IMMUNOHISTOCHEMICAL ANALYSIS OF P53 PROTEIN IN COLORECTAL CARCINOMA AND ITS RELATIONSHIP TO CLINICOPATHOLOGIC FEATURES

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Abstract
Colorectal cancer regarded as one of the most widespread malignant tumor in the world. It is considered the second leading death factor among people in some developed countries. Colorectal cancer comprises several distinct histological types including adenocarcinoma which forms 85%-95% of all colorectal cancer cases. Pathogenesis of colorectal cancer is a multistep process characterized by involvement of many genetic alterations, including p53.
The aim of this study is to detect the expression of p53 protein in colorectal carcinoma and to show its relationship with some clinicopathological features including age, gender, histological types, histological grades and staging.
Forty paraffin-embedded tissue blocks of colectomy specimens were used in this retrospective study. They were collected from the Department of Pathology in Sulaimania Teaching Hospital, Shorsh Hospital and Shehid Saifeeddin Private Clinic from January 2007 to July 2008.
Two sections of 4 micrometer thickness were taken from each paraffin embedded tissue block. First section was taken for hematoxylin and eosin stain and the other one for immunohistochemistry [anti-p53 monoclonal antibody] by using DakoCytomation Envision + Dual Link System-HRP (DAB+). The relationship between p53 over expression and the dependent variable (age, gender, histological types, histological grades, and staging) were evaluated statistically using an analysis of variance (ANOVA) with STATA 8 soft ware (College station, Tx). A positive reaction for p53 was scored on a semi-quantitative base as score 0 (no staining), score 1+ (weak staining), score 2+ (moderate staining), and score 3+ (strong staining).
Staining was negative for p53 (score 0) in 16 cases (40%). Positive cases were scored as (1+) in 4 cases (10.0%); (2+) in 8 cases (20.0%); and (3+), in 12 cases (30.0%). There were no significant relationships between p53 over expression and age (p=0.682), gender (p=0.924), histological types (p=0.30), histological grades (p=0.516), and the stage of the disease (p=0.281). Conclusions: Considering the p53 protein over expression in a relatively high percentage of patients, it seems that p53 mutation may play an important role in the development of colorectal carcinoma. There were no significant relationships between p53 protein expression and some clinicopathologic variables such as age, gender, histological types, histological grades, and the stage of the disease.

Introduction
Cancer of the large bowel continues to be one of the major public health problems, ranking as the second or third cause of cancer related death in many western countries with more than one million new cases diagnosed each year. It is the second leading cause of cancer death in the United State. In 2004, in the U.S. there were 145,083 new cases of colorectal cancer (CRC), and 53,580 people died of the disease. There are several etiologic determinants of CRC,
since multiple steps are involved in its occurrence. Several genetic mutation have been detected, and exogenous factors interact for the development of the acquired ones. Risk factors that have been identified include a personal history of colorectal cancer or adenomas, family history of colon cancer or adenomas, inherited colorectal cancer syndromes, and long standing inflammatory bowel disease. There is convincing evidence that nutrition affects colorectal carcinogenesis, also low levels of physical activity, obesity, smoking and excessive alcohol intake, may play a role in colon cancer development. Approximately 80% of colorectal carcinomas occur sporadically, whereas 20% appear to have a genetic basis. However, the recognized familial colorectal cancer syndromes-FAP and HNPCC account for much less than one third of inherited colorectal cancer, leaving the additional heritable component of this disease unexplained. Genetic changes inactivating tumor suppressor genes (APC, DCC, and p53), activating oncogenes (k-ras), and inactivating DNA mismatch repair genes are implicated in the development of CRC.

The aim of this study is to detect the expression of p53 protein in colorectal cancer and its relationships to the clinicopathologic factors including age, gender, histological types, histological grading, and staging of the disease.

Materials and methods
This retrospective study was performed on 40 paraffin embedded tissue blocks of colectomy specimens diagnosed as colonic carcinoma. These were collected from Department of Pathology in Sulaimania Teaching Hospital, Shorsh Hospital, and Shehid Saifeddin Private Clinic from January 2007 to July 2008. Two sections of 4 micrometers thickness were taken from each paraffin embedded tissue block. First sections were put on ordinary slides for hematoxylin and eosin stain while the second sections were put on the silanized slides for immunohistochemistry using DakoCytomation Envision+Dual Link System-HRP (DAB+). H&E staining slides were examined to confirm diagnosis and to detect the type of carcinoma and its histological grade.

Immunohistochemical Scoring:
The cellular immunoreactions of the tissue samples were scored quantitatively, and were classified into four groups according to the percentage of tumor cell nuclei that stained: Score 0: < 5% positive cells. Score (1+): 5-25% positive cells. Score (2+): 25-75% positive cells. Score (3+): > 75% positive cells.

Statistical Analysis:
Analysis of variance (ANOVA) with STATA 8 software (College station, Tx) was used to evaluate the relationship between p53 expression and the clinicopathological variables of age, gender, histological types, histological grades, and stage of the disease.

Results
Clinicopathological Findings:
Age Distribution: Patient’s age ranged from 20 to 80 years. Most of cases (27.5%) were in the fifth decade of life as shown in table I. The median age was 47.5 years.
Gender: Of the 40 cases, 21 (52.5%) were males and 19 (47.5%) were females, as shown in table I.
Histological Type: There were three histological tumor types (adenocarcinoma, signet-ring carcinoma, and mucinous carcinoma). The frequency distribution is shown in table II, in which the commonest histological type was adenocarcinoma that was found in 34(85%) cases.
Histological Grading: In the present study histological grading was applied only for adenocarcinoma. Frequency distribution of the histological grading is shown in
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table II. The highest numbers of cases were moderately differentiated found in 16(47.1%) cases followed by well differentiated and poorly differentiated adenocarcinoma which found only in 4(11.8%) cases.

Staging: The cases were divided into 4 staging groups according to modified Dukes staging system (A, B, C, and D). The frequency distribution is shown in figure -1. Stage C was found in 21 cases (52.5%), stage B in 15 cases (37.5%), while stage A and D was found in 2 (5.0%) of cases each.

Results of Immunohistochemical Analysis of p53:
Immunohistochemical analysis of the colorectal cancer tissue samples showed nuclear accumulation of altered p53 protein and was recognized as yellow to brown discoloration of the nucleus(Figure -2)

The results of immunohistochemical scoring of p53 showed 16 cases (40%) as score 0 (negative); 4 cases (10%) as score (1+); 8 cases (20%) as score(2+); and 12 cases (30%) as score (3+). Figure-3 showing frequency distribution of immunohistochemical scoring.

Examples of tumors showing the different scores of immunohistochemical p53 expression are illustrated in figures (4-6).

Relationships between p53 Expression and Clinicopathological Features:
Age Distribution and p53 Expression:
Result of our study revealed that 5cases(45.5%) of the score (0) were in the age group 40-49 years. While most of the cases in score (3+) were in age groups 40-49 years and 50-59 years. Statistically there is no significant relationship between p53 and age distribution as shown in table III.

Gender and p53 Expression:
In both males and females most of the cases (42.9%) were males, 7(36.8%) females in score (0), followed by 7(33.3%) males, 5 (26.3%) females in score (3+), 3(14.3%) males, 5 (26.3%) females in score (2+), and 2(9.5%) males, 2(10.5%) females in Score (1+) as shown in figure (6).

Statistically there is no significant relationship between p53 expression and gender.

Histological Types and p53 Expression:
P53 over expression was more frequently seen in adenocarcinoma 12(35.3%) of cases in score (3+), followed by 6(17.6%) and 4(11.8%) of cases in the Score (2+) and (1+) respectively while in mucinous carcinoma only 1(25.0%) of cases in score (2+) and the remaining 3(75.0%) were in score (0). In signet-ring cell carcinoma 1(50.0%) of cases in score (0) and 1(50,0%) was in score (2+) as shown in table IV and figures (7-9).

Statistically there is no significant relationship between p53 expression and histological type.

Histological Grading and p53 Expression:
Most of the cases (50.0%) of well differentiated adenocarcinoma in score (3+), while most of the cases (43.8%) of moderately differentiated adenocarcinoma in score (0), and half of the cases (50.0%) of poorly differentiated adenocarcinoma in score (2+) as shown in table V.

Statistically there is no significant relationship between p53 expression and histological grading.

Dukes Staging and p53 Expression:
Most of the positive cases were found in stage B&C and all of negative cases found also in stage B&C as shown in table VI.

Statistically there is no significant relationship between p53 expression and staging.

Discussion
Mutations of P53 are the most frequently detected genetic alteration in human cancer which is found in more than 50% of all human cancers13-15.

Mutation of the p53 gene alters the conformation of the nuclear protein product, which can inactivate wild-type p53 protein16. The longer half life of the
mutant p53 protein results in the accumulation of this phosphoprotein in the nuclei, facilitating its detection by immunohistochemical analysis\textsuperscript{17}.

Elevated expression of the p53 protein or mutational inactivation of the p53 gene has been shown in various human malignant tumors including carcinoma of colon and rectum\textsuperscript{18,19}, breast\textsuperscript{20}, prostate\textsuperscript{21}, lung\textsuperscript{22}, stomach\textsuperscript{16}, thyroid\textsuperscript{23}, and liver\textsuperscript{24}. In colorectal carcinoma the occurrence of p53 mutation is variable among different series and has been found in 50\% to 70\% of cases\textsuperscript{13,18}.

In the present study, p53 expression by IHC techniques detected in 24 (60\%) out of the 40 analyzed colorectal carcinoma cases. This result is consistent with other studies\textsuperscript{17,25,26}.

Clinicopathological variables:

In this study, the age of patients was 20-80 years with most of the cases being in the 5th decade of life and a median age of 47.5 years. Which occurs in a younger age group than other reported studies done by Ahsan et al\textsuperscript{27} and Neagoes et al\textsuperscript{28} who reported that most of the CRC cases are in the 6th and 7th decades of life.

Regarding gender in this study 52.5\% of patients were males and 47.5\% were females. This is consistent with other studies that showed nearly equal gender distribution\textsuperscript{29,30}.

In the current study shows that adenocarcinoma accounts for 85\% of cases, followed by mucinous (10\%) and signet-ring cell carcinoma (5\%). This is consistent with a study done by Georgescu et al. they reported the same results (85\% of cases were adenocarcinoma followed by mucinous carcinoma in 10\% of cases, and signet ring carcinoma in 5\% of cases)\textsuperscript{17}.

Concerning histological grading of colorectal adenocarcinoma the result of this study showed that 41.2\% were well differentiated, 47.1\% moderately differentiated, and 11.8\% poorly differentiated. While the study done by Valera et al. showed that 57.6\% well differentiated, 39.6\% moderately differentiated, and 2.8\% poorly differentiated\textsuperscript{31}.

Regarding staging in this study, most of the cases were in the stage C (52.5\%), followed by stage B (37.5\%), lastly stage A and D (5\%). While in the study done by Nyam et al showed that 51.9\% of cases in stage B, 40.7\% in stage C, and 3.7\% of cases in both stage A and D\textsuperscript{32}.

Relationships between p53 expression and clinicopathological features:

In the present study, there was no significant relationships between p53 and age, or gender and is consistent with other studies that found no relationships between these variables\textsuperscript{13,33}. While the study done by Slattery et al. detected significant relationships between age and gender and p53 expression which is inconsistent with the results of our study\textsuperscript{34}.

In the present study, there was no significant relationship between p53 expression and histological types but higher frequency of p53 positive cell has been shown in adenocarcinoma than mucinous and signet ring cell carcinoma. This result is consistent with the study done by Kim et al. and Georgesco et al. who found higher frequency of p53 positive cells for adenocarcinom than mucinous and signet ring cell carcinoma but no significant relationship between p53 and histological types\textsuperscript{33,17}, while Asaad et al. found significant relationship between p53 and histological types\textsuperscript{26}, and there is no significant relationship between p53 expression and histological grading which is consistent with the studies done by Georgescu et al. and Asaad et al\textsuperscript{17,26}.

In the present study there is no relationship between staging and p53 expression which is consistent with the results of study done by Demirbas et al. who found no significant relationship between p53 and staging but inconsistent with the result of study done by Jackson.
et al. who found significant relationship between p53 and staging\textsuperscript{35,36}.

The role of p53 as a prognostic factor is still now controversial. In some studies p53 was regarded as a bad prognostic factor\textsuperscript{37}, while in other studies the accumulation of p53 protein might have a favorable prognostic value\textsuperscript{38}.

In conclusion, Sulaimania region showed an unusually young CRC patient age. The median age of 47.5 years was almost 25 years younger than CRC patients in the west. Evidence of p53 mutation are found in the majority of CRC in our clinical population suggesting that chromosomal instability is responsible for most of colorectal carcinomas.

No relationship could be found between p53 expression and the clinico-pathological variables of age, gender, histological types, histological grades, and staging.

**Table I: Frequency Distribution of Age and Gender:**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number</th>
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<tbody>
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<td>Age</td>
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<td>30-39 years</td>
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<td>40-49 years</td>
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<tr>
<td>50-59 years</td>
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<td>15.0</td>
</tr>
<tr>
<td>60-69 years</td>
<td>7</td>
<td>17.5</td>
</tr>
<tr>
<td>More than 70 years</td>
<td>7</td>
<td>17.5</td>
</tr>
<tr>
<td>Sex</td>
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<td></td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>47.5</td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>52.5</td>
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**Table II: Frequency Distribution of Histological types and Histological Grades.**

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<td>Histological type</td>
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<tr>
<td>Adenocarcinoma</td>
<td>34</td>
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<tr>
<td>Signet ring</td>
<td>2</td>
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</tr>
<tr>
<td>Mucinous</td>
<td>4</td>
<td>10.0</td>
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<tr>
<td>Grades of adenocarcinoma</td>
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<tr>
<td>Well differentiated</td>
<td>14</td>
<td>41.1</td>
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<tr>
<td>Moderately differentiated</td>
<td>16</td>
<td>47.1</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>4</td>
<td>11.8</td>
</tr>
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**Figure 1: Pie Chart Showing Frequency Distribution of Dukes staging.**
Figure 2: Nuclear Immunostaining of p53 Protein

Figure 3: Pie Chart Showing Frequency Distribution of Immunohistochemical Scoring.

Figure 4: Colorectal Adenocarcinoma Score (1+)/ IHC X 400

Figure 5: Colorectal Adenocarcinoma Score (2+)/IHC X 400.
Figure 6: Colorectal Adenocarcinoma Score (3+)/ IHC X 400.

Table III: Age Distribution and p53 Expression.

<table>
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<th>Variable</th>
<th>Score</th>
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<tr>
<td></td>
<td>0 N (%)</td>
<td>1+ N (%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 30 years</td>
<td>4(66.7)</td>
<td>1(16.7)</td>
</tr>
<tr>
<td>30-39 years</td>
<td>1(33.3)</td>
<td>2(66.7)</td>
</tr>
<tr>
<td>40-49 years</td>
<td>5(45.5)</td>
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</tr>
<tr>
<td>50-59 years</td>
<td>1(16.7)</td>
<td>2(18.2)</td>
</tr>
<tr>
<td>60-69 years</td>
<td>3(42.9)</td>
<td>2(28.6)</td>
</tr>
<tr>
<td>More than 70 years</td>
<td>2(28.6)</td>
<td>2(28.6)</td>
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Figure 7: Gender and p53 Expression.

Table IV: Histological Types and p53 Expression.

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<tr>
<td></td>
<td>0 N (%)</td>
<td>1+ N (%)</td>
</tr>
<tr>
<td>Adenocarcinoma.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Signet-Ring cell Carcinoma.</td>
<td>1(50.0)</td>
<td>1(50.0)</td>
</tr>
<tr>
<td>Mucinous Carcinoma.</td>
<td>3(75.0)</td>
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Table V: Histological Grading and p53 Expression.

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<td>1+</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>4(28.6)</td>
<td>2(14.3)</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>7(43.8)</td>
<td>3(18.8)</td>
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<tr>
<td>Poorly differentiated</td>
<td>1(25.0)</td>
<td>2(12.5)</td>
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Table VI: Staging and p53 Expression.

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<tbody>
<tr>
<td></td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Stage A</td>
<td>1(50.0)</td>
<td>1(50.0)</td>
</tr>
<tr>
<td>Stage B</td>
<td>8(53.3)</td>
<td>1(6.7)</td>
</tr>
<tr>
<td>Stage C</td>
<td>8(38.1)</td>
<td>3(14.3)</td>
</tr>
<tr>
<td>Stage D</td>
<td>1(50.0)</td>
<td>1(50.0)</td>
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Figure 8: Mucinous Carcinoma Score (2+)/ IHC X 400.

Figure 9: Signet-ring Cell Carcinoma Score (0)/ IHC X 400.
References