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# Role of Mutant P53 Protein Expression in Colorectal Carcinoma

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*Abstract*

***Introduction:Colorectal cancer (CRC) is the third most diagnosed malignancy and the fourth leading cause of cancer-related deaths in the world. P53 protein is a nuclear biomarker that is most investigated for its predictive value in CRCs.Aim: to evaluate the relationship between P53 expression and colorectal carcinoma. Materials and methods:In this study we investigated the expression of P53 in colorectal carcinoma using immunohistochemistry on 60 cases collected retrospectively from Department of Pathology, Faculty of Medicine, Zagazig University.Results:30/60 (50%) of the cases showed positive P53 expression, the commonest Dukes stage was stageA which forms 25/60 (41.7%), There was a high statistically significant difference (p<0.001) between the studied subgroups as regard histopathology, age, lymph node metastasis, distant metastasis and DUKES, a significant difference (p=0.002) between the studied subgroups as regard lymph vascular invasion. Conclusion: P53 overexpression in colorectal carcinoma was associated with DUKES stage, lymph node metastasis, distant metastasis, and lympho-vascular invasion.***

## Introduction

Colorectal cancer (CRC) is the third most diagnosed malignancy and the fourth leading cause of cancer-related deaths in the world, and its hazardous effects are expected to increase by 60% to more than 2.2 million new cases and 1.1 million cancer deaths by 2030**(Lesko et al., 2020)**.In Egypt, the prevalence rate of colorectal cancer is 5.1% in males and 4.7% in females. It was stated that Egyptian patients who have CRC below the age of 30 years havethreefold increase in mortality rate within 5 years contrasted those having CRC over the age of 50 years **(Bader El Din et al., 2020).**About 70% of colorectal carcinomas present as sporadic carcinomas, 25% are familial carcinomas without a specific inherited pattern and less than 10% of patients have inherited syndromes**.** Three eminent molecular carcinogenesis pathways are chromosomal instability (CIN), microsatellite instability (MSI), Cytosine-phosohate-Guanine (CpG) island methylator phenotype (DNA methylation), each account for approximately 85%, 15%, and 17%, respectively **(Ren & Tao, 2018).**P53 protein is a nuclear biomarker that is most investigated for its predictive value in CRCs. p53 is stimulated by cellular stress, including DNA damage, shortened telomeres, hypoxia, aberrant growth signals and chemotherapy **(Oh et al., 2019).**Mutation of P53 is nearly found in 60% of

colorectal cancers, being implicated in the submucosal invasion, promotion of metastasis and poor prognosis **(Nakayama and Oshima, 2019).** p53 protein overexpression is related to expanded proliferative activity and increased rate of lympho-vascular invasion, metastasis to lymph node and distant metastasis. **(Akshatha C, et al., 2016)**

**Materials and Methods:**

**Study design:**

This retrospective cross-sectional study was carried out on a total of 60 cases of colectomy and abdomino-perineal resection specimens in the Department of Pathology, Faculty of Medicine, Zagazig University. From May 2019 to June 2020. The detailed clinical history and results of relevant investigations were collected from the medical records. Specimens obtained were fixed in 10% neutral buffered formalin. Multiple sections were taken from representative area and processed routinely, embedded in paraffin and sections of 5 µm thickness were cut and stained with Haematoxylin and Eosin (H&E) Then evaluated for the tumour histology, lymph node metastasis, distant metastasis and other features. In addition, 4µm sections were cut from a paraffin block of tumour tissue for IHC to detect p53 overexpression. The study was approved bylocal ethical committee Institutional review board (IRB).

**Immunohistochemistry:**

Retreival of monoclonal antibody (IGg) against p53 (Clone DO-7; DakoCytomation, Glostrup, Denmark), the immunohistochemical staining was carried out using EnVision™ FLEX+ system (DAKO, North America Inc, CA, USA), a polymer-enhanced two-step IHC detection system.

**Interpretation and evaluation of p53 immunostaining:**

weak when1–10% of tumor cells showed p53 immunoreactivity, moderate when 10–50% of tumor cells showed p53 immunoreactivity, or strong when ≥ 50% of tumor cells showed p53 immunoreactivity. **(Oh et al., 2019).**

**Statistical analysis:**

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median Significance of the obtained results was judged at the 5% level. The used tests were: Chi-square test (For categorical variables, to compare between different groups) and F-test (ANOVA)(For normally distributed quantitative variables, to compare between more than two groups.)

**Results:**

**Table (1): Clinico-pathological parameters of studied CRC cases.**

| Clinico-pathological parameters | Total (n = 60) | |
|---|---|---|
| | No. | % |
| **Histopathology** | | |

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| | | |
|---|---|---|
| **Conventional** | 52 | 86.7 |
| **Mucinous adenocarcinoma** | 8 | 13.3 |
| **Sex** | | |
| **Male** | 30 | 50.0 |
| **Female** | 30 | 50.0 |
| **Size** | | |
| **<5cm** | 31 | 51.7 |
| **≥ 5cm** | 29 | 48.3 |
| **Age** | | |
| **<45** | 11 | 18.3 |
| **≥ 45** | 49 | 81.7 |
| **Min. – Max.** | 39.0 – 85.0 | |
| **Mean ± SD.** | 58.85 ± 11.47 | |
| **Median** | 60.0 | |
| **Lymph vascular invasion** | | |
| **Absent** | 37 | 61.7 |
| **Present** | 23 | 38.3 |
| **Lymph node metastasis** | | |
| **Absent** | 43 | 71.7 |
| **Present** | 17 | 28.3 |
| **Distant metastasis (liver)** | | |
| **Absent** | 51 | 85.0 |
| **Present** | 9 | 15.0 |
| **DUKES stage** | | |
| **A** | 25 | 41.7 |
| **B** | 18 | 30.0 |
| **C** | 8 | 13.3 |
| **D** | 9 | 15.0 |

**Table (2): Correlation between P53 expression and clinico-pathological parameters**

| parameters | Total (n = 60) | | P53 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Negative (n =30)** | | **Low (n =13)** | | **High (n =17)** | | **Test of Sig.** | **p** |
| | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** | | |
| Histopathology | | | | | | | | | | |
| **Conventional** | 52 | 86.7 | 29 | 97 | 7 | 53.8 | 15 | 88.2 | $\chi^2$= 14.540* | p <0.001* |
| **Mucinous** | 8 | 13.3 | 1 | 3 | 6 | 46.1 | 2 | 11.8 | | |
| Sex | | | | | | | | | | |

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| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Male** | 30 | 50.0 | 15 | 50.0 | 4 | 30.8 | 11 | 64.7 | $\chi^2$= 3.394 | 0.183 |
| **Female** | 30 | 50.0 | 15 | 50.0 | 9 | 69.2 | 6 | 35.3 | | |
| Size | | | | | | | | | | |
| **<5cm** | 31 | 51.7 | 16 | 53.3 | 7 | 53.8 | 8 | 47.1 | $\chi^2$= 0.203 | 0.904 |
| **≥ 5cm** | 29 | 48.3 | 14 | 46.7 | 6 | 46.2 | 9 | 52.9 | | |
| Age | | | | | | | | | | |
| **<45** | 11 | 18.3 | 3 | 10.0 | 8 | 61.5 | 0 | 0.0 | $\chi^2$= 17.599* | p <0.001* |
| **≥ 45** | 49 | 81.7 | 27 | 90.0 | 5 | 38.5 | 17 | 100.0 | | |
| **Min. – Max.** | 39.0 – 85.0 | | 45.0 – 78.0 | | 39.0 – 59.0 | | 50.0 – 85.0 | | F= 17.810* | <0.001* |
| **Mean ± SD.** | 58.85 ± 11.48 | | 59.97 ± 8.96 | | 46.46 ± 8.49 | | 66.35 ± 9.97 | | | |
| **Median** | 60.0 | | 60.0 | | 8.4 | | 70.0 | | | |
| Lymph vascular invasion | | | | | | | | | | |
| **Absent** | 37 | 61.7 | 25 | 83.3 | 4 | 30.8 | 8 | 47.1 | $\chi^2$= 12.742* | 0.002* |
| **Present** | 23 | 38.3 | 5 | 16.7 | 9 | 69.2 | 9 | 52.9 | | |
| Lymph node metastasis | | | | | | | | | | |
| **Absent** | 43 | 71.7 | 28 | 93.3 | 7 | 53.8 | 8 | 47.1 | $\chi^2$= 14.744* | p <0.001* |
| **Present** | 17 | 28.3 | 2 | 6.7 | 6 | 46.2 | 9 | 52.9 | | |
| Distant metastasis (liver) | | | | | | | | | | |
| **Absent** | 51 | 85.0 | 30 | 100.0 | 13 | 100.0 | 8 | 47.1 | $\chi^2$= 22.375* | p <0.001* |
| **Present** | 9 | 15.0 | 0 | 0.0 | 0 | 0.0 | 9 | 52.9 | | |
| DUKES stage | | | | | | | | | | |
| **A** | 25 | 41.7 | 14 | 46.7 | 7 | 53.8 | 4 | 23.5 | $\chi^2$= 37.731* | p <0.001* |
| **B** | 18 | 30.0 | 14 | 46.7 | 0 | 0.0 | 4 | 23.5 | | |
| **C** | 8 | 13.3 | 2 | 6.7 | 6 | 46.2 | 0 | 0.0 | | |
| **D** | 9 | 15.0 | 0 | 0.0 | 0 | 0.0 | 9 | 52.9 | | |

$^2$: **Chi square testF**: **F for ANOVA test**

p: p value for association between **P53** and different parameters

*: Statistically significant at $p \leq 0.05$

Among the high P53 group, there were 15/17 (88.2%) conventional histopathology and 2/17 (11.8%) mucinous histopathology, concerning lymph vascular invasion there were 8/17 (47.1%) absent and 9/17 (52.9%) present, concerning lymph node metastasis there 8/17 (47.1%) absent and 9/17 (52.9%) present, concerning distant metastasis there were 8/17 (47.1%) absent and 9/17 (52.9%) present and about DUKES stage there were 9/17 (52.9%) stage D and 4/17 (23.5%) for both stage A and B.

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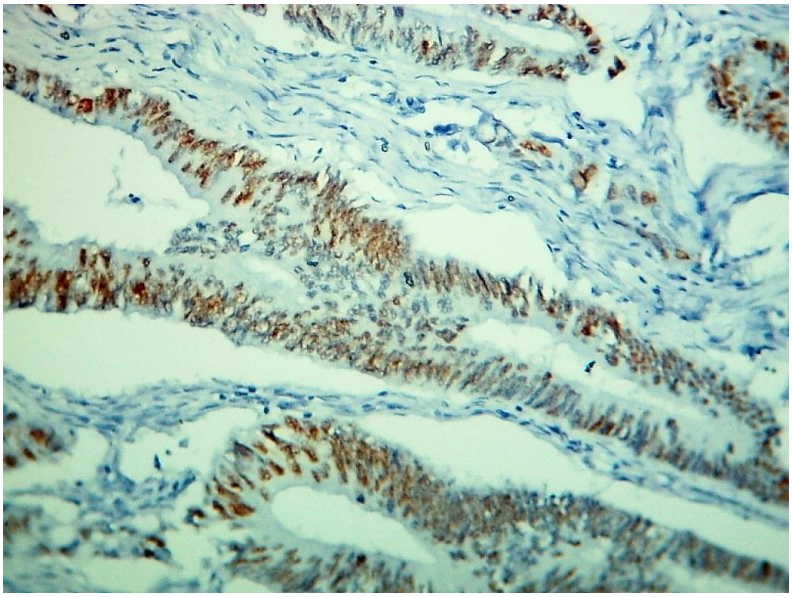

**Fig (1):** Well differentiated adenocarcinoma showing moderate stain of nuclear P53 expression, IHC, original magnification X200

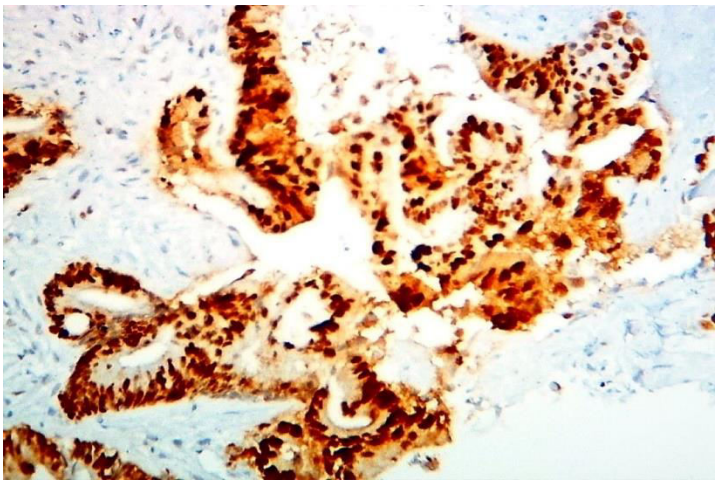

**Fig (2):** Poorly differentiated adenocarcinoma showing strongly positive nuclear P53 expression, IHC, original magnification X200.

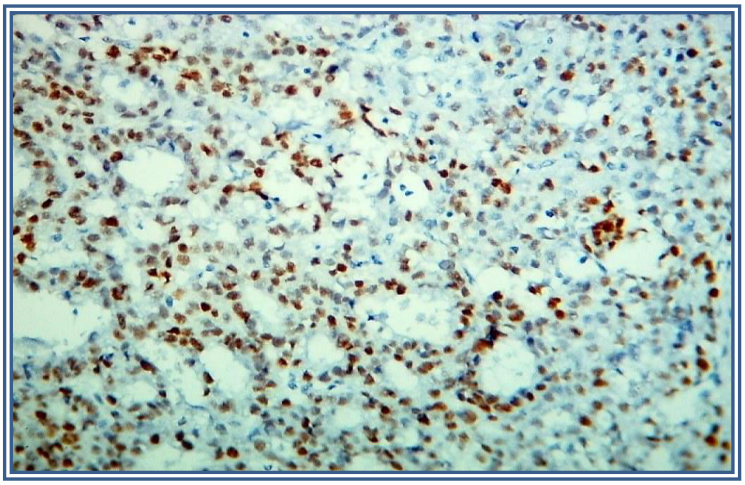

**Fig (3):** A case of poorly differentiated adenocarcinoma showing moderate stain of nuclear P53 expression, IHC, original magnficationX200.

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**Discussion:**

Mutation of P53 is nearly found in 60% of colorectal cancers, being implicated in the submucosal invasion, promotion of metastasis and poor prognosis **(Nakayama and Oshima, 2018).**

P53 expression was observed as staining of the nuclei of tumor cells **(Oh *et al.*, 2019), (Wang *et al.*, 2017)**and**(Nakayama and Oshima, 2019).**

Concerning P53 expression, among a total of 60 colorectal cases, 30/60 (50%) of them showed P53 expression, from these positive cases 17/60 (28%) showed high P53 expression, this was in alignment with the results of **(Azarhoush et al., 2018)** who found the percent of colorectal cases with positive P53 expression to be 58.9%.

Significant associations between P53 and metastasis (P<0.001) lympho-vascular invasion (P=0.002), histopathological types (P<0.001), andage (P<0.001) were detected.

**Powell, Piwnica-Worms and Piwnica-Worms 2014** explained the link between mutant P53 and metastasis on basis of mutations that give p53 a gain-of-function which in turn have a hand in tumorigenesis, invasion, and metastasis.

**Wang *et al.* 2017**noticed a significant difference between P53 expression and vascular invasion (P=0.01) and histological types especially the mucinous type (P=0.03). Finally **Mardi *et al.* 2017** revealed a significant association between P53 expression and age (P=0.006).

On the other hand **Oh *et al.* 2019** disagreed with the point of age when they found that age has been independent of mutant P53 expression.

On the contrary, no significant association was detected between P53 and size of the tumor, and sex of the patients, these results were in line with **(Singh *et al.*, 2019)**and**(Oh *et al.*, 2019).**

**Limitations of the study**:

- The retrospective nature of the study that lacks patients follow up to detect the patient's outcome.
- The limited number of patients.
- One marker only was used in this study.
- P53 expression was assessed using only immunohistochemistry in our study.

## Conclusion

- P53 may be associated with tumor progression in colorectal carcinoma.
- P53 overexpression could be considered as a poor prognostic factor in colorectal carcinoma.

## Recommendations

- To perform a prospective study that is important inpatient's follow-up and detecting the survival and relapse in colorectal carcinoma patients that may help in developing new strategies in colorectal cancer therapy.
- To correlate our findings with patients' response to therapy thereby assessing the predictive role of P53.
- Performing more detailed studies about the molecular basis of CRC for assessing the relation of P53 with the molecular status such as gene expression.

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