضرورة تأمين هؤلاء الفيتامينات. بسبب المستويات المنخفضة بها لعامة الناس. و مع ذلك ينبغي توضيح درجة الإصابة و الدقة التي ثبت وجود علاقة بين نقص الفيتامينات و تكون الأورام.