The Expression of P53 and ki67 in Colonic Polyps

ظهور المعلمات السرطانية p53 و Ki-67 في بولب القولون

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الخلاصة :
خلفية البحث: بولب القولون من أكثر الأمراض انتشارا في العراق ويعد سببا لحدوث سرطان القولون .
ان كل من (ki67&p53) بروتينات خلوية تلعب دورا في حدوث السرطان .
KI-67:بروتين خلوي يظهر في نواة الخلايا المتكاثرة في الجسم في جميع الأطوار التكاثرية للخلية بدرجات متفاوتة ويفقد في الطور الصفري.
P53:هو مورث يقع على الكر وموسوم السابع عشر يسيطر على انقسام الخلية ويعمل كمورث محبط للورم.
هدف الدراسة:تقييم التعبير المناعي لعاملي P53,KI-67 في بولب القولون وإيجاد إمكانية تحول بولب القولون إلى سرطان القولون.
المنهجية : طبقت الدراسة على خمسون مريض لديهم بولب القولون للفترة من ٢٠١١ولغاية ٢٠١٤،تم جمع العينات من مستشفى الصدر التعليمي وبعض المختبرات الخاصة في النجف وكانت اعمار المرضى تتراوح بين ٤-٨٠ سنة استخدمت طريقة ال(En Vison) لتحديد التعبير المناعي النسيجي لP53,KI-67 .
النتائج :
أظهرت النتائج إن هناك علاقة بين كل من العمر ونوع البولب مع زيادة في تعبير كل من المعلمين P53,KI-67 بينما لا توجد علاقة بين المعلمين ونوع الجنس .
أظهرت الدراسة ان كلا المعلمين يرتبطان ببعضهما بعلاقة ايجابية.
الاستنتاجات : من النتائج يمكن إن نستنتج إن العلاقة بين المعلمين P53,KI-67 وكل من العمر ونوع البولب يوكد إمكانية حدوث سرطان القولون مع ازدياد العمر وأيضا إمكانية حدوثه في النوعين الأكثر شيوعا هما Adenomatouse وHyperplastic اللذان تظهر بهما فرط التعبير في المعلمين P53,KI-67 لذا فهما أكثر عرضة لحدوث سرطان القولون .
التوصيات: الكشف المبكر لبولب القولون مع الإسراع باستئصاله مما يقلل إمكانية حدوث سرطان القولون وعلى المرضى الذين يعانون من البولب المتضاعف عليهم القيام بالمتابعة الصحية وإجراء الفحوصات باستمرار لمنع حدوث السرطان.

Abstract:
Background: Colonic polyps are slow-growing overgrowth that projects above the surrounding mucosas of the colon that carry a small risk (< 1%) of becoming malignant. However, because colonic polyps are highly prevalent in the general population (especially with increasing age), they confer an important predisposition to colon cancer and are therefore removed when detected. The distribution of Ki67 and p53 as markers of cell proliferation is very studied in Colorectal Carcinoma. In Iraq, IHC studies to detect p53 & ki-67 expression in GIT malignancy has been conducted.
Aim of the study: To detect the expression of p53 & ki-67 in colonic polyps patient by immunohistochemistry .To assess the possible correlation between p53 &ki-67 in colonic polyps patient.To detect the possibility of conversion of pre-malignant colonic polyps to malignant form.
Methodology: Fifty cases(27 male and 23 female) were selected randomly with different types of colonic polyps were included in this study and using discreptive study. The age of patients were ranging from 4 up to 80 years, with mean age of [46.3] years. Cases were collected from the laboratory of histopathology in Al-sadder Medical city in Al-Najaf and from some private laboratories in the same governorate(Al-Najaf) .the data analysed by qi sequare.
Result: There is a significant correlation between P53 & age ,p53 & type of polyp also there is a significant correlation between Ki-67& age, Ki-67 & type of colonic polyp .There is a correlation between ki67 & p53.
Conclusion :The study represent a trial to understand the probable conversion of polyps to colorectal carcinoma and the role of P53 & KI-67 in genesis of colorectal carcinoma.
Recommendation: Screen and follow up to all patient with colonic polyps specially to patient with multiple polyps. All type of polyps should be removed ,because polypectomy currently constitutes the best strategy for preventing colorectal cancer
Key word :Colonic Polyp, Expression,KI-67,P53.

INTRODUCTION:

There are several types of colon polyps. They include nonneoplastic polyps and neoplastic polyps.

Nonneoplastic polyps - These polyps are not likely to develop into cancer. Hyperplastic polyps, hamartomas, lymphoid and inflammatory polyps are all nonneoplastic polyps.

Neoplastic polyps :These polyps are more likely to develop into cancer. Adenomas are the most common type of neoplastic polyp. while others (hyperplastic or inflammatory polyps) have virtually no chance of becoming cancerous[1].Depending on their characteristics (multiplicity, size, histological features, and grade of dysplasia), these lesions can be associated with a substantial risk of recurrence and the development of advanced neoplastic disease. It has been estimated that 15% of all adenomas measuring >1 cm will progress to carcinomas within 10 years of their detection.[2]

P53 &ki-67 :P53 originally referred to 53-kilodalton phosphoprotein, the product of a 20-kilobase gene on short arm of humen chromosome17. P53 gene is a tumor suppressor gene acting as "guardian of genome " or "master watchman " referring to its role in conversing stability by preventing genome mutation[3].P53 was discover in the end of 1979[4].P53 mutation is the commonest genetic alteration detect in more than half of human carcinomas[5,6,7].

The p53 protein is codificated by gene p53, located on the short arm of chromosome 17p, a frequent site of allelic loss in many tumors. The wild p53 maintains the integrity of genes by detecting mutations and preventing the division of cell with damaged DNA .It blocks the cells in G1 phase of cellular cycle. In Colorectal Carcinoma, the gene p53 may be rearranged and p53 protein may be altered. Therefore, the replication errors and deregulation of cells growth could appear. The 17p deletion

is found in 6%-25% of colonic adenomas and P53 gene mutation found in more than 50% of colorectal carcinoma[8]. The distribution of Ki67 and p53 as markers of cell proliferation is very studied in colorectal carcinoma. More articles reveal that their immunostain could predict the colorectal Carcinoma prognostic.The p53 protein is evidentiated by immunohistochemistry.The monoclonal antibody DO-7 reacts with both wild and mutant type of the p53 protein, being expressed intranuclear.So far p53 Immunhistochemistery staining has been advanced and introduce in clinical and histological assessment of colorectal cancer aggressiveness and prognosis[9].

The Ki67 protein is another immunohistochemical marker utilized for identification of proliferative cells. It is expressed in all phases of cellular cycle, except the G0 phase, so Ki-67 is a nuclear and nucleolar protein.[10] In contrast to many other cell cycle associated proteins like PCNA (Proliferating Cell Nuclear Antigen), the Ki-67 antigen is consistently absent in quiescent cells and is not detectable during DNA repair processes. Thus, the presence of Ki-67 antigen is strictly associated with cell cycle and confined to the nucleus, suggesting an important role of this structure in the maintenance and /or regulation of the cell cycle[11].

The monoclonal antibody Ki67, has the particular feature of recognizing the Ki67 antigen in formalin-fixed, paraffin-embedd materials . IHC studies to detect p53 & ki-67 expression in GIT malignancy has been conducted[12].

Aim of the study:

1-To classify the histological subtypes of colonic polyps.

2-To detect the expression of p53 & ki-67 in colonic polyps patient by immunohistochemistry by using paraffin embedded blokes.

3-To assess the possible correlation between p53 &ki-67 in colonic polyps patient.

4-To detect the possibility of conversion of pre-malignant colonic polyps to malignant form.

METHODS:

Biopsy and fixation :
All biopsies were fixed in 10% formalin and sent for histopathological laboratories.

Staining methods:
Preparation of tissue sections:
The specimens were formalin-fixed, paraffin embedded tissue blocks, from these blocks, 5 micrometer-thick tissue sections were obtained and stained with hematoxylin eosin staining method and immunohistochemical Envision staining method.The following steps were applied for (H and E) and immunohistochemical staining methods.

a)Deparaffinization : This has been performed previously by immersion in the followings:
1.Xylene for 5 minutes
2. Xylene for 5 minutes

(b) Hematoxyline and eosine staining methods: (Deparaffinization as in above). Stain in hematoxylin for 3-10 minutes.Wash well in running tap water. Wash well in tap water until sections regain their blue color. Stain in eosin for 2-5 minutes. Dehydrate slowly through increasing grades of alcohols.Clearing by xylene.Mount with DPX
They were stained immunohistochemically with p53and Ki-67 monoclonal antibody (source: Dakocytomation)

Immunohistochemical staining protocol: The immunostaining method used in the current study was the En vision technique which applied for both Ki-67 and p53 staining and included the followings[13,14]: Four µm sections block with Formalin fixed and paraffin embedded human tissue. Mounted on Silanized slides (S3003). The sections were dried for 1 hour at 60°C.Deparaffinization was done by incubating the sections in an oven at 65c over night, followed by two changes in xylene for 10 minutes each , then rehydration in decreasing grades of alcohol (90-70) to distilled water. -Target Retrieval solution, PH 9)Dako cytomation.Incubate in water bath at 95c for (20 to30 minutes).After cooling wash in Envision™ Flex wash buffer (for 5 minutes). Encircle tissue with Pap Pen .Wipe off buffer 1/2 cm above and below the tissue and draw a line with the Pap Pen. Incubate with Envision ™ Flex Peroxidase Blocking-Reagent for (5 minutes). -Wash in Envision™ Flex wash buffer. Incubate with primary antibody for (20 minutes).10-Wash in Envision™ Flex wash buffer.-Envision ™ Flex/HRP secondary antibody (ready to use) for (20 minutes). Wash in Envision™ Flex wash buffer, incubate for (5 minutes).Incubate with Envision ™ Flex- DAB+ chromogen (for 10 minutes). Wash in Envision™ Flex wash buffer. Envision™ Flex hematoxylin (ready to use) incubate for (5 minutes). Wash slides in deionized water. Wash in Envision™ Flex wash buffer. Wash slides in deionized water. Dehydrate and mount the slides.

RESULTS:

Age distribution: Fifty cases of colonic polyps were included in this study. Their ages were ranging from 4 to 80 years. The mean of age was (46.34) and stander deviation S.D was (20.64).

Table 1: frequencies of patient age groups with their percentage:

Age group	Frequency	Percentage
4 – 20	8	16%
21 – 35	6	12%
36 – 50	15	30%
51 – 65	10	20%
66 and >	11	22%
Total	50	100%

In table (1) The ages distributed into five main group include age from less and equal to 20 with percent (16%), from 21-35(12%),36-50(30%) ,51-56 (20%) and from 66 and more than it with percent (22%).the most age group affect by colonic polyps include age group of 36-50 that have high percentage.

Type distribution:

Table 2 :show the four type of colonic polyps and their percentage.

Type of colonic polyps	Frequency	Percent%
Juvenile	9	18
Adenomatous	16	32
Inflammatory	15	30
Hyperplastic	10	20
Total	50	100

In this current study there are 4 different types of polyps include: Adenomatous polyps patients composed 32%(16cases) of whole samples, where as patients of inflammatory polyps are composed 30% (15cases).

Table (3): The correlation between the ki-67 expression and age of the patient with colonic polyps.

Age group	Ki-67 expression			
	Negative	weakly-positive	Strongly-positive	Total
<= 20	7(12%)	0(0%)	1(2%)	8(16%)
21 – 35	4(8%)	1(2%)	1(2%)	6(12%)
36 – 50	4(8%)	0(0%)	11(22%)	15(30%)
51 – 65	4(8%)	0(0%)	6(12%)	10(20%)
66 and >	1(2%)	0(0%)	10(20%)	11(22%)
Total	20(40%)	1(2%)	29(58%)	50(100%)
	Pearson Chi-Square=23.498[a]		df=8 P. value 0.003	

In this table there is Strongly -positive of ki-67 score seen in patient with age group 36-50 and 66 - more than this age.

Table (4): The correlation between the ki-67 expression and type of colonic polyps.

Type of colonic polyps	Ki-67 expression			
	Negative	weakly-positive	Strongly-positive	Total
Juvenail	8(16%)	1(2%)	0(0%)	9(18%)
Adenomatous	0(0%)	0(0%)	16(32%)	16(32%)
Inflammatory	12(24%)	0(0%)	3(6%)	15(30%)
Hyperplastic	0(0%)	0(0%)	10(20%)	10(20%)
Total	20(40%)	1(2%)	29(58%)	50(100%)
Pearson Chi-Square= 43.195		df= 6	P. value 0.000	

In this table Strongly-positive of ki-67 score in adenomatous(16) and hyperplastic polyps(10).So This reveled that there is highly significant association between the ki-67 tier scoring system and type of colonic polyps $p<0.05$.

The correlation between the p53 tier scoring system and age group of patient with colonic polyps:.

Table(5): The correlation between the p53 tier scoring system and age group of patient with colonic polyps.

Age group	P53 Expression				Total
	Negative	weakly-positive	moderately-positive	strongly-positive	
<= 20	7(14%)	0(0%)	0(0%)	1(2%)	8(16%)
21 – 35	5(10%)	1(2%)	0(0%)	0(0%)	6(12%)
36 – 50	3(6%)	1(2%)	1(2%)	10(20%)	15(30%)
51 – 65	1(2%)	2(4%)	1(2%)	6(12%)	10(20%)
66 and >	2(4%)	0(0%)	1(2%)	8(16%)	11(22%)
Total	18(36%)	4(8%)	3(6%)	25(50%)	50(100%)
Pearson Chi-Square=26.172[a]		df= 12	P. value 0.010		

There is significant correlation between age group of colonic polyps and p53.This correlation which shows Strongly-positive highly seen in age group 36-50 and also seen in older than this age. This reveled that there is significant association between the p53 tier scoring system and age of the patient with colonic polyps as shown in table (5).

The Correlation between the p53 tier scoring system and type of colonic polyps:

Table (6): The correlation between the p53 expression and types of colonic polyps

Type of colonic polyps	P53 expression				Total
	Negative	Weakly-positive	moderately-positive	strongly-positive	
Juvenail	7(14%)	2(4%)	0(0%)	0(0%)	9(18%)
adenomatous	0(0%)	0(0%)	1(2%)	15(30%)	16(32%)
inflammatory	11(22%)	2(4%)	1(2%)	1(2%)	15(30%)
hyperplastic	0(0%)	0(0%)	1(2%)	9(18%)	10(20%)
Total	18(36%)	4(8%)	3(6%)	25(50%)	50(100%)
Pearson Chi-Square= 44.698[a]		df= 9	P. value 0.000		

In this table there is strongly-positive of p53 score were seen in adenomatous type (15) and hyperplastic type (9).

DISCUSSION:

This study represents a trial to understand the probable conversion of polyps to colorectal carcinoma and the role of p53 and ki-67 in genesis of colorectal carcinoma and shows the correlation of these factors with different clinicopatholoical parameters like age,gender and types of colonic poylps.

A colon polyps is a growth on the surface of colon.adenoma are the precursors of most colorectal cancers, where as about 15% of colorectal carcinoma develop through an alternatie morphogenetic pathway from serrated polyps.

The appearance of adenomas and their progression to adenocarcinomas is the reslt of an accumulation of genetic changes in cells of the intestinal mucosa inherited or acquired during life . several proteins have been studied in relation to development and progression of colorectal cancer including tumour protein p53 and antigen identified by monoclonal antibody ki-67 .

The determination of more prognostic factors related to the development of colorectal cancer is of fundamental importance for primary preventive programs. Thus , the expression of p53 and Ki-67 may be useful as prognostic factors for adenoma in association with other known histopathologic features.

In this study, patients age ranges from (less and equal to 20- 66 and more years with a mean of(46.34) and stander deviation S.D was (20.64).The most case were affect by colonic polyps include age group of 36-50 with percent (30%), Our data agrees with a study by Khatibzadeh et al.2005[15] and a study by Andrey and Alexander, 2009 in the Russian Federation[16]. These data also support the claim that patients with adenomas and colorectal cancer have a similar age distribution (generally >50 years)this shows by studies of Brenner et al 2008, Mansmann et al,2008 and Santos et al,2007,This mean that colonic polyps are the most cause of malignancy in this age group. [17, 18,19]

In this current study there are 4 different types of colonic polyps but adenomatous polyps patients composed 32%(16cases) of whole sample this mean that adenomatous polyps is the most prevelance type among other type, This result agree with the opinion of Charrette et al.2010[20], that said; two-third of colonic polyps are adenomatous .This may related to geographical factors, in addition to dietary habits[20]. there is significant association between the ki-67 tier scoring system and age of the patient with colonic polyps (p=0.003).this study agree with Nussrat et al.,2011,that said all adenoma with different type and different age group have high expression of ki-67[20].

This current study agree with study of Gurzu et al.,2007; that reveal The age was strongly correlated with the expression of both p53 and ki-67 antibodies (p<0.0001). So, at the older patients, both antibodies were more expressed.[22]

This study agree with study of Tocantins de Sousa et al,2012 that show The expression of Ki-67 was higher in adenomas and in adenomas with high-grade dysplasia[23]

This study also revealed that there is highly significant association between the p53 tier scoring system and types of colonic polyps (p<0.05) also agree with study of Tocantins de Sousa et al,2012[22].The study of Kambara et al,2006 first suggested that some hyperplastic polyps were precursors lesions for malignancy[24].

CONCLUSION:

1-There is highly expression of P53 & KI-67 in certain type of colonic polyps specially the Adenomatous and hyperplastic type , So these type are more labile to transfer to malignant form.

2-There is significant correlation between age and type of colonic polyps with p53 &ki-67 but there is no correlation of p53 &ki-67 with gender ; that mean there is a possibility of colonic polyp to change to colorectal cancer in old age group.

RECOMMENDATION:

1-Screen and follow up to all patient with colonic polyps specially to patient with multiple polyps.

2-All type of polyps should be removed ,because Polypectomy currently constitutes the best strategy for preventing colorectal cancer .

3-Further study for other type of colonic polyps specially inflammatory type by using other type of markers to give more information about relationship of the disease with cancer.

REFERENCES:

1- Steve A., Lowe J.,Scott I., Damjanov I.:Core Pathology 2009;13:262-266.

2- Winawer SJ, Zauber AG, Fletcher RH, Stillman JS, O'Brien MJ, Levin B, et al.: US Multi-Society Task Force on Colorectal Cancer. American Cancer Society Guidelines for colonoscopy surveillance after polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer and the American Cancer Society. Gastroenterology 2006. :1872-1885.

3- Sidransky D., Hollstein M.Annual Reviews Medicine1996;47:285-

4- Strachan T.,Read A.P.: Human Molecular genetics.*Cancer Genetics1999;***2:18.**

5- Joerger A.C, Fresht A.R.: Structural biology of the tumor suppressor p53 and cancer associated mutant.*Ad.Cancer Research* 2007**;4:161-167**.

6- Hussein M.,: Clinicopathological Study of p53-Over Expression in Colorectal Carcinoma by Immunohistochemisty.2007;2-3.

7- Hussein A.S., Hershel J., Ghada K.,: Correlation of Bcl-2 and histopathologic parameters in colorectal neoplasia. *Pathology Oncology Research1999;***5(4):273-279.**

8- Kanarova E.A,Chamacov P.M. :P53in cancer origin and treatment In. Cowell j.k.ed.Molecular Genetic of Cancer 2ed.Oxford :Bios San Diego, CA: Distributed by Acadamic press2001:195-216

9- Munro A.J, Lain S., Lane D.P :P53 Abnormalities and outcome in colorectal carcinoma a systemic review.*B.J. Of Cancer 2005;92;434-444.*

10- Scholzen T, Gerdes J.: The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000. Mar;182(3):311-322.

11- Dimopoulos MA, Moulopoulos LA, Maniatis A, Alexanian R. :Solitary plasmacytoma of bone and asymptomatic multiple myeloma. Blood 2000. Sep;96(6):2037-2044.

12- Al-Sammak F.F. Colorectal carcinoma:A Seroprevalence of helicobacter pylori CagA in association with immunohistochemical staining of C-Myc and MUC-2.A thesis submitted to colllege of Medecine of Al –Nahrin 2004.

13- Chenoweth D, Key M, ed. Immunohistochemical Staining Methods, 4th ed. DAKO, 2006.

14- Sabattini E, Bisgaard K, Ascani S, Poggi S, Piccioli M, Ceccarelli C. The EnVision™ system: a new immunohistochemical method for diagnostics and research. Critical

comparison with the APAA P, ChemMateTM, CSA, LABC, and SABC techniques. *J Clin Pathol* 1998**;51:506-11.**

15- Khatibzadeh N, Ziaee SA, Rahbar N, Molanie S, Arefian L, Fanaie SA. The indirect role of site distribution in high-grade dysplasia in adenomatous colorectal polyps. *J Cancer Res Ther* 2005**. Oct-Dec;1(4):204-207. doi: 10.4103/0973-1482.19587**.

16- Andrey B, Alexander D. Peculiarities of Vascular Component of Communicative Systems in Rectal Adenomas. *International Journal of Collaborative Research on Internal Medicine & Public Health* 2009**;1(1):12-21.**

17- Brenner H, Hoffmeister M, Haug U. Should colorectal cancer screening start at same age in European countries? Contributions from descriptive epidemiology. *Br J Cancer*. 2008;**99:532-5.**

18- Mansmann U, Crispin A, Henschel V, Adrion C, Augustin V, Birkner B, Munte A. Epidemiology and quality of control of 245 000 outpatient colonoscopies. Dtsch Arztebl Int. 2008;105:434-40.

19- Santos Jr JCM. Ano-rectal-colic cancer. Current Issues II - Colorectal cancer - Risk factors and prevention. Rev Bras Coloproctol. 2007;27:459-73.

20- Charette A, Fletcher R, Thomas j, Moynihan l, Bonis p :Colon polyps. Gastroenterology Consultants of San Antonio,2010:1-3 .

21- Nussrat F, Ali H, Hussein H and Al-Ukashi R. :Immunohistochemical Expression of ki-67 and p53 in Colorectal Adenomas: A Clinicopathological Study. *Oman Med J*. 2011 July; **26(4): 229–234.**

22- Gurzu S., Jung J., Mezei T.,and Pavai Z.,:The correlation between the immunostains for p53 and Ki67 with bcl-2 expression and classical prognostic factors in colorectal carcinomas. *Romanian Journal of Morphology and Embryology* 2007**, 48(2):95–99.**

23- Tocantins de Sousa W; Rodrigues L; Gerônimo da Silva R; Vieira F, Immunohistochemical evaluation of p53 and Ki-67 proteins in colorectal adenomas. *Brazil protocol. Gastroenterol*. vol.49 **no.1 São Paulo Jan./Mar. 2012 . 593.**

24- Goldstein N, :Serrated Pathway and APC (Conventional)-Type Colorectal Polyps. American Society for Clinical Pathology.*Am J Clin Pathol* 2006;;**125:146.**