Biocemical studies of alkaline phosphatase for cancer patients

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الخلاصة

تم قياس فعالية إنزيم الفوسفاتيز القاعدي استنادا لطريقة (آرمسترونك و كنك) في مصول دم المرضى المصابين بالسرطان وتضمنت الدراسة ٣٠٣ مريضا مشخصين بأنواع مختلفة من السرطان و٤٢ شخصا سليما لغرض السيطرة وتم التحليل الإحصائي بطريقة ANOVA لكل من النوع، العمر ،الجنس ،والمرحلة المرضية وأشارت النتائج إلى ارتفاع فعالية الإنزيم في مرضى السرطان بصورة عامة مقارنة مع الأصحاء . وأوضحت النتائج بأنه يمكن اعتبار زيادة نشاط الإنزيم كمؤشر ورمي لتحديد مرضى السرطان خاصة سرطانات العظام والكبد ولتحديد السرطان الانبثاثي لهذه الأعضاء كما أوضحت النتائج إن فعالية الإنزيم تزداد عند الفئة العمرية ٤٠-٥٠ سنة مقارنة مع الفئات العمرية الأخرى التي شملتها الدراسة.
ولم تلاحظ أية فرو قات معنوية لفعالية الإنزيم بين الرجال والنساء وان العلاقة طر دية بين مرحلة المرض وفعالية الإنزيم حيث تزداد الفعالية بتقدم مرحلة المرض . وظهر في ١٠%-٢٠%من مرضى السرطان وكان أكثر ظهورا في المتناظر الإنزيمي Regan المرضى المصابين بسرطان البنكرياس والرحم والمبيض مقارنة مع سرطان المعدة والرئة والثدي .

ABSTRACT

The activity of alkaline phosphates (ALP) was determined by the calorimetric method of kin and Armstrong in serum of 303 cancer patients and 42 normal healthy controls. The ANOVA test of type, age sex and stage of disease ,the results showed that the ALP activity was higher in cancer patients then normal (p=0.000),this incensement could be used as a tumor marker (significant difference p=o.ooo) detected bone & liver cancer and to detect metastases to the organ .
The enzyme activity reached its highest level in the stage group (40-50 years). The results also show that there are no significant differences of ALP activity between males and females while the activity was increased with the development of disease stage.
Regan isoenzyme (RI) of ALP was elevated in about 10%-20% of cancer patients and was higher in pancreas ,cervix ,ovarian than stomach ,lung and breast .

INTRODUCTION :

Historically enzyme had been used as tumor markers before the discovery of oncofetal antigens and the advent of monoclonal antibodies. The abnormality of enzyme as marker for cancer are either the expression of the fetal form of the enzyme (isoenzyme) or the octopi production of enzymes .Enzyme are present in much higher concentration inside then outside the cell, they are released into the systemic circulation as the result of tumor necrosis or the change of membrane permeability of cancer cells(1). By the time enzymes are released into the systemic circulation the metabolism of tumors may have occurred.
Alkaline phosphates (ALP) are a group of plasma membrane and seam enzyme(2,3) which hydrolyzed phosphate esters at alkaline pH. They are present in most tissues(4-7),but are particularly high in the esatoblastes of bones, hepatobiliry tract, intestinal weal, renal tubules and the placenta(8) .

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The recognition of entopic protein production and the expression of fetal antigens by tumor cell have been the subject of intensive study during the last decade[9] .Unfortunately ,it is not yet possible to utilize the appearance of these enzymes, hormones or antigens in screening procedures as the sol diagnostic tests for the presence of cancer.
However, the ability to detect such products can be a valuable aid in the management of patients with malignant disease and the detection of recurrence[10,11]. Serum ALP is rapidly denatured at 56°C but is relatively stable at lower temperature[12] (placental isoenzyme is most stable). Sera kept at room temperature usually show a slightly increasing in activity. An increase in plasma ALP activity had been detected with mainly causes[13-15] such as Pahget's diseases of bone, obstructive liver diseases, Hepatitis, osteomalecia, Malignancy of bone or liver ,hyperparathyroidism and hepatotoxicity caused by drugs, while its activity decreased with cretinism ,vitamin C deficiency ,hypophosphatasia, anemia and vitamin D deficiency[13].
ALP isoenzyme of bone had been observed to be elevated in serum in patients with bone disorders such as Pahget disease, hyperparathyroidism, and rickets and in idiopathic hyperphosphatasia of infancy[16]. The intestinal ALP isoenzyme is seldom encountered in large amount. There are many methods used to detect the isoenzyme such as electrophoreses[17], heart inactivation[18], chemical inhibition[19], and isoelectric focusing gel technique[20].

MTERIALS AND METHODS :

The blood samples were taken from cancer patients of the Hospital of Radiology and Nuclear Medicine, Medicine City Hospital and Baquba General Hospital. After taken all information about the selected patients the blood was drawn by venipuncture. Blood samples were left at room temperature for about an hour then the serum was separated by spinning for 15 minutes at 3500 rpm in a centrifuge at room temperature .The unseparated specimens was discarded.
Determination of total ALP activity :-
The activity of the enzyme was measured in sera according to the method of Kind and Belfield[21] using four solutions as follow:
Solution A : - disodium phenyl phosphate 5mmol/l and carbonate-bicarbonat buffer pH=10 were prepared.
Solution B : -phenol standard equal to 20 kind and king.
Solution C :-amino-4-antipyrine sodium arsenate 60mmol/l.
Solution D :-potassium ferricyanide 150mmol/l.
Serum sample : 2ml of solution A were mixed and incubated for 5min at 37°c then 50 μ l serum was added and reincubated for further 15 min. After that 0.5 ml solution C was added and mixed well preferably in vortex, then 0.5 ml solution D was added, mixed and left stand for 10 min. in the dark. The UV absorption of this solution was measured at 510 nm.
serum blank : 2ml of solution A was mixed and incubated for20 min at 37°c after that 0.5 ml solution C was added ,mixed then 0.5 ml of solution D was added ,50μl serum was then added ,mixed ,left to stand for 10 min in the dark. The absorption of this solution was measured at 510 nm.
Standard solution : 2ml of solution A was mixed and incubated for 5 min at 37°c , then 50ul of solution B was added , incubated for farther 15 min ,0.5 ml solution C then was added ,mixed ,0.5 ml solution D was added ,mixed and left to stand for 10 min in the dark ,its absorption at 510 was measured .
Reagent blank: 2ml solution A was mixed and incubated for 20 min at 37°c then 0.5 ml solution C was added, mixed, 0.5 ml solution D then was added with 50μl distilled water and mixed again ,stored in the dark for 10 min the absorbance then was measured at 510 nm .
The assay procedure is shown in table (1).

Table 1 . Measurement of total ALP activity in serum

solution	Serum sample(1)	Serum blank(2)	Standard(3)	Reagent blank
A	2 ml	2 ml	2 ml	2 ml
incubationFor5 min at 370 c				
Serum	50 μ l	………..	………..	………..
B	………..	………..	50 μL	………..
Incubation for exactly 15 min. at 370 c				
C	0.5ml	0.5ml	0.5 ml	0.5 ml
Mix well preferably vortex				
D	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Serum	………..	50 μ l	………..	………..
Dist. Water	………..	………..	………..	50 μL

ALP assay :-

the assay depend on the reaction of C with B in the presence of alkaline oxidizing agent to produce qinoid substitution product .this product give red color whose intensity was proportion to the phenol liberated . The assay procedure was performed according to the table(2).

Table 2 . ALP assay in serum

Solution	Test	Control	Standard	blank
Carbonate-bicarbonate buffer pH10	1ml	1ml	1.1ml	1.1ml
Disodium phenyl phosphate (substrate)	1 ml	1 ml	----	------
Serum	0.1 ml	-------	-------	-------
Working phenol (1mg/100ml) stander	------	------	1 ml	-------
Distilled water	------	-------	-------	1 ml
Incubation for 15 min at 37 0c				
NaOH (0.5 M)	08 ml	0.8 ml	0.8 ml	0.8 ml
Serum	------	0.1 ml	-------	-----
NaHCO3 (0.5 N)	1.2 ml	1.2 ml	1.2 ml	1.2 ml
4-amino antipyrine (0.6g/l)	1 ml	1 ml	1 ml	1 ml
Potassium ferricyanide (150mmol/l)	1 ml	1 ml	1 ml	1 ml

Read immediately at 510 nm.

$$\text{ALP activity (K.A.U. /dl)} = \frac{\text{Test- control}}{\text{Standard – blank}} * 10 \text{ (in K.A.U./ dl).}$$

K.A.U./100 ml × 7.5 → IU/L

Regan isoenzyme separation :-

The following procedure was used for separation of Regan isoenzyme of ALP in serum and has been found to give reproducible results :-

0.5 ml serum was placed in a small thin walled glass tube and seal with Para film, the tube was placed in a thermostatically controlled water bath stabilized at 56°c. The bath should be large enough have good temperature stability and well stirred to minimize temperature fluctuations. The tube was removed from the bath after 10 min exactly and rapidly placed in ice bath. The ALP activity of the heat specimen is measured and expressed as percentage of the activity of unheated portion of the same serum which has been kept in ice water . Residual activity of 20% or less suggested that the predominant isoenzyme is the bone type. Values between 25-55% are associated with sera in which the predominant isoenzyme is of liver or intestinal origin or both. If the presence of Regan isoenzyme is suspected the incubation was done at 65°c for 30 min any remaining activity is due to Regan isoenzyme.

RESULTS AND DISCUSSION :

The results obtained show that ALP activity in cancer patients is affected by several variables such as :-

1- The effect of type of cancer : the data shows that ALP activity was increased in all types of cancer tissues , however ALP activity show a significant increase only in bone, liver and pancreas tissues as show in table (3) ,our results are in a agreement with those obtained by Whitby and Bechkett(22) ; Lott and Wolf(13) they found that there is a significant association between increased serum ALP activity in patients with malignant diseases. Zilva(11) also conclude that the increase of plasma ALP activity in patients with carcinoma is usually due to osteoblastic secondary deposits in bone or hepatic metastasis giving rise to cholistasis.

Table 3 . The difference of ALP activity between cancers tissue and normal subject

Control (A)	Site of cancer (B)	Mean (A-B)	Std. deviation	Sig. value
Normal	Bile	33.7676	6.959	0.117
=	Leukemia	3.9463	5.0610	0.436
=	Cervix	29.7438	6.5090	0.213
=	Stomach	8.0532	5.857	0.170
=	Lung	16.1921	4.492	0.661
=	Pancreas	36.9726	5.219	0.027
=	Bone	21.6595	4.261	0.000
=	Liver	29.8463	5.061	0.000
=	Colon	7.2458	3.933	0.999
=	Breast	16.5671	3.622	0.197
=	prostate	10.3310	7.543	0.172
=	Kidney	23.7310	7.543	0.218

* Sig .=significant value

2- Effect of patient's age: The results show that there were differences in ALP activity depending on patient age as in table (4). the activity increased with increasing patients age , it may be due to natural damages of body cell with age so the replication or repairing of the damaged cell was very slow comparing to that in younger age in both normal and cancer patients until age of 40-59 years , then the activity began to be constant with the increasing of age (> 60 years) .

Table 4 . The effect of patient's age on ALP activity

Age	No.	Mean	Std. deviation	Std. error
<20	18	15.237	9.336	3.300
20-39	61	18.172	10.381	1.621
40-59	99	26.548	24.408	2.587
>60	59	25.118	18.148	2.592
Total	237	23.853	20.187	1.476

3- The effect of patient's sex: The results show there is no significant differences in ALP activity between sexes in normal volunteers and patients as shown in table (5); these are in agreement with those obtained by Bowers and Comb[23] who studies the effect of sex and age.

Table 5 . The effect of patient's sex on ALP activity

Sex	Number	Mean (k. a.)	Std. deviation	Std. error
Female	183	23.768	18.975	1.777
Male	120	23.901	21.425	2.395

4- The effect of the stage of disease: Our results show in table (6) indicates that the ALP activity increased with the development of the tumor, stage (VI) has the highest activity while stage (I) has the lower value.
The effect of the stage of disease in association with age may contributed to the higher levels in older patients taking in account the severity of the damaged caused to various tissues at latter stages and the weakness of cell resistance at older ages .Several authors obtained similar results, Whitby & Bechkett[22] found that there is a significant association between high serum ALP & high tumor growth.

Table 6 . The effect of stage of disease on ALP activity

Stage	Mean (k.a)	Std.deviation	Std. error
I	11.542	3.661	0.498
II	19.705	8.237	1.121
III	33.382	17.973	2.807
IV	59.477	33.488	7.161
Total	25.224	21.855	1.671

Regan isoenzyme of ALP :

The most remarkable property of placental ALP is its pronounced stability to heat. Incubation of the enzyme at a temperature as high as 56°c for 30 min could be accomplished there fore provides a convenient and specific test for the presence of its isoenzyme. Pronounced heat stability is also shown by the Regan isoenzyme, placental like fetal form of ALP that occurs

in 5-15% of specimen from patients with cancers of various types[24]. Thus heating the sample at 56°c can also be used to detect this abnormal isoenzyme.

The similarity between these two enzyme forms is evidenced by the fact that the Regan isoenzyme react with antibodies raised against the placental Isoenzyme. The proportion of the untreated patients in whom Regan's isoenzyme (RI) was identified is shown in table (7), the categories in which samples from more then 10 patients were tested, cervix, ovarian & pancreatic carcinoma had the highest incidence of Regan isoenzyme, followed by breast, lung and gastric cancer and carcinomas. A number of tumors in which RI was never identified previous studies have demonstrated the presence of an ALP isoenzyme in patients with cancer which was biochemically and immunochemically identical with placental isoenzyme comparative studies have been carried out on patients with elevated ALP and on individuals appearing for reemployment or annual physical examinations. These studies have demonstrated that approximately 14% of cancer patients have detectable RI.
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Table 7 . Incidence of RI in relation to type of cancer

Type of cancer	Total no.of patients	RI	PercentofRI
Breast	36	7	14.6
Colon	27	3	10.2
Gall bladder	6	1	8.2
Leukemia's	13	2	6.4
Stomach	9	2	16.2
Lung	18	5	15.2
Bone	21	4	11.5
Liver	13	3	8.0
Pancreas	12	3	24.3
Cervix	7	2	26.5
Prostate	5	1	13.8
Ovary	15	5	22.5
Kidney	10	3	5.2

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The highest percentage occurred in patients with carcinomas of the lung, ovary pancreas, stomach and breast. Most patients who were Regan positive proved to have greater then 0.8 heat stable ALP units. It could be emphasized on the basis of data in this and other studies[24] that the incidence of RI in the group of patients with benign disease is small and that the quantitative level of isoenzyme tends to be low in comparism with patients with neoplasia.

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