

Determination the Sialic Acid in Colorectal Cancer and its Correlation to Some Enzymes

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Abstract

This study is conducted to determination the serum level of sialic acid(SA), Alkaline phosphatase ALP, Lactate dehydrogenase LDH and Creatine kinase(CK) in 25 patients with colon cancer, 25 patients with rectal cancer and 25 healthy subjects.

Serum SA, ALP and LDH levels in patients with colon and rectal cancer were significantly higher than control groups and serum CK level in patients with colon and rectal cancer was nonsignificantly higher than control.

There were a positive significant correlation between SA and ALP and LDH but there was a positive nonsignificant correlation between SA and CK. The results suggest that the alteration of SA, ALP and LDH levels were related to the pathogenesis of colorectal cancer.

الخلاصة

تم في هذا البحث جمع مصل ٢٥ مريضاً مصاب بسرطان القولون و ٢٥ مريض مصاب بسرطان المستقيم و ٢٥ شخصاً من الاصحاء كمجموعة ضابطة. قيست مستويات حامض السياليك وانزيم الفوسفاتيز الحامضي وانزيم اللاكتيت ديهيدروجينيز وانزيم الكرياتين كايينيز بطريقة طيفية. وجد ان مستويات حامض السياليك وانزيم الفوسفاتيز الحامضي وانزيم اللاكتيت ديهيدروجينيز لها قيم معنوية احصائية يعتد بها اعلى من المجموعة الضابطة اما مستوى انزيم الكرياتين كايينيز فكانت له قيمة معنوية احصائية لا يعتد بها اعلى من المجموعة الضابطة.

لقد وجد ان هناك ترابط معنوي ايجابي بين حامض السياليك وانزيم الفوسفاتيز الحامضي وانزيم اللاكتيت ديهيدروجينيز كما لم يظهر البحث ترابط بين حامض السياليك وانزيم الكرياتين كايينيز. ان النتائج تقترح بان ارتفاع مستويات حامض السياليك وانزيم الفوسفاتيز الحامضي وانزيم اللاكتيت ديهيدروجينيز مرتبطة بوجود مرض سرطان القولون وسرطان المستقيم.

Introduction

Tumor markers, such as sialic acid (SA)^[1], are naturally occurring modified molecules that can be measured in serum, plasma, or other

body fluids and their concentration varied in the presence of cancer.

N-acetylneuraminic (referred to as SA) is a negatively charged nine carbon monosaccharide commonly attached by an glycosidic linkage to

the non-reducing residues of the carbohydrate chains of glycoproteins and glycolipids^[2,3]. Over 20 different naturally occurring SAs have been reported in glycoconjugates of various animal species^[2]. SA participates in the stabilization of the conformation of glycoproteins and cellular membranes^[4,5].

Alterations in the level of membrane-bound SA or a rearrangement of SA in the plane of the membrane could be responsible for some of the altered properties of transformed cells^[6].

Increased sialylation helps malignant cells to disguise their immunogenic sites, to increase the negative charge of the outer cell membrane so that the binding and killing by lymphocytes and macrophages can be impaired, and to hide the receptor sites for IgM antibodies, which kill cells by a complement-mediated reaction^[7].

Changes of serum /plasma SA have been reported in patients with various types of cancers^[8].

Colon and rectal cancers are sometimes referred together as "colorectal cancer". which they are the third most common cancer in adults, accounting for 11% of cancer death. Most cases of colorectal cancer begin with the development of benign polyps, which are relatively common in people over age 50, they can become cancerous, though, with the ability to invade the normal colon and spread to other parts of the body^[9].

Alkaline phosphatase (ALP)(EC 3.1.3.1) :-

Alkaline phosphatase is a Phosphate transfer, a phosphate moiety from one group to another, forming an alcohol and a second phosphate compound^[10]. This enzyme is present in high concentration in the liver, bone and intestinal epithelium. These tissues each contain specific isoenzymes of ALP^[11]. The activity of ALP has been

elevated in the bone and liver disease such as malignant disease and carcinoma with osteoblastic metastasis^[12]. Increases in ALP activity are the result of increased synthesis of the enzyme by cell lining the bile canaliculi^[7].

Lactate Dehydrogenase (LDH) (EC 1.1.2.4):-

Lactate dehydrogenase catalyzes the reversible interconversion of lactate and pyruvate in the presence of reduced nicotinamide adenine dinucleotide (NADH). LDH is found in the highest concentration in the liver followed by, heart, skeletal muscles, and distributed generally in body cells and fluids^[13].

The similar elevations occur with hemolytic anemia. LDH is elevated also in leukemia specially with acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL)^[14]. The level of LDH enzyme was studied in sera of patients with gastric^[15], prostate and testicular cancer where it is used as a tumor marker for diagnosis, staging and follow-up^[16].

Creatine kinase (CK)(EC 2.7.3.2) :-

Creatine kinase is a cytoplasmic and mitochondrial enzyme with a wide tissue distribution and catalyzes the reversible transfer of the phosphate group of phosphocreatine (PCr) to Adenosine diphosphate (ADP), to yield Adenosine triphosphate (ATP) and creatine (Cr)^[17,18].

The CK, PCr and Cr system is present in tissues with high and fluctuating energy demands such as brain, heart and skeletal muscle, and serves as a temporal and spatial "energy buffer" that helps to maintain a high intracellular phosphorylation potential in situations of increased metabolic demand^[19,20].

Creatine kinase has been reported in patients with gastrointestinal tract

malignancies and in particular of colorectal cancer^[21], lung and prostate cancer, metastatic ovarian tumor^[22-24].

Because of the above conflicting data of SA, ALP, LDH and CK in carcinoma parts, the aims of this study were to:-

- Evaluate the serum SA, ALP, LDH and CK levels in patients with colon and rectal cancer.

- Find correlation between SA and ALP, LDH and CK in patients with colon and rectal cancer. .

Subjects and methods

Patients:- The present project was approved by the ethics commission of Baghdad Teaching and AL-Shaheed Adnan Khir Alla hospital.

Blood sampling were drawn from (75) patients, (25) patients with colon cancer (age: 40-67 yr. old), 15 males and 10 females. (25) patients with rectal cancer (age: 37-64 yr. old), 12 males 13 females. (25) normal healthy individuals (age: 27-67 yr. old), 9 males and 16 females as a control group.

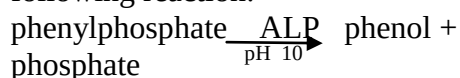
The diagnosis was based on clinical , histological examination and systoscopy test reports by Dr. Lua'ai Edward , Dr ali Al-Hadithi and Dr. Nawal AL-aiash.

Venous blood samples (5 ml) from the control and each patient were transferred into plain tube then blood was left to clot and serum was obtained by centrifugation at 3000 rpm for 10 min then serum was removed and kept at (-20°C) till analysis.

Methods:-

1-Determination of ALP activity:-

Colorimetric determination of ALP activity was obtained according to the following reaction:-

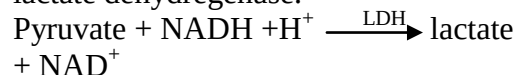


The phenol liberated was measured in the presence of amino-4-antipyrine

and potassium ferricyanide. The presences of sodium arsenate in the reagent is for stopping the enzymatic reaction, ALP was assayed by means of colorimetric method with a commercially available kit^[25].

2-Determination of LDH activity:-

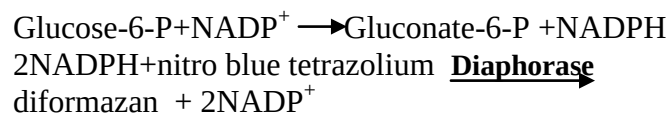
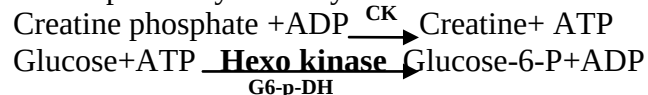
This method is based on the reduction of pyruvate to lactate in the presence of NADH which catalyzed by lactate dehydrogenase.



The pyruvate that remains unchanged reacts with 2,4-dinitrophenyl hydrazine to give the corresponding phenylhydrazone, which was determined colorimetrically in an alkaline medium pH 9-10 with a commercially available kit^[26].

3-Determination of CK:-

Colorimetric determination of CK activity utilizes creatine phosphate as substrate to act as the initial catalyst for a series of reactions resulting in the formation of NADPH as outlined in the coupled enzyme assay:-



The reaction is stopped by the addition of HCL and the blue/violet colored complex of diformazan has an absorption maximum around 560^[27].

4-Determination of TSA:-

Total sialic acid levels were determined according to the thiobarbituric acid method described by Aminoff^[28], to hydrolysis of the protein and lipid-bound sialic acid, were added 5 volumes of 0.1 N H₂SO₄ at 80 °C for 1 h. briefly, 5 ml serum were treated with 0.25 ml of 25 mM periodic acid in 0.125 N H₂SO₄ (pH 1.2) for 30 min in a water bath at 37 °C.

C. The excess of periodate was then reduced with 0.2 ml of 2% sodium arsenite in 0.5 N HCl. Yellow color of the liberated iodine had disappeared (1-2)min, 2 ml of 0.1 M thiobarbituric acid were added and the test sample was covered and heated in a boiling water bath for 7.5 min. The colored solutions were cooled in ice water and shaken with 5 ml of acidic butanol (1-butanol containing 5% v/v of 12 N HCl). The separation of the phases were facilitated by a short, rapid centrifugation and the intensities of the colors in the butanol layer were measured at 549 nm. A calibration curve was obtained by plotting absorbance versus concentration. The TSA concentration of unknown samples was then calculated from calibration curve.

Statistical methods:-

The results were analyzed statistically, and values were expressed as (mean $\pm$ standard deviation). The significance level was determined by employing (student t-test). Only when the (P value) was less than 0.05 was the difference between two groups is considered statistically significant. Pearson correlation coefficient (r) was used to test the relation between two parameters.

Results and Discussion

Mean serum SA, ALP, LDH and Ck levels of patients with colon and rectal cancer, compared to control, table (1) and figure (1), it was found that the mean :-

- Concentration of serum SA with colon and rectal cancer was statistically significant higher than the control groups ($P < 0.05$) and concentration of serum SA in patients with colon cancer was higher than patients with rectal cancer.

Surface glycoproteins and glycolipids of tumor cells have altered carbohydrate composition, that may

contribute to aberrant cell-cell recognition, cell adhesion, antigenicity and invasiveness demonstrated by malignant cells. Since sialic acids are major constituents of glycoproteins and glycolipids, recent studies showed that the values of total bound sialic acid or protein-bound sialic acid were higher in patients with cancer than normal subjects. Lipid-bound sialic acid levels are correlated with stage of the disease, degree of metastatic involvement and recurrence of disease [29,30].

Serum SA has been found to be increased in malignant melanoma^[31], colorectal cancer^[32], gastric cancer^[33], head and neck cancer^[34] and laryngeal cancer^[35], breast cancer^[36], lymphoma and Dalton's lymphoma^[37,16], and higher SA levels were observed in cases of more developed malignancies^[38]. The data of the present study are in agreement with those previously reported which described an increase in the SA content of serum in several types of carcinoma. This assumption is in agreement with others who suggested to use the changes in SA as a marker for response to drug therapy in management of malignancies^[39,40].

- Serum of LDH activity in patients with colon and rectal cancer was statistically significant higher than the control groups ($P < 0.05$) and serum LDH activity in patients with colon cancer was higher than patients with rectal cancer.

It is well known that glycolysis in cancer tissue increase significantly, consequently, as an important enzyme of the glycolytic pathway, LDH may manifest a higher activity in cancer patients; serum and tissues^[41], this result may be explained to be due to the present LDH isoenzyme which possess different molecule constitution, functional differences on the metabolic adjustment to some

extent exit among them, since it is easy for LDH-5 to convert pyruvate into lactate, which benefits anaerobic metabolism, while LDH-1 can convert lactate into pyruvate, and the latter can be oxidized in citric acid cycle, which benefits aerobic metabolism. This suggests that increased activity of LDH in cancer cells was mainly due to the increase in LDH-5 activity, LDH-5 might increase glycolysis in cancer cells, with rise of lactate in cancer cells and adjacent tissues, pH in local tissues decreased and thus invasion and metastasis of cancer cells could be indirectly promoted by enhanced activities of acid hydrolyze^[42]. Serum LDH activity has been found to be increased in gastric, colon, prostate bladder, testicular cancer^[43-45]. Result of our study is in agreement with before studies of same enzyme in the same disease.

- Serum of ALP activity in patients with colon and rectal cancer was statistically significant higher than that of control groups ($P < 0.05$) and serum of ALP activity in patients with colon cancer was higher than patients with rectal cancer.

Most data confirms the elevation of serum ALP activity occurs because of the accelerated de novo synthesis of the enzyme and subsequent regurgitation into the serum^[46,47]. Elevation of serum ALP was reported in patients with colorectal, pancreatic, liver and breast cancer^[48-50]. The data of the present study are in agreement with before studies of same enzyme in the same disease.

- Serum of CK activity in patients with colon and rectal cancer was statistically nonsignificant higher than of control groups ($P > 0.05$) and serum of CK activity in patients with colon cancer was higher than patients with rectal cancer.

Ck is an enzyme still widely analyzed in clinical diagnostics. Higher CK activity observed than the normal samples because there is increase in the expression of CK activity in cancer cells. Serum CK activity has been found to be increased in gastrointestinal tract, hepatocellular carcinoma, ovarian, lung, gastric cancer and colon adenocarcinoma^[22-25,51]. The results of our study is in agreement with before studies of same enzyme in the same disease.

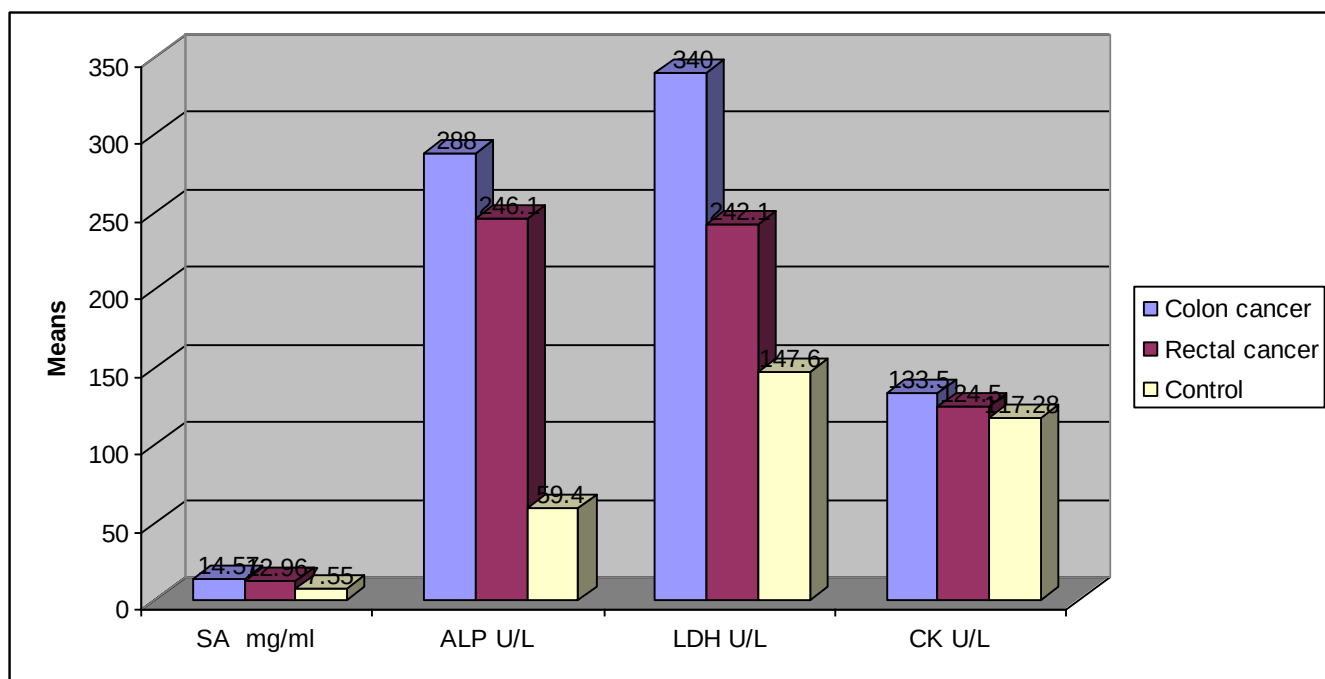


Figure (1):- Means of serum SA,ALP,LDH and CK in patients with colon and rectal cancer and control.

Table(1):-Mean ,SD and p value of serum SA,ALP,LDH and CK in patients with colon and rectal cancer and control.

	Control Mean±SD	Colon cancer Mean±SD	Rectal cancer Mean±SD	P value control- colon	P value control- rectum
SA mg/ml	7.55 ± 0.778	14.57 ± 0.921	12.96 ± 1.309	P<0.05	P<0.05
ALP U/L	59.4±10.49	288±73.6	246.1±50.9	P<0.05	P<0.05
LDH U/L	147.6±15.3	340.3±62.3	242.1±40.1	P<0.05	P<0.05
CK U/L	117.28±17.8	133.5±40.2	124.5±18.1	P>0.05	P>0.05

Table (2) shows the correlations of serum SA and (ALP,LDH and Ck),data observed that :-

- A strong positive correlation was found between SA and ALP in patients with colon and rectal cancer ($p<0.05$).
- A moderate positive correlation was found between SA and LDH in patients with colon and rectal cancer ($p<0.05$).
- A weak positive correlation was found between SA and ck in patients with colon and rectal cancer ($p>0.05$).

In those observations the author can conclude that, in cancer disease the number of cells is increased more than normal cells therefore, membranes of cells are increased too, and SA is one of the components of cells membrane. Elevation number of cells is related to gene expression of some enzymes .Cancer cells require to these enzymes for their metabolism such as LDH and ALP.

Table(2):-Correlations coefficient of serum SA and(ALP,LDH,CK).

	Colon (r),P value	Rectum (r) ,P value
SA and ALP	0.88 , P<0.05	0.78 , P<0.05
SA and LDH	0.56 , P<0.05	0.27 , P<0.05
SA and CK	0.19 , P>0.05	0.04 , P>0.05

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