Sys Rev Pharm 2020; 11(5): 75 – 79
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Immunohistochemical Study of Adenomatous Polyposis Coli Protein in Colorectal Carcinoma and its Precursor Lesions in Iraq

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Article History: *Submitted: 01.03.2020* *Revised: 05.04.2020* *Accepted: 01.05.2020*

ABSTRACT

Globally, colorectal cancer represents the most common cancer. Its incidence was increases with advancement of age. Both men and women face a lifetime risk of 6% to get colorectal cancer. Colorectal cancer represents a major cancer-related health problem in Iraq and significant attribution to morbidity and mortality related to cancer. It ranked as the 7th most common malignant tumor in Iraq and account for about 5.4% of all cancer in this country. Inflammatory bowel disease represent a risk factor for development of colorectal carcinoma and this was related to increased severity and duration of the disease. On other hand colorectal polyp may transform to carcinoma and this risk was more with larger polyps and polyps with sever dysplasia. Adenomatous Polyposis coli gene is a tumor suppressor gene. Its inactivation represents a common genetic alteration in this tumor. This mutation in such gene occur both I sporadic as well as inherited cancers. This study was designed to clarify the role of APC gene and its protein in both colorectal cancer and its precancerous lesion and put the shadow on its possible role in disease development and progression. APC protein was reported in 33 out of 60 cases of colorectal cancer, 16 out of 50 cases of colorectal polyps, and 11 out of 50 cases taken from patients with ulcerative colitis. There was no expression of APC protein in normal looking tissues in all cases. APC expression was showed significant differences when grade and stage were considered. APC gene was altered in colorectal cancer and its precursor lesion and this alteration were correlated well with grade and stage of disease. The alteration plays a role in both disease development and progression.

Keyword: Immunohistochemical, Adenomatous Polyposis, coli protein in Colorectal Carcinoma

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DOI: 10.31838/srp.2020.5.13

INTRODUCTION

Colorectal cancer represent the most common malignant tumor in North American countries [(1)] . It develops slowly over years and starts as polyp which with time acquires more mutations giving rise to carcinoma [(2)]. Colorectal cancer represents an important cause of death of United States. Genetic and environmental factors were implicated in malignant transformation [(3)]. These factors together causing significant damage to the genetic apparatus and accumulation of mutations causing cancer development [(4,5)]. Colorectal cancer ranked as 6th common cancer in Iraqi men and the 5th common cancer in Iraqi women [(3)]. The pathogenesis of colorectal cancer is a complex process involving interplay between several genetic alteration with multistep acquisition of mutation leading finally to cancer development and progression [(7,8,9)]..There were a clear increased in all cancers in Iraq including colorectal cancer especially after the first and second gulf war's [(10)]. Adenomatous Polyposis coli gene encodes APC protein and this is one of commonest mutated gene in colorectal cancer [(11)]. This gene involved in triggering downstream events that involved in multistep tumor development and malignant transformation [(12)]. The majority colorectal cancers arise from adenomatous polyps. As results, colorectal carcinoma is potentially preventable and is highly curable keeping in mind early tumor detection [(13)]. Inflammatory bowel diseases including Crohn disease and ulcerative colitis represent risk factor for development of colorectal carcinoma especially after lond duration and this highlight the importance of surveillance for such patients [(14)]. A study of meta- analysis of population based cohort studies estimate the risk of colorectal carcinoma to be arise from ulcerative colitis in 1%, 2%, and 5% after 10, 20, and > 20 years of disease duration [(15)]. And even the risk is more in increased duration and severity of the disease [(16-18)]. This study was designed to investigate the expression of APC protein in colorectal carcinoma as well as colorectal polyp and ulcerative colitis by immunohistochemical approach, this to highlight its role in neoplastic transformation as well as correlating its expression to pathological parameter to put the light on its importance in disease progression.

MATERIALS AND METHODS

This is a cross sectional study performed in the department of pathology and forensic medicine-faculty of medicine-University of Kufa over a period of one year. The patients

were informed about the study and informed consent was taken. An ethical approval was taken from ethical committee in faculty of medicine and the study followed the tent of declaration of Helsinki. This study include 60 cases of colorectal carcinoma, 50 cases of adenomatous polyps, 50 cases of ulcerative colitis with dysplasia and 50 cases of normal looking colonic mucosa as control group. All cases are formalin-fixed and paraffin embedded tissue. Fresh tissue sections of 4-micron thickness from all these paraffin blocks of colorectal tissue cases were taken for immunohistochemistry using Dako antibody and staining kits [19]. The reaction was labeled as positive when there is brown precipitate in cytoplasm. We estimate the percentage of positive staining in 100 tumor cells at high power field magnification. Each sample was scanned for at least five fields [20, 21]. Statistical analysis were performed using T test, one way ANOVA test and R-regression test at the level of significant alpha <0.05. Statistical packages for social sciences (SPSS 22) and Microsoft excel 2013 were used for analysis.

RESULTS

This is a case control study it include the follow; 50 (23.8%) normal looking colorectal tissue, 50 (23.8%) adenomatous polyp, 50 (23.8%) ulcerative colitis with dysplasia and 60 (28.6%) colorectal carcinoma. Characteristic of studied groups were displayed in table-1. Staging and grading were performed according to TNM staging system and grading according AJCC cancer staging protocol [22] table (3.1)

Table 1: The frequency and types of presented cases in this study

Parameter	No. of cases	Percentage	Total
Types of tissue			
Normal looking (control group)	50	23.8%	
Comparative group			
- Colorectal adenomatous polyp	50	23.8%	210
- Ulcerative colitis with dysplasia	50	23.8%	
Colorectal carcinoma (study group)	60	28.6%	
Age			
21-30	4	6.7%	
31-40	8	13.3%	60
41-50	9	15.0%	
51-60	11	18.3%	
61-70	12	20.0%	
71-80	16	26.7%	
Gender			
Male			60
Female	32	53.33%	
Grade	28	46.67%	60
Grade I			
Grade II	15	25.00%	
Grade III	22	36.67%	
Depth of invasion (T)	23	38.33%	60
T1	21	35.00%	
T2	20	33.33%	
T3	19	31.67%	

The tumor marker (APC) shows sole cytoplasmic accumulation in the malignant cells. None of the normal looking tissue shows immune reaction. There were a significant differences between these groups (P value=0.0001) and (R= 0.598) (Table-2) (fig.1)

Table 2: APC expression in colorectal carcinoma

Colorectal tissue type	APC expression		Total
	Positive	Negative	
CRC	33	27	60
Normal looking	0	50	50
Total	33	77	110

(P value=0.0001, R= 0.598)

A positive immune reaction for APC was displayed in 16 out of 50 case of colorectal polyps white 34 cases showed negative reaction. There were a significant differences in marker expression when compared with normal looking tissues (P value = 0.0001, R=0.436) (Table-3).

Table 3: APC staining in colorectal polyps

Type		APC expression		Total
		positive	negative	
	Colorectal polyp	16	34	50
	Normal looking tissues	0	50	50
Total		16	84	100

(P value = 0.0001 and R=0.436)

The expression of APC protein was present in 11 out of 50 cases of ulcerative colitis while 39 cases showed negative immune expression. There were significant differences when compared with APC protein expression in normal looking tissue (P value = 0.0001, R=0.352) (Table-4)and (fig-3).

Table 4: APC staining in ulcerative colitis

Type		IHC		Total
		positive	Negative	
	Ulcerative colitis with dysplasia	11	39	50
	normal	0	50	50
Total		11	89	100

(P value = 0.0001, R=0.352)

Five out of 15 cases having grade I colorectal cancer showed expression of APC protein, while 9 out 22 in grade II and 19 out of grade III tumor showed positive immunoexpression for such protein. Statistical analysis of these data showed significant differences in regard to APC protein in correlation with tumor grade (P-value=0.002, r=0.410), table (5)

Table 5: Correlation of APC gene staining with grade of tumor

Grade	Immunohistochemical expression		Total
	positive	Negative	
Grade I	5	10	15
Grade II	9	13	22
Grade III	19	4	23
Total	33	27	60

(P-value=0.002, r=0.410)

Adenomatous Polyposis coli protein were expression in 5 out of 21 patients in T1 stage, 13 out of total cases of 20 in patient of T2 stage and fifteen cases out of nineteen in stage T3 tumor. This pattern of expression showed significant differences in respect to stage of tumor (P value= 0.001, r= 0.456) (Table-6)

Table 6: correlation of tumor stage with APC staining pattern

Extent (depth) of Tumor Invasion		Immunohistochemical expression		Total
		Positive	Negative	
	T1	5	16	21
	T2	13	7	2
	T3	15	4	19
Total		33	27	60

(P value= 0.001, r= 0.456)

DISCUSSION

It was clearly outlined from this study the significant differences in APC protein expression between malignancy, polyps and ulcerative colitis when compared with normal looking tissues (P=0.0001) (table 2, 3, and 4). It was clear that expression of such protein was present in benign and malignant cases. This finding denote to the role of this protein in early neoplastic transformation of such tumor specially in progression from adenoma to carcinoma with acquisition of further mutation as a part of multistep theory for malignant transformation. It was stated that APC protein could be expressed in non-malignant tissues Bourroul et. al.(2013)[23]. The APC gene inactivation leads to accumulation of altered APC protein both in cytoplasm and nucleus [24]. The inflammatory process in ulcerative colitis with continuous injury to epithelial cells stimulate their replication and as a result consumption of APC protein

preventing its expression [25]. Neoplastic colorectal polyp showed APC protein expression. This finding support the theory that colorectal carcinoma could arise from colorectal polyp and this finding were supported by other studies Esteller, et al.(2000)[26] , Knudsen, et al.(2003)[27] and Näthke, et al.(2004)[28]. Five out of 15 cases in grade I colorectal cancer showed expression of APC protein, 9 out 22 in grade II and 19 out of grade III tumor express such protein. This finding clearly outlines that expression of such protein increased with increasing tumor grade. This evidence were also notices by others by Smith, K. J. et al.(1993)[29] and Midgley, et al.(1997)[30]. APC protein were expressed in 5 out of 21 patients in T1 stage, 13 out of 20 in T2 stage and 15 cases out of 19in stage T3 tumor(P value =0.001 , r= 0.456) (Table 3.11). This pattern of APC expression with increased frequency in more advanced tumor denote clearly to the role of APC gene in early tumerogenesis as well as its role during the course of disease as it become more advanced. This finding was supported by what was find by others Midgley, et al.(1997)[30].

CONCLUSION

APC protein expression was presents in colorectal cancer and to a lesser extent in colorectal polyp but not in normal looking colorectal tissue. Its expression was more in more aggressive and more advanced tumor. This indicates its role in early malignant transformation and continued involvement with tumor advancement.

ACKNOWLEDGEMENTS

Not applicable

FUNDING

The Authors did not receive any funding for this project

DISCLOSURE OF INTEREST

The authors report no conflict of interest

DATA AVAILABILITY STATEMENT

The data support the findings of this study are available from the corresponding author upon reasonable request.

AUTHORS' CONTRIBUTIONS

ASJ and QMT: conception and work design
ASJ and AHJ: data analysis and draft writing
HSH and AA data analysis and interpretation
AAA and ASJ: Drafting the work or revising it critically for important intellectual content
All authors approved the final version to publish.

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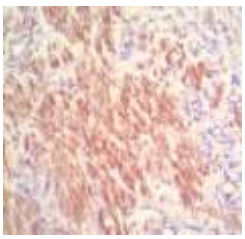

Figure 1: APC staining pattern in colorectal carcinoma (X10)

Figure 2: APC expression in adenomatous polyp (X10)

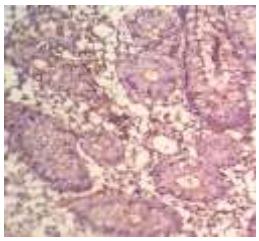

Figure 3: APC expression in ulcerative colitis (X10)