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Immunohistochemical Expression of P53 in Colorectal Tumors among Sudanese Patients

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Abstract: ***Previous studies have suggested that p53 immunostaining may provide useful prognostic data in colorectal cancer. The aim of the study was to detect the immunohistochemical expression of p53 tumor marker in colorectal tumors among Sudanese patients. Retrospective analytical case control study design was used in this study. Formalin-fixed paraffin-embedded tissue block samples from 150 colorectal tumor patients including 100 malignant tissue blocks and 50 benign tissue blocks of colorectal tumors from Ibn Sina Hospital and Soba Teaching Hospital at Khartoum stat were analyzed for P53 using Immunohistochemical detection. The Immunohistochemical expression of p53 was positive in 61% of malignant tumor tissues, while none of the benign tumor samples was positive for this marker with significant relation between immunohistochemical expression and histopathological diagnosis (p= 0.000). The majority of samples with p35 expression were either moderately differentiated tumors or well differentiated tumors (63.5% and 61% respectively) while only 50% of samples were classified as poorly differentiated tumors with no significant relation between the immunohistochemical expression of p53 and cancer grade (p= 0.776). Conclusion: Immunohistochemical expression of P53 was exclusively detected in colorectal malignant tumors providing a useful marker for the carcinogenesis of colorectal tumors. However, immunohistochemical expression of P53 did not affect histological grade of tumors and cannot be used for grading and hence prognosis. Several other markers are needed to be used in combination with p53 to improve colorectal tumor diagnosis among Sudanese.***

Keywords: Immunohistochemical, p53, colorectal tumor

1. Introduction

Colorectal cancer is one of the most common cancers and the third leading cause of cancer death in both sexes. The disease progresses as a multistep process and is associated with genetic alterations [(1)]. In Sudan, colorectal cancer was one of the commonly diagnosed cancers during 2009-2010 [(2)]. TP53 is a tumor suppressor gene on the short arm of chromosome 17 that encodes an important protein in the regulation of cell division [(3)].

p53 protein, encoded by the gene TP53, is involved in the development of many human cancers, in which TP53 gene mutations were found. Under normal conditions, the expression of p53 protein is kept at extremely low level. However, the gene can be activated by genotoxic damage, activation of oncogenes, telomere erosion, loss of stromal support and deprivation of nutrients or oxygen. In response to these multiple cellular stresses, p53 rapidly accumulates in the nucleus. P53 exerts its pro apoptotic function when cellular DNA damage is severe and repair is impossible. In these conditions, p53 commands the cell to enter apoptosis and the cell is removed from the proliferating cell population [(4)]. On the other hand, p53 promotes G1 cell cycle arrest in the early stage of DNA damage response. Thus, for a tumor cell, defected p53 is required for tumor progression. A p53 mutation is a final step in the conversion of adenoma to carcinoma. And the frequency of p53 abnormalities increases with the progression of the lesion [(5)]. Moreover, the expression of this marker was correlated with various clinical features of patients with colorectal cancer.

Immunohistochemistry (IHC) is a method for localizing specific antigens in tissues or cells based on antigen–antibody recognition; it seeks to exploit the specificity provided by the binding of an antibody with its antigen at a light microscopic level [(6)]. The use of immunohisto-chemical markers appeared to be a promising tool in predicting the prognosis of patients with this type of cancer [(7)].

Alterations of this suppressor gene is a common event in colorectal cancer and has been associated with adverse postoperative outcome and poor survival [(8)]. The main objective of this study was to detect the immunohistochemical expression of p53 tumor marker in colorectal tumors among Sudanese patients and to correlate immunohistochemical expression of p53 tumor marker with cancer grades.

2. Materials and methods

Study design

A retrospective analytical case control study was conducted in Ibn Sina and Soba Teaching Hospitals at Khartoum state during the period April 2014 – December 2015 to detect the immunohistochemical expression of p53 tumor marker in colorectal tumors among Sudanese patients.

Study samples

One hundred and fifty blocks of colorectal tumors were used in the study. These included, one hundred blocks previously diagnosed as malignant colorectal tumors and fifty blocks previously diagnosed as benign colorectal tumors.

Ethical consideration
The study was ethically approved by the ethical committee of the Sudan University of Science and Technology, Ministry of health and then by the general managers of the hospital in which the study was performed. All sample blocks were collected anonymously.

Data collection
Data were collected from formalin-fixed paraffin-embedded tissue blocks and patient clinical data were collected from patient's files.

Data analysis
Statistical analyses were performed using the Statistical Package for the Social Science (SPSS) computer software version 11.5. The Chi-square test or Fisher's exact test was used when appropriate, for comparing categorical variables (contingency tables). A p value less than 0.05 was considered statistically significant.

3. Methodology

Immunohistochemical Staining
Sections of 5µm in thickness were obtained from each formalin-fixed paraffin embedded tissue block using rotary microtome. Sections were immune- stained using monoclonal antibodies by an indirect technique. Briefly, sections placed on coated slides were dewaxed in hot plate oven and cleared in two changes of xylene for two minutes. Sections were then hydrated through a series of ethanol concentrations (100%, 90%, 70%, 50%) and a final wash in water for two minutes for each. Slides were retrieved by water bath heat retrieval technique and treated with hydrogen peroxide for fifteen minutes. After that, sections were washed in phosphate buffer saline (PBS, pH 7.4) for five minutes and treated with protein blocker solution for fifteen minutes. Sections were treated with anti-p53 primary antibodies for thirty minutes, and then rinsed in PBS before being treated with secondary polymer conjugate for thirty minutes and rinsed in PBS. Slides were treated with DAB for seven minutes then washed in PBS for five minutes. For the staining step, sections were counter stained in Mayer's hematoxylin for one minute washed and blued in running tap water before they were dehydrated through ascending concentrations of ethanol (50%, 70%, 90% &100%). Sections were finally cleared in xylene and mounted in DPX (9).

4. Results

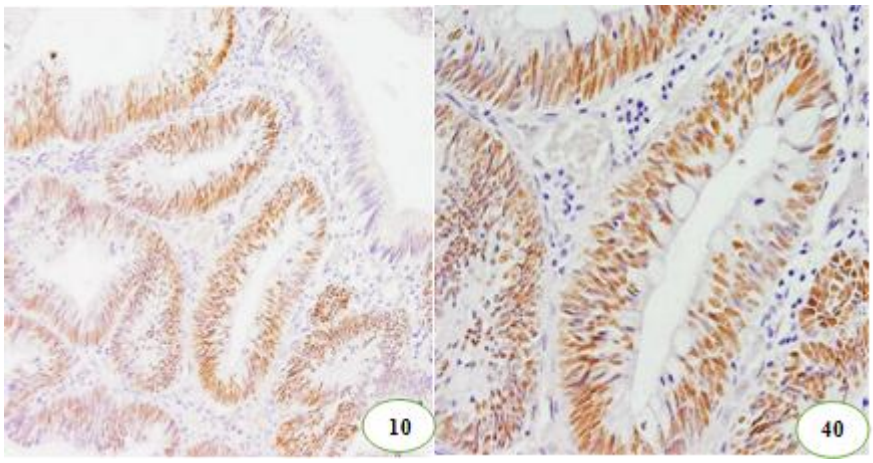
A total of 150 paraffin blocks previously diagnosis as colorectal tumors were collected in the study. According histopathological re-diagnosis, 100 patients were classified as malignant cases and 50 as benign controls. All tissue samples were included in the study and investigated for the P53 by immunohistochemical technique.

Samples with positive immunohistochemical expression of p53 in malignant tissues represented 61% while all samples were negative for this marker in benign tumors. The relation between immunohistochemical expression and histopathological diagnosis was found to be significant (p. value 0.000) (Table 1).

Samples with well differentiated and moderately differentiated tumors showed higher percentages of p53 expression (61% and 63% respectively) with no significant relation between the immunohistochemical expression of p53 and cancer grade (p. value 0.776) (Table 1).

Table 1: Immunohistochemical expression of p53 in the different histopathological diagnostic groups and cancer grades

Immunohistochemical Expression	*Histopathological Diagnosis*		*Cancer Grade*		
	Malignant No (%)	*Benign No (%)*	*Well Differentiated No (%)*	*Moderately Differentiated No (%)*	*Poorly Differentiated No (%)*
Positive P53	61 (61%)	0 (0%)	26 (63.5%)	31 (61.0%)	4 50.0%
Negative P53	39 (39%)	50 (50%)	15 (36.5%)	20 (39.0%)	4 50.0%
Total	100(100%)	50 (50%)	41 (100%)	51 (100%)	8 (100%)
P value	**>0.000**		**0.776**		



Microphotography (1): Well differentiated colorectal cancer showing positive P53 expression (10X and 40X)

5. Discussion

Colorectal cancer (CRC) is the second most common cancer in females and the third in males with 1.2 million annual new cases worldwide [10] .

P53 protein expression has an important role in the progression of CRC; it was considered as an independent predictor of shorter overall survival in patients with completely resected CRC [11]. In the current study, p53 was targeted for immunohistochemical analysis to study the contribution of this marker to colorectal malignancy among Sudanese patients.

In agreement with several other published data, significant positive immunohistochemical expression of p53 was markedly notices in malignant tissues (61%). In concordance, studies by Petrişor et al. [12], Rambau et al. [13] and Shashi Kiran et al [14] evaluated p53 marker in colorectal adenocarcinoma and reported 66%, 56%, and 56.67% positive expression of p53. Also, Qasim et al. [5] reported that the frequency of p53 positive cases was significantly higher in colorectal carcinoma than adenoma.

The reported expression percentage in the current study among Sudanese is within the world wide reported range, indicating the central role of this marker regardless of racial differences. Importantly, the absence of p53 expression in benign tumors indicated that p53 can be considered as a good marker for malignant colorectal tumors among Sudanese.

However, and in harmony with several studies [13,15,14], this marker was not useful for the classification of the different histopathological grades and hence prognosis of the disease. The reported pattern of P53 expression in the different stages has been interpreted into different and sometimes contradicting directions. If we consider that only 50% of poorly differentiated tumors expressed P53, p53 might be correlated with poor outcome and bad prognosis. On the other hand, differences between the different histological grades were not significant abolishing the prognostic value of this marker. This might not be surprising if we know that several studies indicated that *p53* nuclear staining does not always rule out the presence of mutated malfunctioned p53 protein [16]. In fact studies indicated that in approximately 50% of CRC, mutations in tumor-suppressor gene TP53 are found, frequently resulting in overexpression of mutant TP53 protein in tumor cells [17]. Studies that used immunohistochemistry in combination with mutational analysis signified that *p53* staining is common in most point mutations which change the conformation of the protein increasing its stability and hence rendering it easily to be detected by immunohistochemistry. Mutations which produce deletion, truncation or no protein cannot be detected by immunohisto-chemistry. Therefore, conflicting data have been reported regarding the prognostic significance of positive p53 staining. Nevertheless, the presence of a mutation is generally believed to indicate poor prognosis [18]. Theodoropoulos et al. [19] highlighted that the lack of a clear consensus in the literature on p53 prognostic significance despite the fact that it has been one of the most studied prognostic markers in colorectal cancer, may be because of the use of heterogeneous study populations, different antibodies, variations in cut-off values, patient stages included and duration of follow up.

Alterations of P53 gene were also considered to be late events in adenocarcinoma progression which explains the high frequency of positive expression of this marker in all stages in the current study [20].

The lack of significance reported herein might indicate that the defensive role of p53 expression is accompanied by other pathways that define the prognosis and grade of the tumor. K-ras mutations, for example, were one of the targets that support that direction [21].

6. Conclusion

In conclusion, immunohistochemical expression of P53 can be used as a marker for malignant tendency. However, patients with negative P53 expression are either harboring benign tumors or more aggressive malignant tumors due to the absence of p53 protein. The combination of P53 with other markers might provide a refined charecterestic profile for colorectal diagnosis among Sudanese.

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