Original Article

Immunohitochemical expression of p53 in human colocrectal carcinoma

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Abstract:

Background:- Colorectal carcinoma is the most common cancer after the breast cancer in female and bronchus cancer in male. P53 is a tumor suppressor gene, approximately half of colorectal cancers present mutation in p53 gene.

Objectives:- To determine the frequency and the pattern of p53expression in colorectal carcinoma by immunohistochemical technique and to correlate this expression with different clinicopathological parameters.

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Materials and methods:-Thirty cases of colorectal carcinoma were included in this study, these cases were diagnosed in private pathology laboratories in Baghdad / Iraq from January 2015 to Jaune 2015. Clinicopathological parameters such as age , gender , pathological diagnosis , including the tumor site , lymph nodes status , grade and stage of tumor were taken from patients files.Sections of 4µm stained by hematoxylen and eosin stain and immunohistochemical stained for p53.

Results: Nineteen (63.3%) of the cases were males, 11(36.7%) cases were females, with age distribution ranging from (39-89) years with a mean age of 56.5 years . Ten cases(33.3%) located in the cecum , 3(10%) cases from each right colon , sigmoid 8 (26.7%) cases were from left colon and 6(20%) cases were from the rectum. Histologically the tumor grade range from moderately differentiated in 27 (90%) cases, and poorly differentiated in 3 (10%) cases. Regarding pathological staging (TNM system),2 (6.7%) cases were T2 ,24 (80%) were T3 , 4(13.3%) cases were T4. Lymph node involvement found in 14(46.7%) cases, and distant metastasis was found in 3(10%) cases. P53 expression was present in 11(36.7 %) cases, were distributed as follows:- weak in 1(3.3%) case,moderat in 3(10%) and marked in 7(23.4%) cases. There was no correlation between p53 expression and the clinicopathological parameters age , sex , histopathological grade, location , lymph nodes status and tumor stage.

Conclusion: There was no significant statistical correlation between P53 expression by tumor and different clinicopathological parameters in this study.

Keywords: Colorectal cancer, p53.

Introduction:

Cancer is an important problem in public health world-wide colorectal carcinoma is the most common cancer after the breast cancer in female and bronchogenic carcinoma in male(1). The incidence of colon cancer varies widely from country to country throughout the world approximately 10% are considered inherited (2,3,4). The cell cycle controlled by several genes, the main function of which is the synthesis of proteins promote perfectly organized cell multiplication (5,6). P53 is a tumor suppressor gene prevents the accumulation and propagation of cells containing genetic alteration, p53 is the most commonly mutated gene in human cancer and more than 50% of human cancers contain P53(7,8). Studying colorectal carcinoma, showed that over-expression of p53 product is detected in 60-70% of colonic cancer with immunohistochemistry (9,10,11).Physiological function of P53 is essential for preventing inappropriate cell proliferation and maintaining genome (12,13,14).

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Materials and methods:

This study is retrospectively designed, a total of 30 cases of colorectal carcinoma diagnosed in private pathology laboratories in Baghdad / Iraq during the period from January 2015 to Juan 2015, all cases were evaluated in terms of age , gender , pathological diagnosis , including tumor location , lymph nodes situation , stage and grade of tumor.Two Section of 4µm thickness were taken from paraffin blocks of the tumor, one section was stained with hematoxylin and eosin (H and E), and the other dewaxed and processed for immunohistochemical staining using monoclonal antibodies and kits manufacture by DAKO corporation(Dako Denmark A/S) with 3-3'-diaminobenzidine tetrahydrochloride used as chromogene. The dako cytomation,envision© +dual link system-HRP(DAB+) staining protocol was used for immunostaining to detect nuclear p53,using monoclonal mouse anti-human p53 antibody(dilution 1:30, colon Do7,code M7001,Daco,Denmark A/S)(15).Positive controls used included tissue sections from breast carcinoma with a diffuse p53 nuclear immunoreactivity for p53 marker.For negative control a normal rabitt IgG

instead of the primary antibody was applied.The p53 index was calculated as number of positive cells from 100 positive and negative cells in microscopic fieled investigated with x40 magnification.It is interpreted as having a:low,moderate or marked expression,when the percentage of the colored nuclei represented less than 20%,20%-50%,or more than 50%, respectively(16,17,18).

Results:

The 30 cases that are included in this study are divided into different groups according to each study parameter A comparison has been made among the groups in regards to p53expression.

P53 expression :According to the p53 scoring index (as mentioned preriously), nineteen (63.33%) cases expressed a totally negative staining of p53 marker eleven (36.7%) cases stained positive for marker . their score were as follows : 1(3.3%) case low expression, 3(10%) moderate expression, and 7(23.7%) marked expression. as shown in table (1).

Table(1): The distribution of cases according to p53 expression.

P53expression score	Frequency	Percentage %
0(negative)	19	63.3%
Weak(less than20%)	1	3.3%
Moderat(20%-50%)	3	10%
Marked(>50%)	7	23.7 %
total	30	100%

Age distribution:-Age distribution ranging from (39-89)years ,with a mean age of 56.5 years. eleven(36.7%)cases expressed p53,these cases were distributed among different age groups ,there was no significant statistical correlation between age distribution and p53 expression.

Gender distribution: Nineteen (63.3%) cases were males , 11(36.7%) cases were females,5 (16.7%) cases of males and 6(20%)cases of females expressed p53 . No significant statistical correlation was found between p53 expression and gender (p>0.05). as shown in table(2).

Table (2): The distribution of gender in relation to p53 expression .

P53 expression	Gender		total	p.value
	male	female		
0(negative)	14(46.7%)	5(16.6%)	19(63.3%)	
Weak(less than 20%)	1(3.3%)	0(0%)	1(3.3%)	
Moderate(20%-50%)	2(6.7%)	1(3.3%)	3(10%)	p>0.05
Marked(>50%)	2(6.7%)	5(16.7%)	7(23.4%)	
total	19(63.3%)	11(36.7%)	30(100%)	

Distribution according to the grades of the tumor:- Histologically the tumor grades range from moderately differentiated in 27(90%) cases and poorly differentiated in 3(10%) cases as shown in table (3).nine (30%) cases of moderately differentiated,and 2(6.7%),3(10%) cases of poorly differentiated were p53positive. No significant statistical correlation was found between p53 expression and tumor grade .

Table(3):The incidence and frequency of p53 positive cases in relation to grade of the tumor

P53 expression	Grade		total	P value
	moderate	poor		
0(negative_	18(60%)	1(3.3%)	19(63.3%)	
Weak(less than20%)	4(13.3%)	0(0%)	4(13.3%)	
Moderate(20%-50%)	2(6.7%)	1(3.3%)	3(20%)	p>0.05
Marked(>50%)	3(10%)	1(3.3%)	4(13.3%)	
total	27(90%)	3(10%)	30(100%)	

Topographic distribution of the tumor:- Ten (33.3%) cases located in the cecum, 3(10%) cases in the right colon ,and sigmoid each, 6(20%) cases in the rectum, and 8(26.7%) cases located in the left colon. P53 expression was seen in 3(10%) of cecum ,1(3.3%) case in right colon, left colon ,2(6.7%) in sigmoid and 4(13.3%) cases in the rectum.No significant statistical correlation was found between site of the tumor and expression of p53.as shown in table(4).

Table (4): The distribution of the studied cases according to the anatomical site of the colon and corresponded p53 in each site.

P53 expression	site						P value
	cecum	Rt colon	Lt colon	sigmoid	rectum	total	
0(negative)	7(23.3%	2(6.7%)	7(23.3%)	1(3.3%)	2(6.7%)	19(63.3%)	
Weak(less than 20%)	1(3.3%)	0	0	0	0	1(3.3%)	
Moderat(20%-50%)	1(3.3%)	1(3.3%)	0	0	1(3.3%)	3(10%)	p>0.05
Marked(>50%)	1(3.3%)	0	1(3.3%)	2(6.7%)	3(10%)	7(23.3%)	
total	10(33.3%)	3(10%)	8(26.7%)	3(10%)	6(20%)	30(100%)	

Distribution according to tumor invasion (T):- In this study 2 (6.7%) cases were T2 invading the muscular is propria, 24(80%) cases were T3, 4(13.3%) cases were T4 , eleven cases expressed p53 distrbuted as fllows:1(3.3%) case T2,8(26.7%) cases T3,2(6.7%) cases T4,as shown in table(5).

Table (5): Distribution of p53 over expression by the tumor in relation to depth of wall invasion .

P53 expression	T			total	P value
	T2	T3	T4		
0(negative)	1(3.3%)	16(53.3%)	2(6.7%)	19(63.3%)	p>0.05
Weak(less than 20%)	0	1(3.3%)	0	1(3.3%)	
Moderate 20%?-50%)	0	2(6.7%)	1(3.3%)	3(10%)	
Marked(>50%)	1(3.3%)	5(16.6%)	1(3.3%)	7(23.3%)	
total	2(6.7%)	24(80%)	4(13.3%)	30(100%)	

Distribution according to the lymph nodes status (N of TNM):- Sixteen (53.3%) cases showed no nodal involvement, while 14(46.7%) cases presented with nodal involvement, 9(30%) were N1, 5(16.7%) cases were N2. Eleven cases expression p53. Distributed in relation (N) as fllows:4 (13.3%) cases of N0 ,4 (13.3%) cases of N1,3(10%) cases N4. No significant statistical correlation found between nodal stastus and p53 expressed,as shown in table (6).

Table (6): Frequency distribution of p53 over expression in relation to lymph node status.

P53 expression	N			total	P value
	N0	N1	N2		
0(negative)	12(40%)	5 (16.6%)	2(6.7%)	19(63.3%)	p>0.05
Weak(less than 20%)	1(3.3%)	0	0	1(3.3%)	
Moderate (20%-50%)	1(3.3%)	2(6.7%)	0	3(10%)	
Marked	2(6.7%)	2(6.7%)	3(10%)	7(23.3%)	
total	16(53.3%)	9(30%)	5(16.7%)	30(100%)	

Discussion:

Colorectal cancer results from stepwise progression through several genetic alterations, including in the P53 gene (19). This gene is often found to be altered in tumors, and is one of the most frequently inactivated genes in human cancer (20,21). Results of this study showed that there is no significant statistical correlation between age and sex of patients included in the study and p53 expression these findings agreed with Michael odida el.al.(22). And other studies (23,24,25).The relation ship between p53 abnormalities and patient survival is a subject of controversy, in patients with colorectal adenocarcinomas,some studies found that p53 in colorectal tumors as assessed by immunohistochemistry is of limited value in predicting clinical out come as mentioned by Garewall et.al.(23) In contrast to others who stated that evaluation of p53 over expression using a standardized immunohistochemical procedure,could be aclinically usual marker.In this study,tumor situated in the ceacum and rectum showed a high percentage of p53 expression, however there was no significant statistical correlation between p53 expression and site of the tumor,this disagree with many previous studies(26,27).P53 expression in this study was higher in moderately differentiated carcinoma, may be because their number was higher than poorly differentiated carcinoma, however there was no significant correlation between p53 expression and grade of tumor,this result agree with other studies(28,29). In respect to TNM staging p53 expression.There was higher in T3 7(23.3%) cases,and in N0 4(13.3%) cases however then was no correlation between T or N and p53 expression,these results agreed with may other studies(30,31,32,33).Lymph node status is the common indicator of prognosis in clinic ,Zhao et.al.2005,showed that p53 expression and lumph node status of the tumor are significant independent prognostic factors of colorectal adenocarcinoma and no association was found between other patient variables(34.35). In this study p53 expression was seen in 11/30 cases (36.7%) this was weak in 1(3.3%) case, moderate in 3(10%) cases, and marked in7(23.7%) cases,and 19(63.3%)cases showed no p53 expression,these results agreed with other studies(26,34,35).

Conclusion:

Colorectal carcinoma is associated with increased p53 expression ,but there was no significant statistical correlation with p53 expression and any of the studied clinicopathological parameters.

Author contributions:

Tharwa Hadi Hassan AL-Tai: Acquisition of data analysis,interpretation of data and drafing of manuscript.

Sazan abdulwahab Mirza: Study conception,design,interpretation of data and critical revision

Hind Hamid Shaker: Acquisition of data analysis and critical revision.

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