Comparative study of CA19-9 levels as tumor marker in sera and tissues of breast, prostate and thyroid cancer patients

A. H. Al-dujaili, W.F. Al-Taei, K. M.Turky ,G. H. Al-Ubaidi
Department of Chemistry, College of Education, Ibn Al-Haitham, University of Baghdad

Abstract

Carbohydrate antigen 19-9 (CA19-9) levels were measured in sera and tissues of 40 patients with breast cancer (G_1), 8 patients with prostate cancer (G2)and 12 patients with thyroid cancer (G3), by the enzyme-linked immunosorbent assay (ELISA) technique.

The patients were admitted to Medical City Hospitals (Baghdad Teaching Hospital and Nursing Home Hospital). The sera were taken just before surgery, where the specimens were taken immediately after surgery and kept in saline solution at -20°C until the time of homogenizing process.

The results of CA19-9 levels in sera were (16.309±7.143; 31.281±0.766; 11.5±0.707 U/ml respectively compared with serum CA19-9 level of control group G_4 which was 7.74±4.92 U/ml. The results were found to be significantly higher than control group ($p < 0.005$).

The results of CA19-9 levels in tissues' homogenate were (30.554 ± 17.39; 142.375 ± 8.00; 194.5 ± 92.4) U/ml. The results revealed significantly higher levels of CA19-9 in tissue's homogenate of each patients group compared with the serum levels of the same patients ($p < 0.005$).

Introduction

CANCER

Cancer is a genetic disease resulting in the abnormal proliferation of a clone of cells(1). In Iraq, the percentages of incidence in breast, prostate and thyroid cancer were 16.92%, 1.87% and 2.52% respectively(2).

The diagnosis of cancer made by a pathologist is based on tissue and cells submitted for examination. However, difficulties arise when clear histologic and cytologic distinctions are not present. Severely inflamed and markedly hyperplastic tension can mimic cancer, whereas some very well differentiated carcinomas can resemble benign tissue(3).

The pathologist now uses this new genetic information to characterize tumors at the molecular level hoping that the more specific information will allow for better diagnosis, disease prognosis, and treatment(4).

Screening tests for early detection of cancer in asymptomatic individuals include a variety of standard diagnostic procedures. The tests are performed on a regular basis and are age adjusted. Currently, with molecular oncology concept, the search is to identify the cancer in its in situ, when no lymphatic or vascular seeding has occurred(5).

Tumor Markers

Tumor markers are biochemical substance synthesized and released by cancer cells or produced by the host in response to cancerous substance and are used to diagnosis, screening, prognostic, monitoring or identify the presence of cancerous growth(6).

Tumor markers may be present, in blood, body cavity fluids, membranes, and cell cytoplasm. Tumor markers are different from substances produced by normal cells, in quantity and quality(6).

CA 19-9 is among a group of mucin glycoprotein sialosyl lewis antigen (SLA), have come to be recognized as a circulating cancer associated antigens for gastrointestinal cancer(7).

Basic research into cancer causation will be a powerful determinant of interventions in the transformation process reinforcing the need for developing effective molecular tumor biomarkers for early detection of malignant evolution in tissues or organs i.e. breast, prostate and thyroid(5).

Experimental part

Patients Groups

The patients included in this study (their age ranged from 17-75 year), were classified into three groups as the following:

1.The first group was included 40 patients with breast cancer (G1).
2.The second group was included 8 patients with prostate cancer (G2).
3.The third group was included 12 patients with thyroid cancer (G3).
4.The last group was included 30 healthy subjects considered as control group (G4).

The patients were selected, during the period from November 2004 to July 2005, according to the investigation of histopathologist. They were admitted for treatment at Medical City Hospital (Baghdad Teaching Hospital and Nursing Home Hospital) and all surgical operations for all patients were carried out under the supervision of surgeons.

Preparation of Blood Samples

Five milliliters (mls) of venous blood were drown from each patient by veinpuncture just before surgery, left to clot, and then centrifuged at xg 2500 for 30 min. Serum was separated and stored at -20°C until time of analysis.

Collection of Specimens

The tumor tissue was surgically removed from patients. The specimens were immediately kept in normal saline solution and stored at -20°C until the time of homogenizing process.

Homogenization of Tumor Tissues

The frozen tissue was sliced finely scalped in Petridish standing on ice, and then homogenized with three fold volumes of phosphate buffer pH7.4 by the homogenizer.

The homogenate was filtered through nylon gauze to eliminate fiber connective tissues. The filtrate was centrifuged at xg 2500 for 30 min at 4°C in order to precipitate the remaining intact cells and the intact nucleus. The supernatant and precipitate fraction were separated and frozen at -20°C until use.

Determination of (CA19-9) Antigen Using ELISA Assay

Serum and tissue's homogenate of all studied groups were detected by an immunoenzymatic colorimetric method used for quantitative determination of (CA19-9) concentration in serum and tissue's

homogenate. In this essay, the CA19-9 in the samples is bound to an available excess of monoclonal antibodies against CA19-9, which immobilized to the surface of the microtiter wells. After a washing step to remove all foreign substances, the quantification for bound CA19-9 is carried out by adding an enzyme (horseradish peroxidase or HRPO) labeled antibody, which also binds to the CA19-9. The amount of bound enzyme is directly proportional to the CA19-9 content. The substrate is then converted to a chromogenic compound, which can be determined photomerically at 450 nm(8-10).

Result and Discussion

CA19-9 Study

CA19-9 essay measures a tumor related which is produced by adenocarcinoma of pancreas, stomach, gall bladder, colon, ovary and lung, and it is shed into the circulation(11).

The characterization of cancer patients groups and control group included in this study are shown in table (1).

The values for serum CA19-9 of all studied groups and control were shown in table (2) and figure (1).

CA19-9 in sera of G1, G2, G3 and G4 were (16.309 ± 7.143), (31.281 ± 0.766), (11.5 ± 0.707) and (42.625± 33.088) U/ml respectively.

The normal healthy subject has an upper limit of normal serum CA19-9 defined by a cutoff value of 37.0 U/ml(12,13).

The values for CA19-9 control group were within normal values with a range of (1-19) U/ml.

From Table (2) and Figure(1) the values for CA19-9 in sera of breast cancer G1, prostate cancer G2 and thyroid cancer G3 were within the normal healthy subjects, yet signification higher values were reported for the cutoff values which was found in this study.

Table (3) and figure (2) showed the results of CA19-9 in tumor tissue's homogenate of all studied groups.

Breast cancer patients G1 had CA19-9 in the tumor homogenate (30.554 ± 17.39) with range of (8-64) about 30% of the whole patients under

investigations showed lower values than 37.0 U/ml, the rest showed a higher values.

Differentiation of the cystic mass from any malignancy poses some difficulties. The relation of carbohydrate antigen CA19-9 with breast cysts had been investigated with results of elevated CA19-9 level associated with breast tissue (0.0-39.0 U/ml). The serum CA19-9 were higher compared to control, three months after operation reduced to 12 U/ml(14). Pathological examinations on prostate and penile biopsy specimens revealed prostate adenocarcinoma with penile metastasis. The patient was diagnosed as having prostate cancer with bone, lymph node and penile metastasis. After the surgical castration and chemotherapy, the values of CA19-9 and PSA were decrease(15).

A 24-year-old woman presented with anaplastic transformation from papillary thyroid carcinoma with increased serum CA19-9 was detected in the terminal stage. Although there are few clinical reports suggesting a prognostic indicator for anaplastic thyroid carcinoma, CA19-9 may be useful serum marker for this tumor(16).

CA19-9 in tissue's homogenate of breast cancer G1, prostate cancer G2 and thyroid cancer G3 were (30.554±17.39)and (142.375±8.00) respectively.

The elevation in the carbohydrate antigen CA19-9 could be due to the increase in their expression and their ligand protein. A study conducted on the expression of some carbohydrate antigens and their roles in the invasion and metastasis of different cancer types (renal pelvis, ureter and urinary bladder) the results showed that there were differences between the organs with regard to the degree of expression of these antigens were evident and frequently expressed in the tumor cells. Regardless of atypical grade, some of the antigen could be corrected with the stage and grade of tumor, other antigen may be involved in tumor invasion and metastasis(17).

Conclusions

1.Normal levels of CA19-9 were found in sera of breast cancer, prostate cancer and thyroid cancer patients.

2.High levels of CA19-9 were found in tissue's homogenate of prostate cancer and thyroid cancer patients compared to sera of same patients.

3.Tissue's homogenate of breast cancer patients, showed elevation in CA19-9 level in 70% of cases.

Refrences

1-The Human Genome Project, (1994), "Deciphering the Blueprint of Heredity", University Science Book, Mill Valley.

2-Iraqi Cancer Board, (2006), personal communication.

3-Cotran, RS.; Kumar, V. and Collins, T. (eds), (1999),"Robbins Pathology Basis of Disease" 6th ed, W.B. Saunders, Philadelphia.

4- Rosai, J (ed), (1997 – 1998): Atlas of Tumor Pathology, 3rd ser., Armed Forces Institute of Pathology, Washington.

5- Rubin, P. Williams, JP.(2001), "Clinical Oncology" 8th ed, W.B. Saunders Company, USA.

6-Chatterjea, MN. Shinde, R. (1995) "Textbook of Medical Biochemistry" 2nd ed, Jaypee Brother Medical Publisher (P) Ltd, Japan.

7- Steinberg, W. (1990), A merican J of Gastroenterology 85: 350 – 355.

8- Glenn, J. Steinberg, W.M. and Kurtzman, S.H., (1988), J Clin Oncol; 6: 362-468.

9- Hayakaw, T. Kondo, T. and Shibata, T. (1988), Cancer 61: 1827-1833.

10- Malesci, A. Tommasini, M.A. and Bonato, C.(1987)Gastroenterology 92: 60-67.

11- Magnani, J.L. Steplewski, Z. and Kiprowski, H. (1983),Cancer Res; 43: 5489-5492.

12- Del Villano BC, Brennan S, and Brock P, (1983), Clin Chem ,29: 549- 552.

13- Ritts, Jr. RI,; Del Villano,B.C. and Go, VLW. (1984), Int J Cancer , 33: 339-345.

14- Yuksel, B.C.; Ozel, H.; Akin, T.; Avsar, F.., and Hengirmen, S. (2005),Am J Trop Med Hyg 73(2): 368-370.

15- Sawada, A.; Segawa, T.; Nakanishi, S.; Kinoshita, H.; Yamamoto, S.; Kamoto, T.and Ogawa, P. (2005),Hinyokika Kiyo ,51(11):771-773.

16- Ogawa, M.; Hori, H.; Hirayama, M.; Kobayashi, M.; Shiraishi, M.; Shiraishi, T.; Watanabe, Y.;and Komada, Y. (2005) Pediater Blood Cancer ; 45(1): 64-67.

17- Kajiwara, H.; Yasuda, M.; Kumaki, N.; Shibayama, T. and Osamura, Y. (2005),Tokai J Exp Clin Med ; 30(3): 177-182.

Table(1): The information of patients and control groups included in this study

Group	Patients	No.
G1	Breast cancer	40
G2	Prostate cancer	8
G3	Thyroid cancer	12
G4	Healthy subjects (control	30

Table(2): CA19-9 value in serum in all patients groups

Patients Groups	No.	Mean± SD U/ml	Range U/ml
G1	30	16.309 ± 7.143*	7 – 32
G2	8	31.281 ± 0.766*	30- 32.3
G3	12	11.5 ± 0.707*	10.5-12.5
G4	30	7.74 ± 4.915*	1-19

* Significant P value< 0.005

Table (3): CA19-9 values in tissues homogenate in all patients groups

Patients groups	No.	Mean ±CD U/ml	Range U/ml	under cutoff value%	Over cutoff value
G1	40	30.554 ± 17.39 *	8 - 64	30	70
G2	8	142.375 ± 8.00 *	132 - 152		
G3	12	194.5 ± 92.4 *	60 - 316		

* Significant P value< 0.005

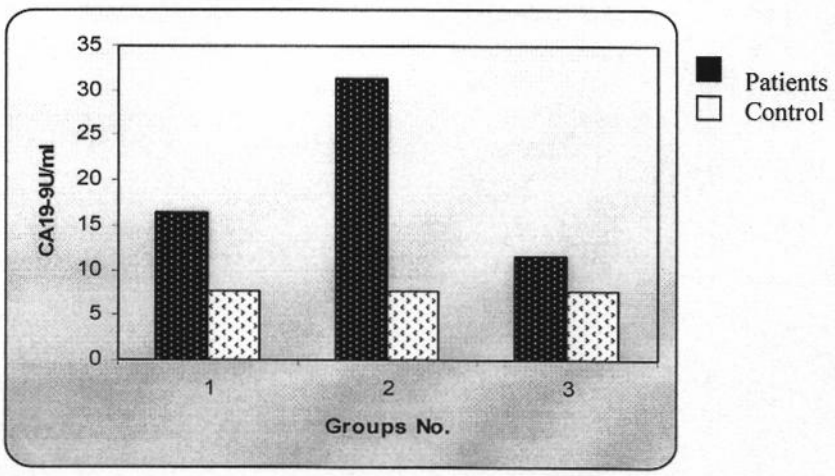

Fig.(1) Serum CA19-9 of the studied groups and control

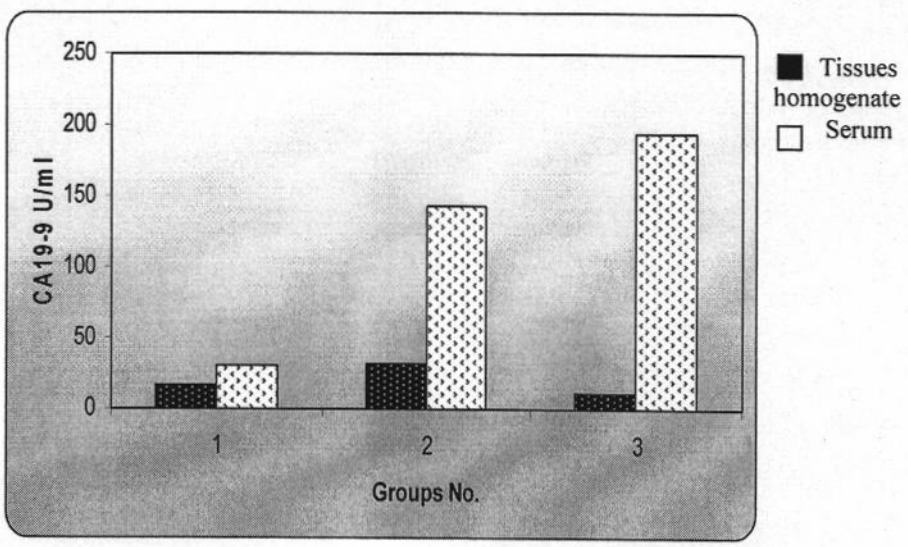

Fig.(2) Tissue's homogenate CA19-9 and serum CA19-9 of all studied groups

مجلة ابن الهيثم للعلوم الصرفة والتطبيقية المجلد22 (1) 2009

دراسة مقارنة لمستويات CA19-9 كدالة ورمية في أمصال وأنسجة مرضى مصابين بسرطان الثدي والبروستات والغدة الدرقية

عمار هاني الدجيلي، وفاء الطائي، قسمة محمد تركي، غيد حسان العبيدي
قسم الكيمياء ، كلية التربية ابن الهيثم ، جامعة بغداد

الـــخـــــلاصـــــة

قيس مستوى المستضد الكاربوهدراتي (CA19-9) في امصال ،40 ثمانية مصابين بسرطان الثدي (G_1)، 8 مصابين بسرطان اليروستات (G_2)، 12 مصابا" بسرطان الغدة الدرقية (G_3)، وثلاثين شخصا من الاصحاء مجموعة سيطرة (G_4) ، بطريقة تحليل الامتزاز المناعي الانزيمي التاصري (ELISA)، تم أخذت النماذج من المرضى الذين ادخلوا الى مستشفيات مدينة الطب قبل اجراء عملية استئصال الورم. اما نماذج النسيج فقد اخذت مباشرة بعد الجراحة وحفظت في المحلول الملحي عند -20°م حتى وقت المجانسة.

كانت النتائج لمستوى المستضد الكاربوهدراتي(CA19-9) في امصال المرضى كمايأتي : (16.309 ± 7.143 , 31.281 ± 0.766 و 11.5 ± 0.707) U/ml لـ (G_1 G_2, G_3 و)مقارنة بمستوى هذا المستضد مجموعة الاصحاء G_4 التي كانت 7.74 ± 4.92 U/ml وقد اظهرت النتائج ان للمستضد قيمة معنوية عالية بالنسبة الى مجموعة مجموعة الاصحاء ($p < 0.005$).

كانت النتائج لمستوى المستضد الكاربوهدراتي(CA19-9) في انسجة المرضى كمايأتي: (30.554 ± 17.39 , 142.375 ± 8.00 و 194.5 ± 92.4) U/ml. اظهرت النتائج قيمة معنوية عالية لمستويات المستضد (CA19-9) في المجانس الانسجة المستئصلة لكل مجموعة من المرضى مقارنة مع المستضد في امصال المجموعة ($p < 0.005$)نفسها .