P53 and heat shock protein 70 expressions in colorectal adenocarcinoma

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ABSTRACT

Objective: To examine the localization and over expression of heat shock protein 70 (HSP70) and p53 in patients with colorectal cancer and compared it with control tissue (including normal colon tissue).

Methods: This was a retrospective study of 60 patients with colorectal adenocarcinoma at the Jordan University of Science and Technology (JUST), Irbid, Jordan from 1997 to 2000. The Pathology Department at JUST is the chief provider of surgical pathology services in the north of Jordan. It receives specimens from both government and private hospitals. Immunohistochemistry was the technique of choice.

Results: The HSP70 was over expressed more highly in colorectal cancers than in the control tissue. Immunohistochemistry showed that over expression of HSP70 had no statistically significant difference with any of the different prognostic factors assessed, mainly the grade and the stage. The p53 was over expressed in 60% of the cases. Control tissue (normal colon) was negative, p53, cell-cycle-related oncogene product, was strongly over expressed in the nuclei of the cancer cells of the cancer tissue. We found no significant difference in terms of size, patient age, lymph node state, and stage. The rate of expression was significantly less in high grade tumors than in intermediate and low grade ones.

Conclusion: The strong expression however, may be valuable in estimating a prognosis for patients with colorectal carcinoma.

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Carcinoma of the gastrointestinal tract has a worldwide distribution,[1] approximately 87,600 people get colorectal cancer every year, which makes it the third most common type of cancer after lung and breast.[2] Carcinoma of the colon is the disease of the elderly in western countries, whereas it affects the younger age group, in developing countries.[3-5] There is slight male predominance.[6] The cause and pathogenesis of colorectal carcinoma are related to both environmental and genetic factors.[7] The former are largely dietary, in the form of fats and animal proteins. All living cells, prokaryotic and eucaryotic respond to varieties of physical and chemical stresses by increasing the synthesis of a family of proteins collectively known as stress response proteins (SRPS). The SRPS term is recently used more than the previously more popular, but less inclusive term "heat shock proteins" (HSP). The SRPS are among the most highly conserved and abundant proteins in nature. Studies on the regulation of the synthesis of SRPS have shed light on the mechanisms regulating their gene expression. The results show that SRPS play an important role in a wide variety of physiologic and pathologic cellular processes.[8] These proteins have become an area of interest to molecular biologists and to specialists in various fields of medicine including oncology,[9] autoimmunity, infectious diseases, embryology, neurology

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aging ischemic lesions, and endocrinology.[10] The interest in these proteins in relation to cancer is gaining more momentum not only due to their implications for diagnostics, pathogenesis, and therapeutics,[11] but also due to their recent relation with tumor suppressor genes.[12] In this topic the area of relationship between SRPS and cancer is of a paramount interest. Various review articles summarized the state of the art in that field.[10,13,14] In addition, a large number of original articles investigated the relationship between SRPS and cancers of various organ systems. The list includes; breast,[15] colon,[16] adrenals,[17] bone marrow,[18] soft tissue,[19] brain,[20] kidney,[21] urinary bladder,[22] prostate,[23] lymph nodes,[24] endometrium,[25] pancreas,[26] pituitary,[27] liver,[28] and eye globe.[29] The p53 is a tumor suppressor gene located on chromosome 17PB-1; it is a potent transcriptional regulator of genes, which are involved in many cellular activities including cell cycle arrest, apoptosis, and angiogenesis. Homozygous loss of the p53 is found in virtually every type of cancer.[11] Wild type p53 protein is labile whereas mutated forms have longer half life, and can be detected immunohistochemically more easily.[12] The K-ras and p53 are 2 important genetic events implicated in colon carcinogenesis.[30] Mutation of K-ras is detectable at earlier stages, while mutation in p53 is detectable at later stages of colon carcinogenesis.[30] In addition, Yang et al,[31] found that the serum level of the p53 antigen was significantly increased in cancer patients as compared to its concentration in patients with benign tumors, or in patient with non-cancer disorders. This may allow the use of serum levels of p53 antigen in the follow up, or may be the early detection of colon cancer. Jordan is a Middle East developing country; recent reports from the Jordan Cancer Registry indicated that colorectal carcinoma ranked fourth in males and second in females.[32] The incidence of colorectal cancer in the Jordanian people is considerably lower than the western populations.[32] Despite this fact, studies on colorectal cancer in Jordan are limited. In addition, no studies exist on p53 and heat shock proteins (HSP) expression in colorectal cancer. The aim of this study is to examine the localization and over expression of heat shock protein 70 (HSP70) and p53 in patients with colorectal cancer and compared it with control tissue (including normal colon tissue).

Methods.

This was a retrospective study of colorectal adenocarcinoma at the Jordan University of Science and Technology (JUST), from 1997 to 2000. The Pathology Department at JUST is located in Irbid and is the chief provider of surgical pathology services in the north of Jordan. It receives specimens from both government and private hospitals.

Histopathologic studies. We reviewed the paraffin blocks of 60 patients with colorectal carcinoma from 1997 to 2000. Slides were histologically reexamined by a histopathologist and a senior resident. The diagnosis, clinical data, tumor grades, and tumor stages were confirmed. Modified Astler and Coller staging system was used in this study. We exclude any doubtful cases such as false stain and patients with no documented history and confirmed grade of differentiations of the cancer.

Immunohistochemical studies. New sections were cut from corresponding paraffin blocks and blocking endogenous antigen retrieval was carried out using the pressure cooker technique. The p53 specific monoclonal antibody (DO-7; DAKO-Denmark) was applied in concentrations of 1/50 for 30 minutes at room temperature. Followed by secondary biotinylated antibodies for 10 minutes, then by horseradish peroxide for 10 minutes. The detection system is called Dako LSAB2 system, USA. After that a chromogen (diaminobenzidine) is applied up to 2 minutes (the time by which the brown color has developed). We used Mayers hematoxylin as a counter stain for 30 seconds. Positive sections from known positive colonic carcinoma for p53 were included in each patch. We also included the negative control sections (non–immunized serum was applied instead of primary antibody). The same procedure was used for HSP70 using DAKO-Denmark (Rabbit anti-HSP70), which cross reacts with HSP72 and HSP73 Kda in a dilution of 1/250. We studied HSP70 and p53 on 60 cases of colonic adenocarcinoma in relation to both tumor grade and stage. For p53 evaluation nuclear staining was regarded as positive. Strong positivity was concluded if more than 30% of the tumor cell nuclei expressed the staining.[33] The degree of staining by HSP70 and p53 was analyzed in relation to the tumor grade and stage. Student t-test was used in this study, and for a relation to be significant, a *p*-value of less than 0.05 must be obtained. Statistical analysis was carried out using Statistical Package for Social Sciences and Microsoft Excel.

Results.

Table 1 shows the immunohistochemical results of p53 and HSP70 staining and the percentage of cells positive for p53 in patients with colonic carcinoma. In **Table 2**, the HSP70 percentage of cells positive in colonic carcinoma showed no significant relationship with the tumors grade ($p>0.05$), and it can be seen that as the percentage of positive cells for HSP70 increases, the number of patients increase especially grade II. **Table 3** specifies the intensity of p53 staining in colonic carcinoma.

Discussion.

There are many types of heat shock proteins. Some, such as HSP70, are found in normal cells and are involved in the process of protein synthesis. Other types, such as HSP70 and

Table 1 - Immunohistochemical results of p53 and HSP70 staining and percentage of cells positive for p53 in colonic carcinoma.

Histologic grade	No. of cases	No. of p53		No. of HSP70		Grade	No. of grade case	<30%	30-50%	51-75%	>75%
		Positive	Negative	Positive	Negative						
Well differentiated	8	7	1*	5	3	1	8	1	1	2	4
Moderate differentiated (G2)	40	26	14*	35	5*	2	40	16	2	7	15
Poorly differentiated (G1)	12	5	7	5	7	3	12	7	0	0	5

*p value <0.05 was considered significant. HSP - heat shock protien

Table 2 - Percentage of cells positive for heat shock protein 70 in colonic carcinoma and intensity of heat shock protien70 staining in colonic carcinoma.

Grade	No. of cases	Positive	<30%	30-50%	51-75%	Grade	No. of case	Negative	+1	+2	+3
1	8	2	1	0	2	1	8	2	5	1	0
2	40	8	3	4	10	2	40	8	13	16	3
3	12	5	1	2	1	3	12	5	5	1	1
Total	**60**	**15**	**5**	**6**	**13**	**Total**	**60**	**15**	**23**	**18**	**4**

HSP72 appear in disease conditions and may have a protective function, for example limit tissue necrosis after myocardial infarction.[34] A number of HSP or stress proteins have been shown functionally as potent tumor rejection antigens, despite the fact that there are no single tumor-specific mutations in these proteins.[35] The HSP70 and tumor necrosis factors (TNFs) are potentiating factors in the immune response against the tumor cells.[36] The HSP70 stress protein may confer a new mechanism of nuclear factor kappa B (NF-KB) regulation in cells affected by elevated temperature or other factors related to the cellular response to stress.[37] Immunostaining of HSP70 in polypus and malignant tissue changes shows more expression of the acidic isoforms of HSP70 in comparison to normal tissue,[38] which is similar to our findings. However, there was no significant relation between the percentage and the intensity of HSP70 expression and any of the prognostic variables studied. The p53 is a tumor suppressor gene that is frequently mutated in different human cancers.[39] It is a suppressor gene, which suppresses cell proliferation. Absence of the functional p53 gene product can lead to increased p53 accumulation in cancer cells.[40] Shumacher[41] used the p53 over expression (as detected by immunohistochemistry) as an indicator of p53 mutation.[41] The p53 tumor suppressor gene is a potent transcriptional regulator of genes involved in many cellular activities including cell cycle arrest, apoptosis, and angiogenesis. In colon cancer, the inhibition of NF-KB activity is a plausible mechanism for apoptosis induced by the wild type p53 gene.[42] Both wild and mutated forms of p53 protein can be isolated with extremely high accuracy for the pathologic diagnosis of cancer (87-93%).[43] In our study, we detected the incidence of positivity of p53 in the nucleus of cancer cells in 36 out of 60 cases studied (60%), which is lower than a previous study carried out in 34 cases and showed 70.5% of p53 over expression in the tumor cell nuclei.[44] There was no significant correlation with the tumor size, stage or lymph node involvement. These findings were compatible with the previous study carried out on 471 cases of colonic adenocarcinoma and showed that p53 positive rates show no significant correlation with the age, gender, growth configuration, modified Astler-Coller stage, growth pattern, tumor size, and lymph node involvement.[45] In addition, a study carried out in

Table 3 - Intensity of p53 staining in colonic carcinoma.

Grade	No. of grade cases	<30%	30-50%	51-75%	>75%
1	8	2	2	1	4
2	40	8	10	9	5
3	12	5	1	1	3
Total	**60**	**15**	**13**	**11**	**12**

Egypt showed no significant difference in p53 expression between stages B and C of colonic adenocarcinoma.[45] The p53 positively rate in our study differs according to the histological grade, being lower in high grade tumors (41.6) than in low and intermediate grade ones. Kim et al[33] found the lowest percentage of p53 expression in poorly differentiated and mucinous colonic adenocarcinoma, which is similar to our study. Furthermore, they stated that the common loss of wild-type p53 in many colorectal cancers may play a role in the clinical resistance of these high grade tumors to anticancer drugs.[33] No significant relation exists in our study between HSP70 and p53 expression.

In conclusion, we detected p53 overexertion in patients with colorectal carcinoma. More than half of our patients (36 out of 60) show strong positive staining (>30% of the tumor cells). These do not seem to differ from the others in term of size, patient age, lymph node state, and stage. However, the rate of expression is significantly lesser in high grade tumor than intermediate and low grade tumors. Hence, strong expression may have a value in estimating a prognosis for patients with colorectal carcinoma. The HSP70 expression is found in carcinomatous tissue and not in the adjacent benign tissues examined. There was no statistical significant difference in its expression with any of the different prognostic factors assessed, mainly the grade and the stage.

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