Original Article

Expression of Beta-Human Chorionic Gonadotropin as a Prognostic Factor in Colorectal Carcinoma

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Abstract

Background: Colorectal carcinoma (CRC) is a common malignancy in Iraq and carries a high mortality rate. It has been shown that CRCs express high level of beta-human chorionic gonadotropin (β-HCG) as detected by immunohistochemistry. Objectives: The aim of this study was to assess the expression of β-HCG in CRC and to study the association between β-HCG expression and pathological prognostic parameters. **Materials and Methods:** This study was carried out on 35 colonic specimens consisting of 30 CRC selected from files of Pathologic Laboratory of Al-Kadhymia Teaching Hospital and Teaching Laboratories of the Medical City, Baghdad. Paraffin blocks were cut sections stained with H and E stain and sections for streptavidin-biotin immunohistochemical staining using monoclonal antibody against β-HCG. Immunohistochemical sections were examined for positive or negative staining and positivity assessed as: occasional, focal, and diffuse tumors. **Results:** Of the 30 CRCs, 60% were male and 40% were female. The most common site of large bowel malignancy was the rectum 13 (43.3%). Most of the tumors were moderately-differentiated adenocarcinoma (83.3%). More than half of the cases were Stage T3, followed by Stage T2, then Stage T4, 3 cases (10%), and no case in Stage T1. Lymph node metastases were found in 19 cases (63.3%). Twelve cases (40%) of CRC show immunoreactivity to β-HCG. β-HCG expression was more frequent in the left side of the large bowel and more common in poorly-differentiated adenocarcinoma (80%). β-HCG was more positive in Stage T4, and there was significant association with lymph node metastasis. **Conclusion:** Expression of β-HCG in CRC is not an infrequent phenomenon, and no stain was demonstrated in benign and normal colonic specimens, while β-HCG expression associated with poor differentiation, greater local invasion, and regional lymph node metastasis.

Keywords: Beta-human chorionic gonadotropin, colorectal carcinoma, expression, prognostic factor

Introduction

Colorectal carcinoma (CRC) is a frequent and widespread malignancy in Iraq.[1] According to a recent report of cancer registry in Iraq, the percentage of CRC was 5.35% and represented seventh cancer among all body malignancies.[2] It represents the third most commonly occurring visceral malignancy in the United States and the third leading cause of death from cancer in both males and females.[3] In the UK, it represents the second leading cause of death from cancer next to lung cancer.[4] There is instantly growing awareness over the last decade to identify prognostic factors for CRC. Human chorionic gonadotropin (HCG) is one of these factors. HCG is a glycoprotein hormone which is biochemically composed of two polypeptides subunits (alpha and beta) with attached carbohydrate side chain.[5] The hormone is normally produced by the placental syncytiotrophoblast. It is also produced by trophoblastic neoplasms, and the evaluation of beta-HCG is quite a good tool for the diagnosis and control of patient with gestational and nongestational trophoblastic tumors.[6-10] However, it is now well established that production of HCG is not restricted to gestational tumors. A specific sensitive radioimmunoassay technique has allowed the detection of this placental hormone in a wide variety of nontrophoblastic

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neoplasm, but the biological significance of HCG production by nontrophoblastic tumors is not well understood.[10]

Several reports have shown that the production of this hormone by a neoplasm is associated with a more aggressive behavior.[11] However, other studies indicate that HCG detection is of limited value in the management of nontrophoblastic tumor.[12,13] Gastrointestinal (GI) tumors have been shown to be associated with a high incidence of HCG production specifically;[14,15] the serum detection of this hormone in large bowel carcinoma has been estimated to occur between 5% and 41% of the cases.[16] Immunohistochemical studies are more scarce have detected this hormone in 45%–50% of CRCs. However, the relationship between HCG production and biological behavior of these tumors has not been assessed sufficiently.[17]

This immunohistochemical study is to investigate the expression of β-HCG in CRC and to study the association between β-HCG expression and pathological prognostic parameters (type of tumors, grade, stage, and lymph node metastasis).

Materials and Methods

This study was carried out on 35 colonic specimens (randomly selected), consisting of 30 cases of CRC, 3 cases colonic villous adenoma, and 2 normal colonic mucosae. All cases were selected from files of Pathologic Laboratory of Al-Kadhymia Teaching Hospital and Teaching Laboratories of the medical city for the period from October 2004 to June 2005. Clinical information was collected from pathological files. Paraffin blocks of the 35 cases were cut into 5-μm thick sections for H and E stain and 4 μm sections for streptavidin-biotin immunohistochemical staining using monoclonal antibody against β-HCG. H and E sections were examined. Histological type, grade, and stage of the tumors were evaluated. Immunohistochemical sections were examined for positive or negative staining. The primary monoclonal antibody used in this study was anti–beta-HCG (1 mg/ml) (IgG2a/rat) (Immunotech Acoulter, France).

For the avidin-biotin complex, detection system (secondary detection system), alkaline phosphatase method was used in this study, and the immunorecipient visualized as red color. This was purchased from Chemico International. Chromogen reagents used in this study were Fast Red Chromogen A and Fast Red Chromogen B.

The results of immunohistochemical assays should show the location desired colored pigment with consistency that denotes specificity.

In dealing with Fast Red A and Fast Red B chromogen reagents, the presence of a red reaction product in the cytoplasm of tumor cells was indicative of positive reactivity. Counterstain was pale-to-dark blue coloration of nuclei.

Negative control – replacement of primary antibody by phosphate buffer saline (PBS). Positive control: placenta (trophoblastic tissue) were used as positive control in this study.

HCG-B-positive results were classified as occasional, focal, and diffuse, according to following criteria: Occasional: tumors in which only some isolated positive cells were identified. Focal – tumors in which clusters of positive cells were seen in some areas of the tumor, but other regions were negative. Diffuse – tumors in which isolated and/or clusters of positive cells were found throughout most areas of the tumor.

Statistical analysis

Statistical analysis was performed with the SPSS 10.01 Statistical Package for the Social Sciences. The data were presented as mean + standard deviation, Student's *t*-test was used to compare between different groups. The difference was considered significant statistically when $P < 0.05$.

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patient s verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee (Al-Kadhymia Teaching Hospital, Baghdad Health directorate).

Results

A total of thirty cases of CRC, three cases of benign villous adenoma, and two cases of normal colonic mucosa were enrolled in this study. Age and sex distribution of patients was analyzed [Table 1], and there was male predominance (18 cases) representing (60%) of the total cases of CRC with male:female ratio (1.5:1). Three (10%) of the patients were below the age of 40 years and 12 (40%) of the patients were 50–59 years old. Their age range was 27–80 years, with an overall mean age (53.7) years. The rectum was the most common site of carcinoma 13 cases (43.3%) followed by sigmoid 7 cases (23.3%) and then rectosigmoid 3 cases (10%) [Table 2]. Regarding the histological grade [Table 3], the most common grade was moderately-differentiated adenocarcinoma (25 cases) account for 83.3% followed by poorly-differentiated carcinoma (5 cases 16.6%). Considering the depth of infiltration, T3 was the most frequent (16 cases 53.3% followed by Stage T2 [11 cases]) 38.66% then T4 (three cases) 10% and no case in T1 [Table 4].

Table 1: Age groups (years) and sex distribution of patients

Age groups (years)	Sex		Total count (%)
	Male count (%)	Female count (%)	
20-29	1 (3.3)	0	1 (3.3)
30-39	1 (3.3)	1 (3.3)	2 (0.067)
40-49	2 (0.067)	1 (3.3)	3 (10)
50-59	6 (20)	6 (20)	12 (40)
60-69	5 (16.7)	2 (0.067)	7 (23.3)
70-79	3 (10)	1 (3.3)	4 (13.3)
80-89	0	1 (3.3)	1 (3.3)
Total	18 (60)	12 (40)	30 (100)

Table 2: Human chorionic gonadotropin expression in relation to carcinoma site in the large bowel

Carcinoma site in large bowel	HCG		Total count (%)
	Positive count (%)	Negative count (%)	
Caecum	1 (50)	1 (50)	2 (100)
Ascending colon	0	2 (100)	2 (100)
Hepatic flexture	0	1 (100)	1 (100)
Left colon	1 (33)	2 (66.7)	3 (100)
Sigmoid colon	2 (40)	3 (60)	5 (100)
Rectosigmoid	3 (75)	1 (25)	4 (100)
Rectum	5 (38)	8 (62)	13 (100)
Total	12 (40)	18 (60)	30 (100)

HCG: Human chorionic gonadotropin

Table 3: Human chorionic gonadotropin expression in relation to the grade of the tumor

Grade	HCG		Total count (%)
	Positive count (%)	Negative count (%)	
Well differentiated	0	0	0
Moderately differentiated	8 (32)	17 (68)	25 (100)
Poorly differentiated	4 (80)	1 (20)	5 (100)

HCG: Human chorionic gonadotropin

Table 4: Human chorionic gonadotropin expression in relation to the depth of invasion

Depth of infiltration	HCG expression				
	Positive count (%)			Total positive (%)	Negative (%)
	Diffuse	Focal	Occasional		
T1	-	-	-	-	-
T2	1 (9)	1 (9)	-	2 (18)	9 (82)
T3	4 (25)	4 (25)	-	8 (50)	8 (50)
T4	2 (67)	-	-	2 (67)	1 (33)

HCG: Human chorionic gonadotropin

Table 5: Human chorionic gonadotropin expression in relation to lymph node involvement

Lymph node invasion	HCG expression				
	Positive count (%)			Total positive (%)	Negative (%)
	Diffuse	Focal	Occasional		
Positive	5 (26)	4 (21)	-	9 (47)	10 (53)
Negative	2 (18)	1 (9)	-	3 (27)	8 (73)

HCG: Human chorionic gonadotropin

Lymph node status cases showed no lymph nodes metastases, while cases were positive for lymph node metastasis [Table 5].

Positive staining was localized in the cytoplasm of tumor cells as fine red granules [Figure 1]. Positive staining was found in 12 (40%) of 30 cases of CRC. No staining was demonstrated in normal mucosa and adenoma.

Pattern of staining was variable. It was focal in five cases (41.66%), diffuse in seven cases (58.3%), and no occasional staining. Regarding the site of CRC, β-HCG expression was more frequently positive in the left side of the large intestine, 11 out of 25 cases (44%) and the results were statistically not significant [Table 2] ($P \leq 0.05$). β-HCG was expressed in 4 of 5 poorly-differentiated adenocarcinoma (80%), and in the moderately-differentiated adenocarcinoma, it was positive in 32% of the cases, and the results were statistically significant ($P = 0.046$) [Table 3]. Regarding the depth of infiltration, β-HCG was more frequently positive in T4 (67%), as compared to T3 (50%), T2 (18%), and T1 (0%) and the results were statistically not significant ($P = 0.197$) [Table 4]. β-HCG was demonstrated in 9 of 19 (47%) with lymph nodes metastases, but only in 3 of 11 (27%) without lymph nodes metastases. The results were statistically significant ($P = 0.029$) [Table 5].

Discussion

CRC is one of the most common malignancies worldwide. Several clinical, biological, and genetic parameters have been used to assess the prognosis and to help the clinician in optimizing therapies for CRC patients. Studies indicate that the most important prognostic variable is the tumor stage; however, patients who are apparently at the same pathological stage often have different outcomes.[18]

Efforts have focused on identifying new prognostic factors that predict the clinical outcome of CRC patients, with the goal of providing a rational approach for planning specific therapy. β-HCG expression has been studied in many tumors, including CRC. The identification of β-HCG as a sensitive prognostic marker may allow the use of adjuvant therapy in subset of patients with worse prognosis, with a resultant improvement in their survival.[18] In Iraq, the reports of Iraq cancer registry showed that colorectal cancer incidence increased and exceeding gastric cancer to become the leading GI tract cancer for the past 15 years.[19] Colorectal cancer is not uncommon in Iraq as has been believed and the incidence could well be increasing, especially since the lifestyle is rapidly becoming more Westernized.[1]

Male-to-female ratio in West countries is nearly equal,[4] while this study showed higher incidence of the disease in males (male:female ratio – 1.5:1) which is agreed by Al-Bahrain and Al-Hadithi study that showed similar ratio (male: female ratio – 1.4:1).[19] The sex ratio in other series in Iraq and Arab countries also reported higher male ratio.[20-22] This finding represents difference in sex ratio between Arab and West population, although it may be a function of referral focus.

The peak age incidence on this study was 50–60 years, nearly similar to that of Al-Bahrani and Al-Hadithi study and one decade younger than that of other Iraqi and Arabian studies. The rectum was the most common site for colorectal cancer in this study (43.3%), similar to Al-Bahrani and Al-Hadithi study in Iraq.[19] A similar rectal preponderance has been reported by others.[18]

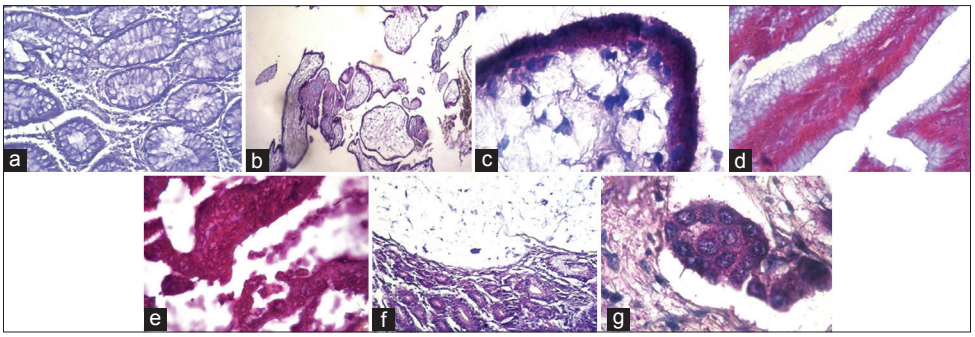

Figure 1: Human chorionic gonadotropin expression immunohistochemical staining, alkaline phosphatase method). (a) Normal colonic mucosa shows negative beta-human chorionic gonadotropin ×400. (b) Trophoblastic tissue shows positive beta-human chorionic gonadotropin × 100. (c) Trophoblastic tissue shows positive beta-human chorionic gonadotropin ×1000. (d) Poorly-differentiated colorectal carcinoma shows positive beta-human chorionic gonadotropin ×400. (e) Moderately-differentiated colorectal carcinoma shows positive beta-human chorionic gonadotropin × 1000. (f) Mucinous carcinoma of colon, positive staining ×200. (g) Moderately-differentiated colorectal carcinoma showing intracytoplasmic distribution of red granules, positive staining, ×1000

Moderately-differentiated carcinoma was the most frequent grade in this study (83.3%) and this finding was agreed by other studies.[19-21]

β-HCG positivity was expressed in 12 of 30 cases of CRC (40%). This incidence is similar to that reported in other studies, Buckley and Fox[12] found β-HCG-positive cells in 26 of 60 (43%) large bowel carcinoma. β-HCG immunoreactivity was negative in all benign colonic lesions and normal tissue, Buckley and Fox[12] also did not observe β-HCG in benign colonic tumor and normal tissue. However, other studies have shown small quantities of β-HCG immunoreactivity in normal colonic mucosa and in other normal tissues including the stomach, kidney, lung, liver, testis, and other.[23,24]

β-HCG expression was more frequently in the left side of the large intestine 11 of 25 cases (44%), which is agreed by other studies.[12,25] The present study reveals that β-HCG expression was more frequently expressed in poorly-differentiated adenocarcinoma (80%), which is agreed by other study.[25] The relationship between β-HCG immunoreactivity and depth of invasion of tumor is shown in Table 4. Eleven of 19 (57.9%) adenocarcinoma that penetrated the full thickness of the bowel wall (T3 and T4) were β-HCG positive cells. On the other hand, only 1 of 9 (11.11%) of T1 and T2 (tumors limited to the submucosa or muscularis properia) were positive, the same findings were found in other study,[12] while Campo *et al*. found that 67% of the adenocarcinoma that penetrated the full thickness of the bowel wall containing β-HCG immunoreactivity and only 30% of the tumor limited to the submucosa or the muscularis properia.[17] The difference noticed could be explained by small sample size.

β-HCG was demonstrated in 9 out of 19 cases (47.36%) with lymph node metastases, but only 4 out of 11 (27.27%) without metastasis [Table 5]. These results are in disagreement with the results of Campo *et al*.,[17] who found β-HCG immunoreactive is 79% in cases with lymph node metastasis and other studies also show significant relation of β-HCG expression with lymph node metastasis.[25] Other studies showed that the production of β-HCG by nontrophoblastic neoplasm can be associated with a more aggressive biological behavior, Tormey *et al*.[26] reported that breast cancer patients with high serum level of β-HCG had poor response to chemotherapy and short remissions. Ito and Tahara have observed a tendency for patients with advanced gastric cancer containing β-HCG to have a lower survival than the β-HCG negative cases.[24] Thus, the presence of β-HCG immunoreactive cells was associated with poorly-differentiated carcinoma, a greater local invasion, and presence of metastasis.

The reasons for association between β-HCG and a more aggressive tumoral behavior are not well understood. Several clinical and experimental studies have indicated an immunosuppressive effect of this hormone.[27] McManus *et al*. have proposed that the β-HCG present on tumor cell surface would suppress the action of the lymphocytes on the tumor antigens favoring the proliferation and invasion of tumor cells.[28] On the other hand, the authors have suggested that the β-HCG in the epithelia may be a marker of the proliferative stem cells and β-HCG expression by epithelial cancers inhibit apoptosis and contributes to the aggressive phenotypes.[29] This highlights β-HCG importance as a therapeutic target.

Overall, we conclude that the expression of β-HCG in CRC was found (40% of CRC revealed positive β-HCG reaction) and no reactivity was demonstrated in benign and normal colonic specimens. Furthermore, the expression of β-HCG is associated with poorly-differentiated carcinoma, greater local invasion, and presence of metastases to the regional lymph nodes. The above data support a model, whereby the immunohistochemical expression of β-HCG is a biological marker of malignancy in colorectal tumor and may also help in determining the prognosis of patient with CRC.

Further studies on larger number of cases with a long follow-up are needed to assess the possible usefulness of β-HCG detection in predicting the evolution of CRC. Preoperative immunohistochemical studies of β-HCG on biopsies of large bowel neoplasms may be of value in the planning of surgical procedures.

CONCLUSION

This study shows a significant association between β-HCG expression and poor prognostics of colon cancer parameters.

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Conflicts of interest

There are no conflicts of interest.

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