Detection of Carbohydrate Antigen CA19-9 Levels in Sera and Tissues' Homogenate of Breast and Thyroid Benign Cases

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Abstract

The aims of the present study are to evaluate the levels of CA19-9 in sera and tissues' homogenate of breast and thyroid benign patients in order to assess its use as an early diagnostic parameter in differentiation between malignant and benign cases. The study was conducted on 8 patients with breast benign tumor and 8 patients with thyroid benign tumor, by the enzyme linked immunosorbent assay (ELISA) technique. The results of CA19-9 levels in sera were (15 ±1.58 and 10.67 ±2.08)U/ml respectively compared with serum CA19-9 levels of control group which was 7.74 ±4.92 U/ml, the results were found to be highly significantly in breast tumor patients and non significantly in thyroid tumor patients than control group. The results of CA19-9 levels in tissues' homogenate were (356.2 ±173.75 and 20 ±14.4)U/ml respectively. The results were found to be highly significant in tissues' homogenate of breast tumor patients and non significant in thyroid tumor patients of higher compare with the it's serum levels of the same patients groups.

Key words: Carbohydrate antigen CA19-9, Benign Cases and CA19-9, Breast tumor and CA19-9, Thyroid tumor and CA19-9.

Introduction

The term "polyp" is clinical description of any evaluated tumor which may be found in tissues as a projection in the lumen as polyp referred as benign tumor. Tumor markers in general are substances present in or produced by a tumor or by the tumor host in response to the tumor's presence that can be used to differentiate a tumor from normal tissue or to determine the presence of a tumor based on measurements in the blood or secretions. Although increased levels of serum tumor markers are often associated with the presence of cancer, marker concentrations may also rise in a number of benign conditions. Chemically, CA19-9 is a tetrascarheride derived from Lewis blood group antigens that are not exclusive to erythrocytes, but they can be found in different tissues and organs. Carbohydrate antigen CA19-9 after its discovery was first described as tumor associated antigen and used as tumor marker for colon and pancreas cancer. In addition, a previous study demonstrated that the level of serum CA19-9 is dependent on the severity of the bile duct obstruction and the degree of cholangitis. An increase in the serum level CA19-9 can be detected even in benign bile duct diseases. It has been reported that CA19-9 was elevated in breast malignant cases only, while the level of CA19-9 was within normal value (37U/ml) in the benign cases. A recent study claimed that CA19-9 levels in sera of thyroid cancer was found to be significantly higher than that for control group. The aims of the present study are to investigate CA19-9 levels in sera and tissues homogenate of patients with benign breast and thyroid tumors by enzyme linked immunosorbent assay ELISA technique, then to assess the differences in the levels of CA19-9 in the serum of benign tumor patients and control, also to correlate the serum and tissue homogenate levels of CA19-9 in patients with breast and thyroid, such determinations might be useful to predict whether or not the tumor marker is related to the benign tumor.
Materials and methods

Patients

The patients included in this study with age ranged 17-75 years, were classified into three groups as follows:

1. The first group included 8 patients with benign breast tumor (G1).
2. The second group included 8 patients with benign thyroid tumor (G2).
3. The last group included 30 healthy subjects considered as control group (G3).

The patients were selected, according to the histopathological investigation. They were admitted for treatment at Medical City Hospitals (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 drawn from each patient by vein puncture just before surgery, left to clot, and then centrifuged at 4000 r.p.m. 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 scalpied 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 4000 r.p.m 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

A basic ELISA work follows simple steps according to included manufacturer’s instructions of CA19-9 Kit supplied by Dia Metra Company, Italy.

Statistical analysis

Comparison between variables was performed by using student's t-test. P values ≤ 0.05 considered significant and ≤ 0.001 considered highly significant.

Results and discussion

The mean ± standard deviation (SD) for serum levels of CA19-9 of control group was found to be 7.74 ± 4.92 U/ml from table (1)

Table 1: CA19-9 values in sera of patients groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast benign</td>
<td>15 ±1.58</td>
<td>0.0027</td>
</tr>
<tr>
<td>Thyroid benign</td>
<td>10.67 ±2.08</td>
<td>0.32</td>
</tr>
<tr>
<td>Control</td>
<td>7.74 ± 4.92</td>
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</tbody>
</table>

A highly significant elevation in the level of CA19-9 in sera of benign breast patients, but a non-significant elevations were found in sera of thyroid patients compared to control was observed. The results are in agreement with other reported data stated that elevated serum CA19-9 levels were found in 61% of benign cases, while CA19-9 serum levels remain within normal range in 50% of malignant cases. A study performed on 91 adults to determine validity of CA19-9 among other cancer antigens as tumor markers, which claimed that CA19-9 showed statistical differences in the serum and ascitic fluids both of malignant and benign compared with control. A research conducted to evaluate the etiology of elevated CA19-9 serum level in 353 subjects, 2.8% were diagnosed with malignancies, 27.5% with benign and 69.7% were non tumor patients, the authors concluded that CA19-9 should not be used as a screening tool, also in cases of a persistently elevated CA19-9 levels, further work-up for determining the etiology should be done. A recent study has confirmed the role of estrogens as the cause of endometrial thickening through hormonal imbalance leading to elevated CA19-9 among other tumor markers in the serum of benign gynecological conditions may be a source of misdiagnosis of malignant diseases. Table 2 shows the results of the mean ± SD of serum and tissue homogenate of breast and thyroid benign patients. A highly significantly elevation were found in tissue homogenate compared to serum in both studied groups. A study conducted on patients with different malignant diseases and benign conditions postulated that CA19-9 may be used as fairly reliable diagnostic tool, but cannot be used to predict survival. Some authors have reported that the elevation in CA19-9 could be provided by differences in the cellular and humoral immune response to hydatidosis. The theories about these findings include substance that may be synthesized by the host in response to infection which lead then to conclude that the possibility of a hydatidosis should be born in mind in
differential diagnosis of palpable mass and elevated CA19-9 levels in the breast especially in endemic areas.\(^{(13)}\)

**Table (2):- CA19-9 value in sera and tissues of patients groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Mean ± SD</th>
<th>Tissues Mean ± SD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast benign</td>
<td>15 ±1.58</td>
<td>356.21 ±173.75</td>
<td>4.7*10^-4</td>
</tr>
<tr>
<td>Thyroid benign</td>
<td>10.67 ±2.08</td>
<td>20 ±14.4</td>
<td>0.187</td>
</tr>
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</table>

**Conclusion**

A conclusion could be drawn that the tumor marker CA19-9 was elevated in benign conditions like breast and thyroid benign tumors, so it couldn't be used as peculiar detective, screening and diagnostic tool for malignant cases.

**References**