Immunohistochemical Study of CA19-9 Overexpression In Colorectal Adenocarcinoma and Its Correlation With Some Pathological Parameters

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Abstract:
Background: Colorectal carcinoma is third most frequent cancer in the world in both sexes and the third most frequent cause of cancer related deaths. Its incidence and mortality rates vary markedly around the world. The risk of developing colorectal carcinoma increasing with age. Most cases occur in the sixty and seventy years old, while cases before age fifty years old are uncommon unless there is a family history of early colon cancer. The pathogenesis of colorectal carcinoma is complex and developed in multisteps process in which several gene mutations will occur and coordinate with each other in genotyping and phenotyping outcome.

Aim of the study: To assess CA19-9 over expression in colorectal adenocarcinoma and its correlation with some pathological parameters.

Materials and methods: this study was included forty cases of colorectal carcinoma, collected randomly from the period of February 2010- May 2012 from Al Hilla Teaching Hospital. 40 patients (30 male and 10 females) of colorectal adenocarcinoma ,their median age 50 years range from (30 – 70) years . A group of 10 patients with nonspecific colitis were included as a control group. A manual avidin biotin peroxidase complex procedure(ABC) system was used in the imunohistochemical analysis (Dako Cytomation Copenhagen ,Denmark).

Results: CA19-9 over expression was positive in (70%) of colorectal adenocarcinoma, while there was no expression in benign colonic lesions. CA19-9 immunohistochemical staining was positively correlated with grade and stage of colorectal adenocarcinoma.

Conclusion: These finding support the role of CA19-9 in carcinogenesis of colorectal adenocarcinoma regarding behavior and aggressiveness, and thus CA19-9 could be considered as a poor prognostic parameter in colorectal adenocarcinoma.

Keywords: colorectal carcinoma, A manual avidin biotin peroxidase complex procedure(ABC) system, CA19-9.

Introduction: Colorectal carcinoma is the third most frequent cancer in the world in both sexes and the third most frequent cause of cancer related deaths (Jon,2009).
Its incidence and mortality rates vary markedly around the world. CRC is the third most commonly diagnosed cancer in males and the second in females, rates are substantially higher in males than in females (Jemal et al., 2011).

The risk of developing colorectal carcinoma increasing with age. Most cases occur in the sixty and seventy years old , while cases before age fifty years old are uncommon unless there is a family history of early colon cancer ,history of Familial adenomatous polyposis (FAP) which carries a near 100% risk of developing colorectal carcinoma and a history of chronic ulcerative colitis (WHO, 2006).

The pathogenesis of colorectal carcinoma is complex and developed in multisteps process in which several gene mutations will occur an coordinate with each other in genotyping and phenotyping outcome ,98% of the cases is adenocarcinoma type (Satya and Deodutta, 2003).

Carbohydrate antigen(CA 19-9) is a tumor-associated mucin glycoprotein antigen that is related to the Lewis blood group protein, this antigen is present in epithelial tissues of the pancreas, biliary ductular cells, stomach, gall bladder, colon, endometrium, salivary glands, prostate, normal pancreatic juice, bile (in benign conditions), and even seminal fluid contain CA 19-9(Steinberg, 1990). However, approximately 5% of the population are Lewis antigen A- B- and do not produce the CA 19-9 antigen ,this assay cannot be used in these patients(Toft, et al 1991).

Carbohydrate antigen(CA 19-9) is associated antigen defined by murine monoclonal antibody NS 19-9 which was established from colon cancer cell line SW 116 (Haglund, et al., 1992).

This sialylated Lewis A blood group antigen is identified by a radioimmunometric assay CA 19-9 (Villano, 2000). Normal level in serum up to 37 unit/ml. may be elevated in healthy patients as well as in patients with benign and malignant condition ( Nishihara et al.,1994).

The monoclonal antibody-defined, tumor-associated antigen Ca 19-9, chemically identical with the sialylated Lewisa-carbohydrate determinant of a monoganglioside and a mucin, was demonstrated by radioimmunoassay to be present in large amounts as component of fucose-rich sialoglycoproteins, which had been extracted from human seminal plasma of healthy donors. The carbohydrate antigen of these glycoproteins (m greater than 205 kDa and m 115 kDa)(Kouri, et al 1992). Sialyl Lewis a, as well as its positional isomer sialyl Lewis x, serves as a ligand for vascular cell adhesion molecule E-selectin and facilitates hematogenous metastasis through mediating adhesion of circulating cancer cells to vascular endothelium. Patients having both strong sialyl Lewis a expression on cancer cells and enhanced Eselectin expression on vascular beds are at a greater risk of developing distant hematogenous metastasis and also that plays a role in the process of tumor progression as an adhesion molecule(Reiji Kannagi et al , 2007).

Materials and Methods:
This study was included forty paraffin embedded samples (30male and 10 females) from patients with colorectal adenocarcinoma were collected randomly from the period of February 2010- May 2012 from Al Hilla Teaching Hospital.

The clinical informations were collected including age, sex, site of the tumor, histological type ,stage and grade of the colorectal carcinoma from the clinical reports of the hospital, the cases were classify according to the tumor site to 14 (35%)colon cancer,26 (65%) rectal carcinoma, ,the median age 50 years range from (30–70) years.

The cases according to modified Dukes staging system (Dukes and Bussey 1958) classified as Dukes stage A, stage B and as Dukes stage C, and no case recorded as
Dukes stage D, the cases according to the grade of the tumor were classify into three grades as grade I, grade II and grade III. A group of 10 patients with benign colonic lesions (non specific colitis) was used as a control group. Tissue sections of 5-Mm thickness from formalin–fixed, paraffin–embedded blocks were taken for immunohistochemistry.

The Avidin Biotin Peroxidase Complex procedure (ABC) (Vlrika VM 1994) was used for immunohistochemical detection of CA19-9.

The criterion for positive reaction was dark brown staining in the cytoplasm and or at the cell membrane. The scoring percentage were assessed by counting the percentage of positive cells in 100 malignant cells at 40x based on the total magnification for at least 5 fields, four scaled scoring system were chosen in this study (Afdhal et al 1987):

**CA19-9 Scoring system (N H Afdhal et al scoring system)**

- Grade 0  no stain
- Grade I  revealed weak stain less than 5% localized to the periphery.
- Grade II  focal intense stain between (5-70%) of section.
- Grade III  diffuse stain.

Considering Grade 0 and Grade I are negative, Grade II and Grade III are positive. The results were statistically evaluated by the help of SPSS version 10 software using Chi-square test.

**Results:**

In all sections of benign control colonic lesions (colitis), none of them revealed a positive overexpression for CA19-9. While in this study group, CA19-9 overexpression was reported in 28 (70%) out of 40 cases of colorectal adenocarcinoma, with highly significant difference in comparison with control group , (P < 0.05) (Table 1).

In colorectal tissue, it has been found that CA19-9 overexpression was positive in 10(71,42%) of colonic carcinoma and 18(69,23%) of rectal carcinoma without highly significant difference between these two sites (p > 0.05) (table 1).

In our study according to the grade distribution, CA19-9 overexpression in relation to grade of tumor revealed that positive CA19-9 was reported in 10 (55,55%) of grade I, 10(71,42%) of grade II, and 8(100%) grade III, with a positive correlation between the detection rate of CA19-9 immunostaining and grade of tumor, i.e., as tumor grade increase more CA19-9 overexpression, ( P < 0.05 ) (table 1) figure 1,2.

The intensity of immunostaining of CA19-9 antigen in relation to the grade of tumor show that, there is no significant difference between the intensity of CA19-9 and the grade of tumor ( P > 0.05) (table 2).

According to the stage of the tumor, CA19-9 overexpression was positive in 3(37,5%) cases in stage A, 8(66,66%) of stage B, and 17 (85%) of stage C, so there was increase in the detection rate of CA19-9 from stage A-C with significant differences between these stages, ( P < 0.0 5) (table 1).

The intensity of immunostaining of CA19-9 overexpression in relation to the tumor stage, revealed that there is significant difference between different tumor stages and the intensity of CA19-9 (P< 0.05) (table 2).
Table(1): CA19-9 overexpression in relation to type of tissue ,site of cancer , grade and stage of colorectal adenocarcinoma.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign(colitis)</td>
<td>0</td>
<td>10 (100%)</td>
<td>10</td>
<td>P&lt;0,05</td>
</tr>
<tr>
<td>Malignant(adenocarcinoma)</td>
<td>28 (70%)</td>
<td>12 (30%)</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td><strong>Site of cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colonic carcinoma</td>
<td>10(71,42%)</td>
<td>4(28,57%)</td>
<td>14(35%)</td>
<td>p&gt;0,05</td>
</tr>
<tr>
<td>Rectal carcinoma</td>
<td>18(69,23%)</td>
<td>8(30,76%)</td>
<td>26(65%)</td>
<td></td>
</tr>
<tr>
<td><strong>Grade</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>10(55,55%)</td>
<td>8(44,44%)</td>
<td>18(45%)</td>
<td></td>
</tr>
<tr>
<td>Grade II</td>
<td>10(71,42%)</td>
<td>4(28,57%)</td>
<td>14(35%)</td>
<td></td>
</tr>
<tr>
<td>Grade III</td>
<td>8(100%)</td>
<td>0</td>
<td>8(20%)</td>
<td></td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage A</td>
<td>3 (37,5%)</td>
<td>5 (62,5%)</td>
<td>8 (20%)</td>
<td>P&lt;0,05</td>
</tr>
<tr>
<td>Stage B</td>
<td>8 (66,66%)</td>
<td>4 (33,33%)</td>
<td>12(30%)</td>
<td></td>
</tr>
<tr>
<td>Stage C</td>
<td>17 (85%)</td>
<td>3 (15%)</td>
<td>20 (50%)</td>
<td></td>
</tr>
</tbody>
</table>

Table (2): The percentage score of CA19-9 overexpression in relation to grade and stage immunostaining in colorectal adenocarcinoma.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CA19-9 Intensity (score)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grade</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>3(16,66%) 5(27,77%) 4(22,22%) 6(33,33%)</td>
<td>18(45%)</td>
</tr>
<tr>
<td>Grade II</td>
<td>2(14,28%) 2(14,28%) 5(35,71%) 5(35,71%)</td>
<td>14(35%)</td>
</tr>
<tr>
<td>Grade III</td>
<td>0 0 3(37,5%) 5(62,5%)</td>
<td>8(20%)</td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td></td>
<td>P&gt;0,05</td>
</tr>
<tr>
<td>Stage A</td>
<td>2(25%) 3(37,5%) 1(12,5%) 2(25%)</td>
<td>8(20%)</td>
</tr>
<tr>
<td>Stage B</td>
<td>2(16,66%) 2(16,66%) 3(25%) 5(41,66%)</td>
<td>12(30%)</td>
</tr>
<tr>
<td>Stage C</td>
<td>0 3(15%) 7(35%) 10(50%)</td>
<td>20(50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P&lt;0,05</td>
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</tbody>
</table>
Discussion:

Immunohistochemistry is widely used in a basic research to understand the distribution and localization of biomarker in the tissue. However, it is widely used in diagnosis and treatment of cancer because specific molecular tumor markers are characteristic of particular cancer types (Boenish, 2001). CA 19-9 can be positive in colorectal, pancreas, gastric, breast as well as in liver cancers (Yoshikawa, et al 2000), in present study, the importance features of CA19-9 staining in colorectal adenocarcinoma were analysed as followed:

CA19-9 overexpression was detected in 70% of colorectal carcinoma and this agree with (Callen, et al 1987), this study has been found that CA19-9 overexpression was 75% and also agree with (Afraa, 2011), this study was found that overexpression of CA 19-9 was 69.23% in colorectal carcinoma, these results also agree with
(Afdal, et al in 1987) had clarified that CA19-9 overexpression was 75% in colorectal carcinoma and agree with (Nakayama, et al 1997) that has been found that CA19-9 overexpression was 71%.

Our results of this study disagree with (Delvillano, et al 1983), was found that the CA19-9 overexpression were 53% of colorectal carcinoma, also disagree with (Shimono, et al 1994) was found that the CA19-9 overexpression were 56% of colorectal carcinoma.

In the present study CA19-9 overexpression was absence from benign colonic lesions (colitis), these results agree with (Afdhal et al in 1987) had been found that CA19-9 overexpression was absence in normal mucosa and also agree with (Afraa, 2010), also we don't find any research disagree with our finding.

These variations in reported rates of CA19-9 overexpression is may be due to differences in the applied scoring criteria for the assessment of CA19-9 overexpression. It also could be attributed to tumor heterogeneity, sampling, methods used, the type of antibody and the antibody delusional rate.

In our study, according to grades of tumors, there was positive of CA19-9 staining in 0(55,55%) of grade I, 10(71,42%) of grade II and 8(100%) of grade III so there was increase in the rate of CA19-9 as the grade increase and this agree with (Afdhal et al 1987), they had clarified that CA19-9 expression was expressed more frequently in poorly differentiated adenocarcinoma rather than well or moderately differentiated types without significant difference between the percentage of the grades (p>0.05), and this disagree with (Sakmoto, et al 1987), (Armin et al, 2006) and (Afraa, 2010) that found their studies no relationship between the grades of tumor and CA19-9 immunostaining.

The results of this study have revealed that CA19-9 overexpression in colorectal adenocarcinoma was increasing as the depth of tumor increased (37,5% in A, 66,66% in B, and 85% in C), with a significant difference between stages of the carcinoma, (p< 0.05), so this agree with (Uras et al 1996) and with (Afraa, 2010), in their study had mentioned that CA19-9 was correlated very well with stages of colorectal adenocarcinoma.

Most of conducted studies were carried out about the detection of this marker serologically because elevated serum level of CA19-9 associated with poor prognosis and more aggressiveness of the disease (Henning Putzki et al 1987).

A study done by (Waaga, et al 2005), was carried out to evaluate the prognostic significance of p53 compared to CEA and CA 19-9 to determine the predictive value of the molecular markers and with tumor specimens were assessed by immunohistochemical staining, the expression of CA-19-9 and p53 as well as CEA in the tumor was dependent on the stage of the patients (CA 19-9 I/II 37,5%, III 80%) and combined with worse prognosis therefor this study suggests that preoperative CEA level combined with CA 19-9 and additional p53 in the tumor are suitable markers for stage III colorectal cancer patients, indicating that these patients should be considered as high risk group. Patients with a high score for p53, CEA, and CA 19-9 in this group may be the best candidates for an aggressive adjuvant therapy.

Another study that done by (Nakayama, et al 1997) to evaluate the prognostic value of CA19-9 tumor expression and CA19-9 preoperative and postoperative serum levels in colorectal cancer patients treated by complete resection was found that 71% CA19-9 tumor expression was identified by immunostaining of primary carcinomas. Positive CA19-9 serum levels (>37 U/ml) were restricted to cases with positive tumor expression. Positive tumor expression, positive preoperative serum level, positive postoperative serum level were all predictive of increased cancer mortality. Patients
with three negative parameters had no recurrences and 97.1% 5-year survival, whereas patients with three positive parameters had 62.5% recurrence and 42.8% 5-year survival.

Regarding the intensity (score) of CA19-9 in relation with the depth of tumor, it has been found that the intensity of CA19-9 was increasing as the depth of tumor increased in colorectal carcinomas (p<0.05). For the best knowledge of the researcher, no published paper regarding this correlation was found.

Monoclonal antibodies to CA19-9 selected for patients based on immunohistochemical staining of biopsies may allow a greater antibody uptake in tumor tissue and improved tumor targeting for therapy in patients with colorectal carcinoma.

Conclusion:

CA19-9 detection in tumor tissue is a marker of aggressiveness of colorectal carcinoma, thus it may be used as a prognostic marker but not as a screening tool due to its low sensitivity and we recognize the patients with high overexpression for high cancer recurrence and death and may be useful in selecting patients for adjuvant therapy.

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