TP53 Over expression In Colorectal Carcinoma By Immunohistochemistry

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الخلاصة:-
اهدف الدراسة هو البحث عن التعبير المناعي النسيجي الكيميائي لجين أل P53 كمؤشر للتغيرات الجينية الطارئة في ألخلايا السرطانية لأورام القولون ومقارنته في النسيج الحميد ولمعرفة ترابط هذا التغير مع ثوابت أخرى مثل درجة تمايز الورم، موقع الورم في الأمعاء الغليضه وأخيرا درجة انتشاره. منذ بداية كانون الثاني ٢٠٠٧ وحتى حزيران ٢٠٠٧ تم جمع ٥٠ عينة من مرضى مصابين بسرطان القولون (٢١أنثى و ٢٩ ذكور) تراوحت أعمارهم بين ١٦ و٨٠ سنه مع معدل عمر ٥٦,٤٤ سنه ،وقد قورنت هذه المجموعة مع مجموعه أخرى (عشرون حاله) مصابين بأمراض القولون الحميدة. أظهرت الدراسة المناعية النسيجية أن تعبير أل- TP53 كان موجبا في ٧٨% من سرطان القولون، بينما لم يلاحظ أي تعبير لهذا ألجين في النسيج الطبيعي (قيمة ألفا < ٠,٠٥). كان تعبير أل- TP53 أكثر في المرضى في القولون البعيد أكثر منه في القولون المستقيم والقولون القريب ، وفي أعلى مراحل الانتشار [Dukes C&D] و في الأورام ذات التمايز الضعيف (الدرجة الثالثة) عنه في الأورام ذات التمايز القوي والمتوسط (الدرجة الأولى والثانية). مع عدم وجود ترابط بين ألتعبير المناعي النسيجي ل TP53 مع موقعه ودرجة انتشاره (قيمة ألفا > ٠.٠٥) كما انه توجد علاقة واضحة لتعبير أل- TP53مع درجة التمايز (قيمة ألفا < ٠.٠٥).

Abstract:-
Fifty paraffin blocks of colorectal carcinomas cases included in this study were randomly selected from January 2007 to Juan 2007 from the histopathological department in AL-Sadr Teaching Hospital.Of the fifty paraffin blocks of the studied colorectal carcinomas' cases; 29 cases (58%) were males and 21 cases (42%) were females. Their ages range was (16-80) years with an overall mean age (56.44±13.558) years , (58%) cases in distal colon (26%) cases were rectal , well differentiated carcinoma in (40%) cases , their stages by Dukes they were (36%) Dukes C followed by (30%) Dukes B then (18%) Dukes D and (16%) Dukes A. Fifty cases were submitted for immunohistochemical stain for P53 antibody, 39 cases (78%) were positive , and positive cases classified as weak positive in 18 cases (36%) and strong positive in 21cases (42%) ,also as faint staining pattern in 15 cases (38.4%) and dense staining pattern in 24 cases (61.6%).TP53 expression was more in distal colon cases (82.7%) , higher stages Dukes C (83.3) and D (88.8%) and poorly differentiated cancer (94.7%) , With no significant correlation of TP53 expression and site and stage of tumor but with significant relationship with grade of tumor

Aim of the study:
To assess the TP53 expression from sections of paraffin blocks of patients with colorectal carcinoma by immunohistochemistry and to evaluate the possible correlations between TP53 expression in colorectal carcinoma and some of its pathological parameters.

Key ward :- *colorectal carcinoma , P53 , IHC.*

Introduction :-

Cancer of colon and rectum is a common malignancy [1]. In Iraq, the incidence of colorectal cancer was 4.55% of whole body malignancy, and represents the seventh cause of death from cancer [2]. It represents the third most common visceral cancer in the United States and the second leading cause of death from cancer in both males and females [3].

P53 :-Is a tumor suppressor gene acting as a transcription factor that plays a key role in the regulation of cell cycle at G1/S regulatory point and the prevention of cancer development [4]. P53 has been described as the "the guardian of genome" or the "master watchman" referring to its role in conversing stability by preventing genome mutation [5]. it was originally discovered in the end of 1979 [6]. The first TP53 mutation was identified in cancer (loss of allele) in 1989. two years later, it was reported as a critical part of a signal transduction pathway that helped cell responding to DNA damage after one year, P53 was voted a molecule of the years by a science magazine[5], in 1999, the number of P53 mutation that described in cancer was 10.000 [7]. TP53 is located in chromosome 17 p13.1 and encodes a 53.000-dalton nuclear phosphoprotien [7-8]. TP53 play an important role in cell cycle regulation and DNA repair and also the induction of apoptosis [9-10]. TP53 mutation is the commonest genetic alteration detected in more than half of human carcinomas [7, 11-14]. The 17p deletion is found in 6%-25% of colonic adenomas and TP53 gene mutation found in more than 50% of colorectal carcinoma [7, 14, 16] and associated with poor survival rate [17-20] .Also TP53 activation may be important in colorectal cancer liver metastasis [21].

Material and method:-

This study included fifty cases of colorectal carcinoma studied prospectively, collected randomly in a period from January 2007 through Juan 2007 from AL-Sadr Teaching Hospital as well as private hospitals & clinics in AL-Najaf City. Their samples including excisional biopsies were collected as paraffin embedded blocks. Patients were grouped according to:

1- The site of the tumor in the colorectum into [23] Proximal site cancer: Includes cancer in the ileo-coecum junction, coecum, ascending colon, hepatic flexure, and right half of the transverse colon ; distal site cancer: includes cancer in the left half of the transverse colon, spleenic flexure sigmoid and descending colon and rectal site cancer: Includes cancer in recto-sigmoidal junction until the anal region not included: ten patients.

2. Histological grades: (according to the WHO classification) [24]: Well differentiated; Moderately differentiated and Poorly differentiated.

3.Stages of tumor: (according to Modified Dukes staging system) into [24]:

i. Dukes A. ii. Dukes B iii. Dukes C. iv. Dukes D.

A control group of 20 patients with non specific colitis disease was also selected randomly from the same center in the same year. A manual avidine-biotin-peroxidase complex procedure was used in the immunohistochemical analysis (Dako Cytomation, Copenhagen, Denmark);the expression is appeared as cytoplsmic staining[25].

The intensity of TP53 over expression staining was either negative, weak positive or strong positive according to the number of cells showing positive expression in comparison to scoring by Sophia K. et al 1999 [26] where by:-

Score zero: none of the cells (or < 5%) were stained
Score +1: less than 10% stained cells
Score +2: 10% - 25% stained cells
Score +3: 26% - 50% stained cells
Score +4: >50% stained cells.
The pattern of TP53 expression was according to qualitative assessment in to faint and dense staining pattern. Statistical analyses of all results were performed according to Chi square test at the level of significant P value ≤ 0.05 and standard deviation depending on Danial 1983 [27] and the help of SPSS statistical package (verge 10&13).

Result :-
All 20 cases of normal colonic tissues (non specific colitis) revealed negative TP53 immunohistochemical over expression in nucleus or cytoplasm.
TP53 over expression was detected only with nuclear localization. (Figure 1A and figure 2 in A&B) . Out of the 50 cases , 39 cases (78%) give positive I.H.C staining and 11 cases (22%) give negative I.H.C staining there were significant relationship between the P53 expression and colorectal carcinoma tissue P value < 0.05 ,(table 1). The positively staining cases were 18 cases (36%) with weak positive as [14 cases score +1 and 4 cases score +2] and 21 cases (42%) were with strong positive as [9 cases score +3 and 12 cases score +4] , (table 2) . Out of 39 positive cases there were 15 cases (38.4) with faint staining pattern , figure (2A) & 24 cases (61.6) with dense staining pattern figure(2B). There were significant relationship between the intensity and pattern of TP53 immunoexpression, (table 3).
Out of the 29 cases at distal site, there were 24 cases (82.7%) positive and out of the 13 cases of rectal site, there were 10 cases (76.9%) positive and out of 8 cases of proximal site, there were 5 cases (62.5%) positive, (table 4). Weak positive nuclear staining was reported in 13 cases (72.2%) of distal site and 2 cases (11.1%) of rectal site and 3 cases (16.6%) of proximal site ,while the strong positive recorded in 11 cases (52.3%) of distal site ; 8 cases (38.1%) of rectal site and 2 cases (9.6%)of proximal site , (table 5). Faint staining pattern was in 7 cases (29%) of distal colon cases, 5 cases (50%) of rectal colon cases & 3 cases (60%) of proximal colon cases and the dense staining pattern was in 17 cases (71%) of distal colon cases, 5 cases (50%) of rectal colon cases & 2 cases (40%) of proximal colon cases, (table 6). There was no significant relationship between the site of tumor and the TP53 expression, intensity & pattern (P value >0.05).
Out of the 20 cases of grade I ,there were 12 cases(60%) positive and out of the 11 cases of grade II ,there were 9 cases (81.8%) positive while in grade III there were 18 cases (94.7%) out of the 19 cases were positive , (table 7) .Weak positive nuclear staining was reported in 4 cases (33.3%) in grade I, 4 cases(44.4%) in grade II and 10 cases (55.5%) in grade III ;while the strong positive nuclear staining recorded in 8 cases (66.7%)in grade I ; 5 cases (55.6%) in grade II and also 8 cases (44.5%) in grade III ,(table 8) . Faint staining pattern was 3 cases (25%) in grade I, 4 cases (44.4%) in grade II & 8 cases (44.4%) in grade III while dense staining pattern was 9 cases (75%) in grade I, 5 cases (55.6%) in grade II & 10 cases (55.6%) in grade III,(table 9).There was a significant relationship between the grade of tumor and the TP53 expression (P value < 0.05) but not with the intensity and pattern of TP53 expression (P value > 0.05).
Out of 8 cases reported in Dukes A, there were 5 (62.5%) positive cases and out of 15 cases reported in Dukes B there were 11 (73.3%) positive cases and out of 15 cases of

group reported in Dukes C, there were 15 (83.3%) positive cases and out of 9 cases reported in Dukes D there were 8 (88.8%) positive cases, (table10).Weak positive nuclear staining was reported in 5 cases (71.5%) in Dukes A , 8 cases (75%) in Dukes B, 4 cases (28.6%) in Dukes C and 1 case (12.5%) in Dukes D ;while the strong positive nuclear staining recorded in 2 cases (28.5%) in Dukes A , 2 cases (25%) in Dukes B, 10 cases (71.4%) in Dukes C and 7 cases (87.5%) in Dukes D , (table11) . Faint staining pattern was reported in 2 cases (28.5%) in Dukes A , 8 cases (75%) in Dukes B, 4 cases (28.6%) in Dukes C and 1 case (12.5%) in Dukes D while dense staining pattern was recorded in 5 cases (77.5%) in Dukes A , 2 cases (25%) in Dukes B, 10 cases (71.4%) in Dukes C and 7 cases (87.5%) in Dukes D ,(table12). There was no significant relationship between the stage of tumor and the TP53 expression (P value > 0.05) while significantly related with the intensity and pattern of TP53 expression (P value < 0.05).

Table 1: IHC expression of TP53 of the colorectal carcinoma in comparison to benign group

Types of tissue	TP53 immunostaining				Total	
	Positive	%	Negative	%	NO.	%
Colorectal Carcinoma	39	78	11	22	50	71
Colorectal Normal	0	0	20	100	20	29
Total	39	55.8	31	44.2	70	100
P value < 0.05						

Table 2: The intensity of TP53 immuno expression in colorectal carcinoma patients.

Scores	*NO.*	*%*
Negative score	*11*	*22*
Weak positive score	*18*	*36*
Strong positive score	*21*	*42*
Total	*50*	*100*

Table 3: The correlation between the pattern and intensity of TP53expression in colorectal carcinoma patients

Intensity of TP53 staining	Pattern of TP53 nuclear expression				Total	
	Faint	%	Dense	%	NO.	%
Weak positive	10	55.5	8	44.5	18	46
Strong positive	5	23.8	16	76.2	21	54
Total	15	38.4	24	61.6	39	100
P value < 0.05						

Table 4: The percentage of TP53 expression in relation to tumor sites.

Sites of tumor	TP53 expression				Total	
	Positive		Negative			
	No.	%	No.	%	No.	%
Proximal	5	62.5	3	37.5	8	16
Distal	24	82.7	5	17.3	29	58
Rectal	10	76.9	3	23.1	13	26
Total	39	78	11	22	50	100
P value > 0.05						

Table 5: The intensity of TP53 expression according to the sites of tumor.

Sites of tumor	*TP53 intensity*				Total	
	Weak	*%*	*Strong*	*%*	NO.	%
Proximal	3	60	2	40	5	12.8
Distal	13	54.1	11	45.9	24	61.5
Rectal	2	20	8	80	10	25.7
Total	18	46	21	54	39	100
P value > 0.05						

Table 6: The pattern of TP53 expression according to sites of tumor.

Sites of tumor	*Pattern of TP53 expression*				*Total*	
	Faint	*%*	*Dense*	*%*	*NO.*	*%*
Proximal	3	60	2	40	5	12.8
Distal	7	29	17	71	24	61.5
Rectal	5	50	5	50	10	25.7
Total	15	38.4	24	61.6	39	100
P value > 0.05						

Table 7: The percentage of TP53expression according to grades of tumors.

Grades of tumor	P53 Expression				Total	
	Positive		Negative		NO	%
	NO.	%	NO	%		
Grade I	12	60	8	40	20	40
Grade II	9	81.8	2	18.2	11	22
Grade III	18	94.7	1	5.3	19	38
Total	39	78	11	22	50	100
P value < 0.05						

Table8: The intensity of TP53 expression according to the grades of tumor.

Grades of tumor	*TP53 intensity*				*Total*	*%*
	Weak	*%*	*Strong*	*%*		
Grade I	4	33.3	8	66.7	12	30.7
Grade II	4	44.4	5	55.6	9	23.2
Grade III	10	55.5	8	44.5	18	46.1
Total	18	46	21	54	39	100
P value > 0.05						

Table 9: The pattern of TP53expression according to grades of tumor.

Grades of tumor	Pattern of TP53 expression				Total	
	Faint	%	Dense	%	NO.	%
Grade I	3	25	9	75	12	30.7
Grade II	4	44.4	5	55.6	9	23.2
Grade III	8	44.4	10	55.6	18	46.1
Total	15	38.4	24	61.6	39	100
P value > 0.05						

Table 10: The percentage of TP53expression according to staging groups

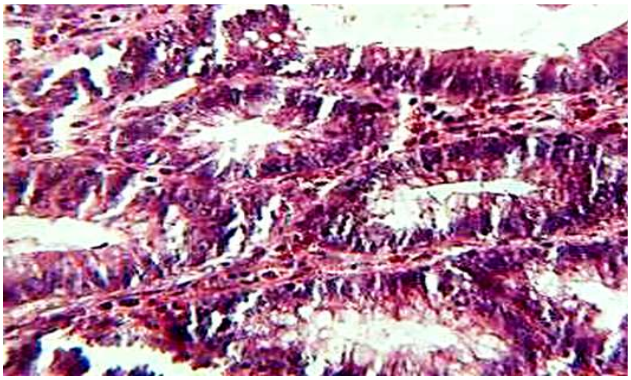
Staging groups	TP53 Expression				Total	
	Positive		Negative		No.	%
	No.	%	No.	%		
Dukes A	5	62.5	3	37.5	8	16
Dukes B	11	73.3	4	26.7	15	30
Dukes C	15	83.3	3	16.7	18	36
Dukes D	8	88.8	1	11.2	9	18
Total	39	78	11	22	50	100
P value > 0.05						

Table 11: The intensity of TP53 expression according to the staging groups.

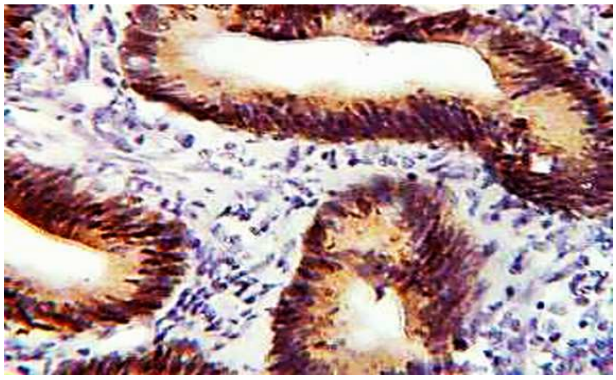
Staging groups	TP53 Expression				Total	
	Weak Positive		Strong positive		No.	%
	No.	%	No.	%		
Dukes A	5	71.5	2	28.5	7	17.9
Dukes B	8	75	2	25	10	25.6
Dukes C	4	28.6	10	71.4	14	35.9
Dukes D	1	12.5	7	87.5	8	20.6
Total	18	46	21	54	39	100
P value < 0.05						

Table 12: The pattern of TP53 expression according to staging groups.

Staging groups	TP53 Expression				Total	
	Faint staining pattern		Dense staining pattern		No.	%
	No.	%	No.	%		
Dukes A	2	28. 5	5	71. 5	7	17. 9
Dukes B	8	75	2	25	10	25. 6
Dukes C	4	28. 6	10	71. 4	14	35. 9
Dukes D	1	12. 5	7	87. 5	8	20. 6
Total	15	38.4	24	61.6	39	100
P value < 0.05						



Figur1:-A-negative nuclear staining of P53 in well differentiated colorectal carcinoma [40X].



B- Nuclear staining of P53 protein expression in well differentiated colorectal carcinoma by IHC [40X].

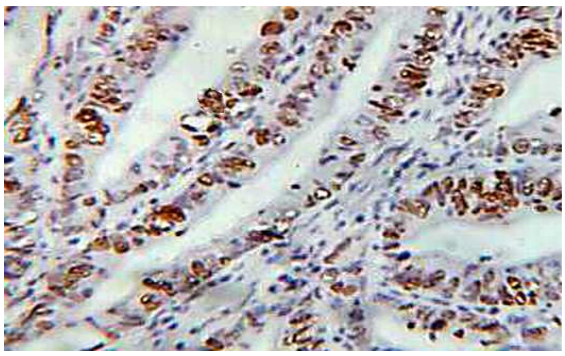
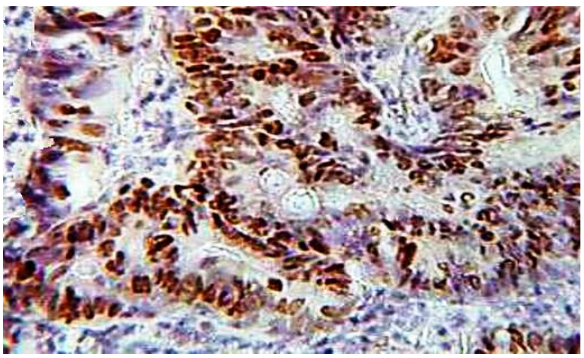


Figure 2:A-Faint nuclear staining pattern (low intensity) of P53expression in well differentiated colorectal carcinoma [40X].



B- Dense nuclear staining pattern (high intensity) of P53 expression in well differentiated colorectal carcinoma [40X]

Discussion :-

The incidence of colorectal cancer shows marked variation throughout the world. In Iraq, the reports of ICR showed that colorectal cancer is rising & exceeding the gastric cancer to become the leading GIT cancer of death for the past 15 years [28].The incidence of colorectal cancer was 4.55% of whole body malignancy, & represents the seventh cause of death from cancer [2]. Colorectal cancer is not as uncommon in Iraq as has been believed & the incidence could well be increasing, especially since the life style is rapidly becoming more westernized [30]. In the United States, colorectal cancer is the second most common visceral cancer in females (15.4% of all cancers) after breast and the third most common cancer in males (14.4% of all cancers) after prostate & lung carcinoma [31] .The P53 gene is mutated and deleted in more than one half of colorectal tumors, leading to inactivation of the P53 protein, and most of the mutations occur almost within its DNA binding domains.[32-35] .The ability of P53 to promote PCD in response to mitogenic oncogenes was vital for its tumor suppressor function.[36]**.** The P53 is a tumor suppressor protein which plays a key role in the regulation of the cell cycle and cell death and involves in cell differentiation, DNA repair, senescence, and angiogenesis, wild type P53 is a labile protein with a short half-life, accumulation and activation of this protein can be triggered by a variety of stress signals including DNA damage, hypoxia, nucleotide deprivation, viral infection, heat shock, and mitogenic or

oncogenic activation [37,38]. The transcription factor P53 lies at the center of a protein network that controls cell cycle progression (growth arrest in cell cycle stages G_1 and G_2) and commitment to PCD. [39] In addition, the P53 tumor suppressor can trigger cell senescence, and DNA repair [40].

In our study no IHC over expression of TP53 could be detected in all sections of 20 cases of normal colorectal tissue. This fact has been supported by Abudolameer K.L 2006; Bakus et al 2002 ; Meek et al 1999; Oren et al 1999 [52, 54, 53, 48], This was explained by that in normal (non stressed) cells TP53 is present at low level & latent (wild type P53) , which is a labile rapidly degraded protein with short half life, accumulation & activation of this protein can be triggered by a variety of stress signals including DNA damage; hypoxia; nucleotide deprivation; viral infection & mitogenic or oncogenic activation [55, 56]. Our results have clarified that (78%) of the CRC cases were expressing TP53 I.H.C. nuclear stain in their histological section. (42%) of them had ≥ 25% immunoexpression score (strong positive) & (61.6%) of them had dense staining pattern. With significant relationship between TP53 expression and colorectal carcinoma tissue and also between the intensity and pattern of TP53 immunoexpression .This result is lower than Abudolameer K. 2006[48], Yamac E. et al 2002[46], Royds J.A. et al 2000[57], In agreement with Karaman G. et al2004[43], Kandiolar et al 2002[58], Moisier et al 1996[59] and Higher than Judith et al 2001[60] , Bakus et al 2002[54]and Kanavaros et al 1999[61] .These results can be explained by that the difference in samples numbers may lead to differences in expressions percentage. The TP53 gene is mutated & deleted in more than (75%) of CRC, leading to inactivation of P53 protein & most of the mutation almost within its DNA binding domains. Also the fundamental positions of P53 as the guardian of the genome reflect its central role in the DNA damage response [47,62].

In present study P53 over expression was higher in distal & rectal cancers than in proximal cancer. Also intensity of P53 expression in distal site CRC cases take the higher percentage than the rectal & proximal; And the staining pattern was denser in distal colon cases than the other site ; with no significant relationship between the site of tumor with P53 over expression , intensity & staining pattern. This is in agreement with Antonio Russo et al 2005, Alexander et al 2003, who found that over (90%) of CR tumor with TP53 over expression were located in the distal colon & rectum [51, 65] Wade .S.et al 2001 observed that TP35 mutation was significant & more common in distal colon tumor,[66] while Papat.S.et al 2006 observed that TP53 expression correlates significantly with the rectal site[67] . Samowit.et al 1997, Soong et al 2000& Diez et al 2000 documented that I.H.C.TP53 expression is associated with distal colon.[64,68,69] . This was explained by that certain dietary associated risks which are the strongest in distal colon & in the rectum .The presenting of these mutagenic agents for long period might have more pronounced effect on P35 mutation & caused the observed association with more aggressive clinic pathological features also they have found significant higher rate of P53 mutation in the left colon than right colon explained by different molecular path to carcinogenesis between right & left sided CRC[41]. Abudoalameer L. 2006 & Maria N. et al 2003 have no significant correlation between TP53 expression & site of CRC [48, 45]**.** In our study the percentage of P53 expression is increased with the dedifferentiation of the tumor. Also the intensity of P53 expression was more in poor differentiated tumor and the staining pattern was denser in well differentiated than other grades. This matches many other studies; Claudia V. et al 2007 who identified that P53 over expression rate was increased with dedifferentiation of CRC [36], Change

W.F.et al 2001 identified that TP53 expression was (33%) in grade I ;(43%) in grade II & (66%) in grade III [50], Abeezar.I.et al 2001 identified that TP53 expression was (50%) for poorly differentiated cancer & (67. 4%) for other grades of tumor [49] & Antonio Rusee et al 2005 &Maria et al 2003; Kyu.Eun.Lee et al 2003 identified that differentiated cancer express more P35 (49. 3%) than undifferentiated cancer (38.9%) [51, 45,42]. In our results, there is a significant relationship between TP35 expression & grades of tumor but not with its intensity or staining pattern; this is not agreed by Abudoameer. L. 2006, Maria.N.et al 2003, Mohammed. R. et al 2007& Abeezar I. et al 2001 [48,45,41,49] .while Karaman G.et al 2004 & Kyu Eun Lee et al 2003 identify that P53 expression has significant correlation with grades of tumor [42,43] . In this study, P53 over expression was higher in advanced stages than in earlier stages. Also the intensity was stronger & the staining pattern was denser in higher stages Dukes C and D than the lower stages. There was no significant correlation between staging group of cancer and TP53 expression while significant relationship with the intensity and staining pattern. This result was in agreement with Yamac E et al 2002, C S Leung. et al 1997, Antonio Russe et al 2005 & Wades et al 2002 [46,62,51,66] . Mohammed Reza et al 2007 identified TP53 expression (52.8%) in B, (65.5%) in C& (88.8%) in D.[66] . Abudolameer2006; Maria N et al 2003; Martha L et al 2002; Morgan M et al 2002& Abeezar I et al 2001 identified no significant relationship [48, 45,47,44,49] .This can be explained by the different in samples size and the cases finding.

Conclusions:- TP53 over expression was significantly noted in colorectal carcinoma tissue, while normal epithelial and benign colorectal tissue did not express TP53 protein.

Recommendations:- Larger epidemiological studies of colorectal carcinoma are warranted for further definition of TP53 gene mutation and to elucidate the effect of TP53 gene mutation on both the survival rates & the response to treatment in Iraq.

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