Biochemical studies of thyrotrophin,L-fucose,cathepsin B and some parameters in blood serum of patients suffering from breast tumour

Sabah hussian khorsheed college of education -Tikrut University

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Abstract

A developed methed was established to 70 patients suffering from breast tumour(negative axilliary node) from kirkuk city to determine the level of total fucose (TF),protein bound fucose (PBF),iron(Fe),total iron binding capacity(TIBC),unsatuted iron binding capacity(UIBC),transferrien,transferrien saturation,alkalin Phosphate(ALP),total protein(TP),albumin(ALB),glubuline(GLUB). The study also determines the level of thyrotropin (TSH),cathepsin B ,cholestol(CH),high density Lipoprotein cholestol (HDL-c),low density lipoprotein(LDL) in sera of three groups(benign, pre-post menopausal malignant of breast tumour. The results obtained showed highly significant increase in the level of TF,PBF, ALP,TP,GLUB,TIBC,and for(15-25),(36-45)age group for Fe and transferrien saturation,while there are highly significant decrease in UIBC,TIBC,Transferrien,ALB concentration in sera of breast tumour patients compared with control group. The results obtained about TSH,cathepsin B showed that there is significant decrease in sera of women affected by benign tumour and significant increase in sera of premenopausal breast tumour ,while in postmenopausal breast there is more significant increase of cathepsin B and more significant decrease of TSH,and also there is elevation in CH,LDL, and decrease in HDL-c for benign to pre to Postmenopausal breast tumour.

دراسة بايوكيميائية لثايروتروبين , ل-فيوكوز,كاثابسين وبعض المتغيرات الكيميوحيوية في امصال اورام الثدي

ا.م.د صباح حسين خورشيد كلية التربية جامعة تكريت

المفتاح : اورام الثدي ,ل فيوكوز, ثايروتروبين ,كاثلبسين ب

الخلاصة

استخدمت طرق مطورة ل 70 مرضى يعانون من الاورام الثدي (negative axilliary node) في محافظة كركوك لقياس مستويات الفيوكوز الكلي TF ,فيوكوز المرتبط بالبروتين PBFالحديد FE ,سعة ارتباط الحديد الكلي TIBC ,سعة ارتباط الحديد غير مشبع UIBC ,ترانسفيرين,ترانسفيرين المشبع, الفوسفيت القاعدي ALP ,البروتين الكلي TP ,الالبومين ALB ,الكلوبيولين GLUB وكذالك تم دراسة قياس مستوى ثايروتروبين TSH,كاثابسين-ب ,كولستيرول,البروتينات الدهنية عالية الكثافة HDL ,البروتينات الدهنية واطئة الكثافة LDL في امصال مرضى لثلاث مجاميع (الحميدة ,الخبيثة ما قبل انقطاع الطمث,الخبيثة ما بعد انقطاع الطمث) لاورام الثدي.

اظهرت النتائج ارتفاع معنوي عالي في مستويات الفيوكوز الكلي ,البروتين المرتبط بالفيوكوز ,الفوسفيت القاعدي ,البروتين الكلي ,كلوبيولين ,السعة الارتباط الحديد الكلي وللاعمار (15-25) و(36-45) لترانسفيرين المشبعة بينما هنالك انخفاض المعنوي عالي لتراكيز سعة ارتباط الحديد غيرمشبع ,سعة ارتباط الحديدالكلي, ترانسفيرين والالبومين

في مرضى امصال الاورام الثدي مقارنة مع مجموعة السيطرة.اما بالنسبة لثايرويروبين وكاثابسين –ب فاظهرت النتائج انخفاض معنوي في امصال المرضى باورام الحميدة وارتفاع المعنوي في امصال اورام الثدي ما قبل انقطاع الطمث اما في مرضى اورام الثدي ما بعد انقطاع الطمث فعنالك ارتفاع معنوي عالي لكاثابسين-ب وانخفاض معنوي عالي لثايروتروبين ,وزيادة لكولستيرول والبروتينات الدهنية واطئة الكثافة وانخفاض في البروتينات الدهنية عالية الكثافة من الحميدة الى الخبيثة ما قبل انقطاع الطمث الى الخبيثة ما بعد انقطاع الطمث الاورام الثدي.

Introduction

The important roles of fucosylated glycans have been demonstrated in a variety of biological settings, several of which are reviewed shortly[(1)] . However, because of the diversity of fucose-containing glycoconjugates and the difficulties inherent in studying the biological function of carbohydrates, it is likely that many additional functions for fucosylated glycans remain to be uncovered[(2)]. Fucosylated glycans have been implicated in the pathogenesis of several human diseases[(3)] . Two prominent examples of altered glycosylation in cancer involve fucose-containing oligosaccharides. First, expression of A and B blood group antigens is lost in many tumors with concomitant . Second, up-regulation of sialyl Lewisx and sialyl Lewisa have been demonstrated in numerous cancers, and these increases are also associated with advanced tumor grade and poor prognosis[(4)].

Breast cancer is a hormone-dependent neoplasm.Conflicting results regarding the clinical correlation between breast cancer and thyroid diseases have been reported in the literature, many studies showed that thyroid diseases are common among women with breast cancer, where as other reports did not confirm that.The aim of the present study was to determine the prevalence of thyroid diseases in patients with breast cancer as compared with that in the general female population[(5)] .The early diagnosis of breast cancer has yielded a larger increase in node-negative patients diagnosed with invasive carcinoma of the breast, , alarge body of literature has accumulated ,demonstrating that lysosomal proteinases cathepsin B ,D,L-are involved in the process of cancer invasion and possibly facilitate metastatic pathways[(6)]

Total protein :-the clinician can obtain information regarding disease states in different organ systems[(7)] . The measurement of protein is done on serum, which is the fluid that remains after plasma has clotted, thus removing fibrinogen and most of the clotting factors . Total protein content provides some information regarding a patient's general status ; more clinically useful data are obtained from fractionating the total protein . The normal serum protein level is 6 to 8 g/dl . Albumin makes up 3.5 to 5.0 g/dl, and the remainder is the total globulins . These values may vary according to the individual laboratory [(8)].

Iron is an essential nutrient with limited bioavailability. When present in excess, iron possess a threat to cells and tissues, and therefore iron homeostasis has to be tightly controlled. Iron’s toxicity is largely based on its ability to catalyze the generation of radicals, which attack and damage cellular macromolecules and promote cell death and tissue injury[(9)] .

Material and Methods

Sampling (Subjects)

In a plane tube (no anti coagulant),10 mL of venous blood placed, which was taken from the groups, left for (15 min) at room temperature, then centrifuged (at 3000 rpm for 10min) to get the serum, which is stored at (-20^{o}C) unless used immediately .

Collection of blood

The blood samples were collected from two groups as follow:

1- Control group: include (54) healthy with, with no previous diseases which may interfere with the parameters analyzed in this study.
2- brain tumors group: include (50) benign breast tumours and (35) malignant breast tumours

Determination of total fucose according to Dische and Sheetels methods:- This method depends on a direct reaction of concentrated sulphuric acid with serum components. The reactants combine with cysteine, and the colored product was measured at (390 and 430nm). The differences in absorbance were directly proportional to α-L-fucose content of the solutions[10] .

Determination of Protein bound fucose according to Dische and Sheetels methods:- These described the estimation of protein bound fucose leading to the determination of methyl pentose in serum. Specifically for methyl pentose which has been achieved by determining the optical densities at two wavelengths in order to correct for color developed by other sugars[11] .

Determination of protein bound hexose:- The hexose moiety of protein-carbohydrate conjugate was precipitated by 95% ethanol at room temperature, and determined by the orcinol reaction which measured at 520nm[12] .

Determination of total proteins (TP) according to Biurete methods:- This method depends on the reaction of peptide bond of the protein with copper ion (Cu^{++}) in alkaline medium to form colored products, which absorbance is measured at (540nm)[13].

Determination of Albumin(ALB) in Blood Serum :-The method is based on the specific binding of bromocresol green (BCG), an anionic dye , and the protein at acid pH with the resulting shift in the absorption wavelength of the complex .the intensity of the color formed is proportional to the concentration of albumin in the sample[14].

Determination iron concentration in blood Serum :-Iron is dissociated from transferring – iron complex in weakly acid medium .liberated iron is reduced into the bivalent form by means of ascorbic acid .ferrous ions give with ferrozine a coloured complex[15] .

Determination of Total- Iron Binding Capacity(TIBC) in Blood Serum:- precipitated unbound iron by basic magnesium carbonate .

And we can determination unsaturated binding capacity UIBC by Formula :-

UIBC = TIBC – Serum iron concentration

Determination of transferrien and transferrien Saturation from Formula

Transferrien (µg\dl) = 0.7 x TIBC (µg\dl)

$$\% = \frac{\text{Iron conc.}}{\text{TIBC conc.}} \times 100$$

Determination of thyroid stimulating hormone in blood serum:

A total of consecutive women with breast cancer and age-matched control serum thyroid stimulating hormone levels were measured using aimmunoradiometric assay designed for measurement of TSH in serum using Coat-A-Count kit containing radioactive

I^{125} –polyclonal anti-TSH.i

Determination of serum cathepsin B:

Cathepsin B determined by using the modified method of Barrett(16)which depend on amount of B-Naphthylamine release from analysis of N-Benzoyl-DL-Arginin-B-Naphthylamide.

Statistical Analysis :
Results were analyzed statistically using (t) and(f) tests by using statistical program Minitab. Averages were compared to calculations of the characteristics of the application Duncan's Multiple range test by probability level p 0.05 .

Results and Discussion
Table 1 showed a highly significant increase ($p \leq 0.01$) in the levels of total fucose(TF) , protein bound fucose (PBF) in sera of breast tumors patients compared with control group and no significant difference in total fucose (TF) , protein bound fucose (PBF) , protein bound hexose (PBH) concentration in sera of breast tumors compared between ege group .

In the present study, serum total fucose levels were found to be elevated in most of the intracranial tumor patients.L-fucose is found in many glycolipids and glycoprotein including several families of blood group antigen. The elevated levels of fucose have been attributed to tissue destruction and tissue proliferation or many arise from liver, reflecting a process of protein synthesis. However ,various views have been forward by several workers in support of increase in serum glycoprotein in malignancy and other diseases.The increase in level may be due to local synthesis and liberation of the glycoprotein by tumor cells in to the blood or it is a manifestation of the generalized effects of the tumor on the body metabolism. changes have been detected in the fucosylation pattern of these molecules in the tissue of cancer patients,due to fucosyl transferase activity, which is especially high in the serum of patients suffering from high malignant or metstatic tumors, such as colon carcinoma ,breast, and liver cancer[17] .

It is of interest that the inflammatory response in cancer patients is not only associated with a reduction in circulating albumin concentrations . Low serum albumin is used to identify protein-calorie malnutrition in hospital patients . Feeding protein to human

volunteers was reported to increase albumin synthesis in the liver, but yet not to increase serum albumin levels acutely . In addition, serum albumin was associated with skeletal muscle mass but not related to protein intake [18].

these data reveal that the activity of acid phosphatase tends to be low in such aspirates, furnishing a possible control to evaluate the patient alkaline phosphatase is a cell-membrane enzyme. Therefore, an estensive multiplication of cells and catabolism of their membranes may lead to elevation in alkaline phosphatase levels in body fluids (19)

Table 2 showed a highly significant increase ($p\leq0.01$) in the levels of iron (Fe) ,in (15-25) , (36-45) age group and a highly significant decrease ($p\leq0.01$) in (45-55) , (56-65) age group compared with control group . and a highly significant increase ($p\leq0.01$) in the levels of iron (Fe) and %saturation in (15-25) , (36-45) age group and a highly significant decrease ($p\leq0.01$) in (45-55) , (56-65) age group compared with control group . %saturation ,transferrine, UIBC and TIBC concentration in sera of control, breast tumours between ege group .while a significant increase ($p\leq0.01$) in the levels of Transferrin in (15-25) , (56-65) age group and a significant decrease ($p\leq0.01$) in (36-45) , (46-55) age group compared with control group .

also Table 2 showed A highly significant increase ($p\leq0.01$) in the levels of UIBC ,in (46-55) , (56-65) age group and A highly significant decrease ($p\leq0.01$) in (36-45) , A significant decrease ($p\leq0.01$) in (15-25) group compared with control group

the present study, Several epidemiological studies have shown an association between excess iron and cancer. There are several mechanisms by which iron can provoke DNA damage and lead to carcinogenesis. Iron binding sites on macromolecules serve as centers for repeated production of hydroxyl radicals generated via the Fenton reaction. Iron and oxygen together constitute a biologically highly damaging mixture due to increased formation of free radicals . Normally, chances of these are reduced by sequestration in storage or transport proteins and action of ‘acute-phase’ proteins such as ceruloplasmin, haptoglobins, etc., involved in iron metabolism.. Free radical mediated damage is known to be the root cause of many inflammatory, degenerative and neoplastic diseases. Iron excess plays a definitive role in these processes [20].
Elevated serum transferrin levels are associated with decreased survival, even after controlling for factors such as comorbid conditions (eg, hypertension, diabetes, cancer), smoking, and elevated choles iron overload diseases has been associated with cirrhosis and other liver diseases, diabetes mellitus, and cardiomyopathy. These findings provide new information on the impact of elevated serum transferring saturation [21].

In table 3 all parameters shown as a general without age group there is significant decrease of TSH in bengin breast tumer and more decrease in postmenopausal breast tumour while there is significant increase in premenopausal, as shown in table(4): The in cidence of breast cancer have been attributed of difference in dietary lodine intake,and an

effect of lodine on the breast cancer,the possible interaction between breast tissue and thyroid gland are based on the commen property of the mammary and thyroid epithelial cell to concentrate ilodine by a membrane active tranport mechanism as well as on the presence of TSH receptors in fatty tissue which is abundant in the mammary gland.[22][23]

Also in the table(4)there is significant decrease cathepsin B in benign breast tumour, while there is increase in premenopausal breast and more significant increase in postmenopausal,it may be result from change in protien which occurs in metabolism(caltbolism and anabolism)with ages which make decrease anabolism with increase catabolism and pretein turnover process[24].

Also result shows increase in LDL with decrease in HDL from benign to premenopausal to postmenopausal breast respectively it may result from increase in cholesterol or decrease in receptors of LDL which make slowly transport which agreed with Brown et al.[25]

Conclusions:

1-In group one and group two (benign,premenopausal breast tumour) there were decreased TSH and Cathepsin B in sera patients rather than control,while there were Increased inpostmenopausal.

2-From group one to group two to group three(benign ,pre,postmenopausal)there

3-In breast tumour there were increased TF,PBF,ALP,TP,GIUB,TIBC,Fe in patients rather than control,while decreased UIBC,TIBC,ALB in comparision with control.

Table 1 :-Total fucose(TF) , total protein ,protein bound fucose (PBF) , albumine, alkaline phosphatase (ALP), glubuline concentration in sera of control and breast tumour in four age group

Age (year)	Mean ± SE

	Group	TF (mg\dl)	PBF (mg\dl)	TP (g / dl)	ALB (g / dl)	GLUB (g / dl)	ALP (U\L)
15-25	control	13.433 ±0.067	8.1 ±0.2	8.350 ±0.15	4.1 ±0.10	3.600 ±0.10	45.0 ±10
	Tumor	19.33** ±0.88	11.00** ±0.58	9.667** ±0.33	3.00 ±0.058	6.167** ±0.27	90.33** ±1.2
36-45	control	14.5 ±0.5	8.7 ±0.4	7.067 ±0.3	3.467 ±0.29	4.25 ±0.05	73.0 ±7
	Tumor	25.0** ±6.2	11.33** ±1.5	9.067** ±12	2.900** ±0.31	6.667** ±0.33	93.00** ±1.2
46-55	control	14.075 ±0.35	8.775 ±0.28	7.975 ±0.28	3.950 ±0.13	4.025 ±0.15	71.7 ±7.7
	Tumor	20.5** ±2.5	10.5* ±1.5	9.05** ±1.5	3.200** ±0.10	5.850** ±0.15	89.50** ±0.5
56-65	control	13.5 ±0.5	9.00 ±	7.05 ±0.05	4.200 ±0.10	2.85 ±0.05	89.00 ±2
	Tumor	25.33** ±6.2	12.33** ±1.5	9.717** ±1.7	2.967** ±0.042	6.650** ±0.21	94.67* ±1.5

Table 2 :-The of iron (Fe) , %saturation ,transferrine, uIBC and tIBC concentration in sera of control and breast tumours in four age group

	Mean ± SE

Age (year)	Group	Fe (µg\dl)	%Saturation	Transferrine µg\dl	UIBC (µg\dl)	TIBC (µg\dl)
15-25	Control	40.3 ±0.35	16.10 ±2.2	180.1 ±22	218.0 ±33	285.3 ±32
	Tumor	109.0** ±6.2	36.17** ±4.8	214.7* ±15	197.7* ±27	306.7** ±21
36-45	Control	121.0 ±29	40.2 ±9.6	210.0 ±1	169.5 ±39	300.5 ±0.5
	Tumor	162.7* ±7.8	58.9 ±6.1	136.0* ±9	116.7** ±13	279.3 ±15
46-55	Control	131.8 ±14	38.28 ±4.1	242.3 ±19	209.7 ±18	346.5 ±27
	Tumor	87.5** ±25	26.8 ±7.8	229.7 ±2.4	240.0** ±18	327.5 ±3.5
56-65	Control	154.5 ±5.5	40.45 ±0.05	168.0 ±18	227.5 ±8.5	382.0 ±14
	Tumor	102.5** ±17	27.9** ±5.3	262.6** ±9.9	272.5** ±26	375.2 ±14

Table 3 :- Total fucose(TF) , total protein ,protein bound fucose (PBF) , (PBH), albumine,alkaline phosphatase (ALP),glubuline , iron (Fe) , %saturation ,transferrine, UIBC and TIBC concentration in sera of control and breast tumour

	Mean ± SE

Parameters		
	Tumor	Control
TF (mg\dl)	23.29 ± 1.7	13.682±0.2
PBF (mg\dl)	11.57±0.5	8.682±0.24
TP (g / dl)	9.471±0.2	7.536 ±0.19
ALB (g / dl)	2.993±0.063	3.9±0.12
GLUB (g / dl)	6.436±0.15	3.6 ±0.15
ALP) (U\L	92.64±0.9	65.9±6.0
Fe (µg\dl)	114.6±11	101.2±15
TIBC (µg\dl)	333.1±13	321.4±19
UIBC (µg\dl)	218.5±21	218.4±12
Transferrin µg\dl	220.3±18	200.9±20
%Saturation	36.1±4.4	30.3±3.6

Table (4) levels of TSH , Cat B , cholesterol high density lipoprotein ,iow density lipoprotein, very density lipoprotein in sera of different groups of patiens and control samples

Groups	No of cases		Age		Serum TSH u.u./ml		Calt / B U/L		HDL mg/ DL		Ch mg/DL		LDL mg/D L		VLDL mg/D L	
	Control	Patient	Control	Patient	Control	Patient	Control	Patient	Control	Patient	Control	Patient	Control	Patient	Control	Patient
Benign breast tumour	39	31	26.55	25.53	1.O9+ 1.3	0.84+2.5	219+2.1	193+3.2	51	45	191	240	99	120	31	42
Premenopau-sal breast tumour	29	27	29.39	27.41	1.25+0.11	1.73+1.5	219+2.1	301+5.1	51	39	191	305	99	190	31	59
Postmenopa-usal breast tumour	33	29	33.29	54.5	2.61+2.1	0.99+1.3	219+2.1	445+6	51	36	191	401	99	243	31	63

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