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Angiogenesis, p53 and Bcl_2 in Colorectal Carcinoma

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ABSTRACT

This study was an attempt to evaluate, immunohistochemically, the angiogenesis activity as well as the expression of p53 and Bcl_2 proteins in patients on a series of 52 surgically operated colorectal carcinoma cases. The angiogenic activity was assessed based on microvessel density using CD34 marker while the expression of p53 and Bcl_2 proteins was studied using monoclonal antibodies. Active angiogenesis, demonstrated as high microvessel density, was seen in 61.5% of cases while p53 expression was observed in 46.2% of cases; both were significantly higher than Bcl_2 expression (9.6%). Despite a trend showing a decrease toward right sided colon cancers and with worsening of both histological grade and tumor stage, none of active angiogenesis, p53 or Bcl_2 expression had any significant association with the clinicopathologic findings. In conclusion, ccolorectal carcinoma is associated with active angiogenesis and increased p53 expression compared with Bcl_2 although their potential use in the clinical setting appears to be of limited value.

Keywords: Angiogenesis, p53, Bcl_2, colorectal carcinoma

1 INTRODUCTION

COLORECTAL carcinoma (CRC) exhibits several genetic alterations of which p53 abnormalities are frequent, occurring in more than half of cases.[1-5] Immunohistochemical detection of mutated p53 protein, which is metabolically more stable than its wild-type, stands at the top of the examined parameters in the search context for carcinogenesis of CRC and other cancers.[6-8] As well, Bcl_2, gene protein involved in the process of apoptosis, has been argued to play an important role in colorectal carcinogenesis.[1,4,9] Both p53 and Bcl_2 protein overexpression confers cancer cell immortalization by inhibiting the apoptotic cell death machinery.[1,10] Angiogenesis, the process by which new blood vessels are formed, has a crucial role in the survival of malignant cells. The activity of angiogenesis, evaluated by intratumoral microvessel density (MVD), is believed to be associated directly with the ability of cancer cells to infiltrate the normal anatomic structures locally, entrance of cancer cells into the systemic blood circulation and even the establishment of blood-borne metastases in distant organs.[11-13] The increment of MVD in colorectal adenomas and cancer has been reported.[13-15] It has been anticipated that the increased expression of p53 is correlated with the increased microvessel count in cancers of the lung, colon and stomach.[6,12,15-17] Moreover, Bcl_2 overexpression may enhance the synthesis of hypoxia-stimulated angiogenic growth factor production in colon cancer.[18]

In an attempt to evaluate the impact of alterations of p53 oncoprotein, the antiapoptotic Bcl_2 and angiogenesis on CRC, we investigated comparatively the nuclear p53 and cytoplasmic Bcl_2 immunoexpressions as well as the angiogenic activity in patients with CRC. The association of these parameters with

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the clinicopathological findings was also evaluated.

2 METHODS

This study was conducted at histopathology department, Central Laboratory, Duhok/Iraq, during a period extended from January 2007 to March 2008. A total of 52 surgically resected primary colorectal adenocarcinoma specimens were examined. No patient had received chemotherapy or radiation therapy before surgery. Information pertaining to the age at presentation, gender and tumor location was obtained from the patient records. The location of tumors was divided into 2 major sites, right (beginning from the caecum extending to the hepatic flexure and transverse colon) and left (starting from the splenic flexure extending to the sigmoid colon and rectum).

2.1 Histopathology

Three mm-thick tissue sections were taken from the tumor, tumor margins, lymph nodes and any other available tissue. Sections were fixed in 10% formalin overnight at room temperature, processed and embedded in paraffin wax. Four µm sections were cut, deparaffinized and stained with Hematoxylin and Eosin (H&E) stains. Tumors were graded according to the modified WHO classification criteria into low grade (well and moderately differentiated) and high grade (poorly differentiated and undifferentiated) colorectal adenocarcinoma.[10] Tumor staging was based on the naked eye inspection of the surgical specimen as well as the microscopic examination of the the primary tumor with the bowel wall, all the lymph nodes and adjacent fat, mesentery and any associated structure if available, this in addition to the clinical data concerning distant lymph node and organ metastases. Pathological staging was done according to the pathological (pTNM) staging system (I-IV).[10]

2.2 Immunohistochemistry

Blocks representing the tumor (with no necrosis and not much mesenchymal tissue) were selected for IHC. For the sake of demonstrating angiogenesis, sections were selected from the tumor margin with the uninvolved bowel wall. Unstained 3-µm sections from formalin-fixed, paraffin-embedded tumors were used, and the IHC technique applied was Streptavidin-biotin method using monoclonal antibodies and kits manufactured by DAKO Corporation (Dako Denmark A/S) with 3-3'-diaminobenzidine tetrahydrochloride used as a chromogen. The Dako Cytomation, Envision®+Dual link system-HRP (DAB+), staining protocol was used for immunostaining to detect nuclear p53 using monoclonal mouse anti-human p53 antibody (dilution 1:30; clone Do7, code M7001, Dako Denmark A/S). Cytoplasmic CD34 expression was demonstrated, as a vascular endothelial marker, using monoclonal CD34 antibody (ready to use, clone BIRMA-k3, and code F7081, Dako Denmark A/S). Antigen retrieval was done according to the recommendations provided by the manufacturer's (Dako Denmark A/S) and as described previously by Pity.[19] For Bcl_2, we applied the fully automated immunostaining instrument, Ventana Benchmark (Ventana Medical System Inc., Cell Margue, Ventana, Rocklin, Calif.) using monoclonal mouse anti Bcl_2 antibody (dilution 1:80; clone 124, code M0887, Dako Denmark), and a standard DAB detection kit (Ventana) was used according to instructions supplied by the manufacturer's (Ventana Medical System Inc., Cell Margue, Ventana, Rocklin, Calif.) and as described previously by Pity and Baizeed.[20] All slides were manually counterstained with Hematoxylin and mounted in DPX solution. Positive controls used included tissue sections from breast carcinoma with a diffuse p53 nuclear immunoreactivity for p53 marker, normal lymph node sections for Bcl_2 marker and pyogenic granuloma for CD34 marker. For negative controls, a normal rabbit IgG, instead of the primary antibody, was applied. The expression of the markers

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was assessed according to the percentages of immunoreactive cells in a total of 1000 cells (quantitative analysis). Cells were considered positive only when displayed a distinct nuclear p53 and cytoplasmic Bcl_2 immunostaining in at least 10% of cells.

The microvessels density (MVD) was assessed following the first experimental design reported by Weidner and Colleagues (1991).[21] Sections were scanned at low power (magnifications, ×40) then microvessel counting was done on 10 representative high power fields (magnifications, ×400) selected from the highest vascular density areas (hot spots). Vessels with a clearly defined lumen or well-defined (but not single endothelial cells) were taken into account for microvessel counting. Each field was scored thrice and the average microvessel counts were obtained from these 10 fields for each case. Then we adopted a two-scale grading system of MVD (low and high) using one cut-off point (the median microvessel count which was 31). Tumors with less than 31 were considered as low MVD while those with equal or higher values were considered as high MVD.

2.3 Statistical Analyses

The software package SPSS 12.0 (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses. For testing associations between categorical tumor parameters, Chi square test and Fisher exact test were applied. Differences at the p level of less than 0.05 were considered as statistically significant.

3 Results

Of the 52 patients studied, male: female ratio was 0.6: 1 with a mean age of 50.2 years (range, 20-80 years). Majority of tumors were left sided (73.1%) and of low grade (80.8%), and the pTNM staging system indicated that most patients had the disease at stage II. No stage I CRC was noticed among our patients (Table 1).

TABLE 1
CLINICOPATHOLOGICAL PARAMETERS

Variable		Number (%)
Age group		
	20-39	9 (17.3)
	40-49	8 (15.4)
	50-59	14 (26.9)
	≥ 60	21 (40.4)
Gender		
	Male	20 (38.5)
	Female	32 (61.5)
Site		
	Left	38 (73.1)
	Right	14 (26.9)
Grade		
	Low grade	42 (80.8)
	High grade	10 (19.2)
TNM stage		
	Stage II	37 (71.2%)
	Stage III	10 (19.2)
	Stage IV	5 (9.6)

The expression of p53 was demonstrated in 24 (46.2%) cases (Figure 1 and 2) while Bcl_2 expression was found in only 5 (9.6%) cases (Figure 3). The average microvessel count observed was 37.7 (range, 14-48) with a median of 31. Active angiogenesis, demonstrated as high MVD (≥ 31), was noticed in 32 (61.5%) cases (Figure 4) while the remainders (38.5%) showed a low MVD (Figure 5). Despite a trend showing a decrease toward right sided colon cancers and with worsening of both histological grade and tumor stage, none of active angiogenesis, p53 or Bcl_2 expression had any significant association with the clinicopathologic findings. The p-value was more than 0.05 (Figure 6-10).

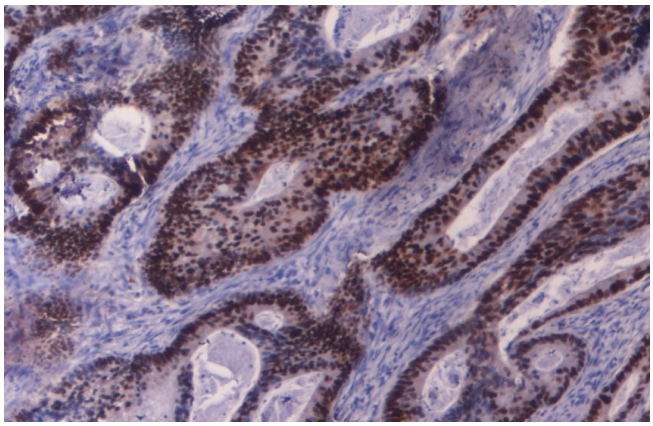

Figure 1. Nuclear p53 expression in well differentiated adenocarcinoma (x 100).

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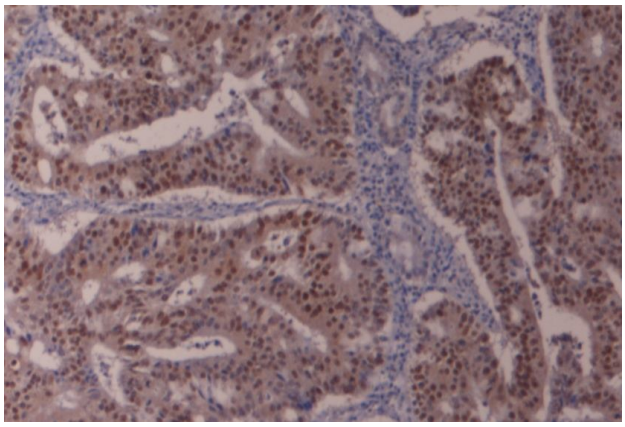

Figure 2. Nuclear p53 expression in moderately differentiated adenocarcinoma (x 100).

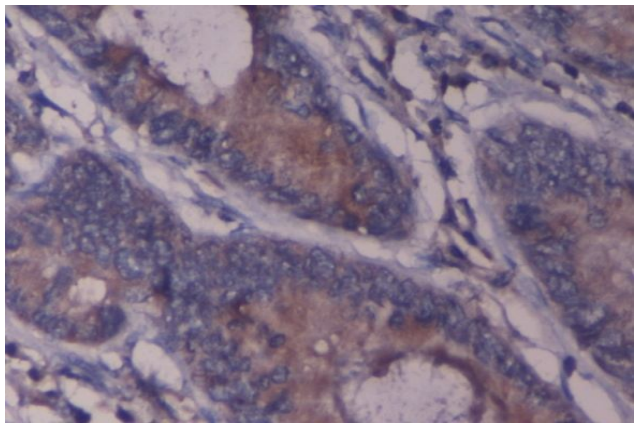

Figure 3. Cytoplasmic Bcl_2 expression in moderately differentiated adenocarcinoma (x 400).

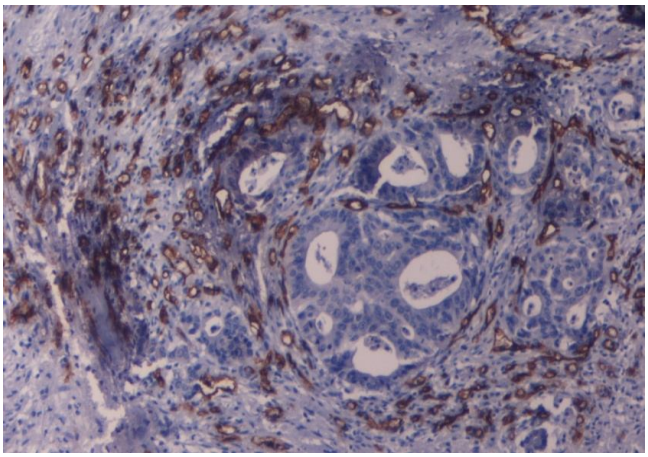

Figure 4. High microvessel density in moderately differentiated adenocarcinoma (x400).

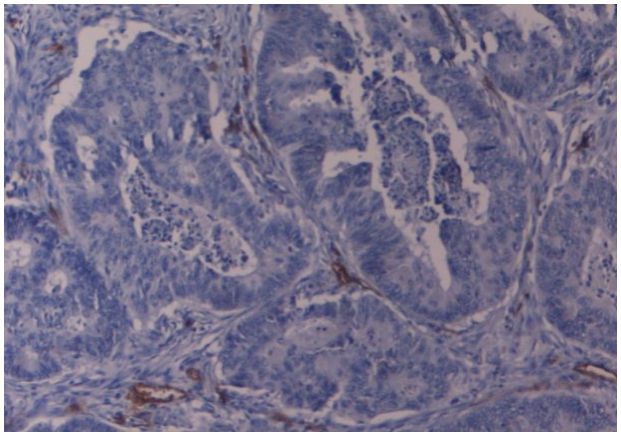

Figure 5. Low microvessel density in moderately differentiated adenocarcinoma (x400).

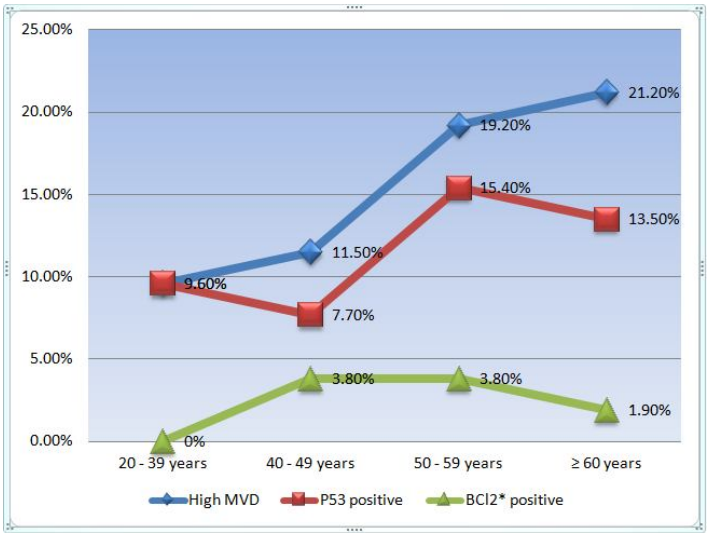

Figure 6. Association of high microvessel density (MVD), p53 and BCl_2* with age group. p= {MVD: 0.325, p53: 0.314, Bcl_2: 0.566}, X^2 used, * Fisher Exact test used.

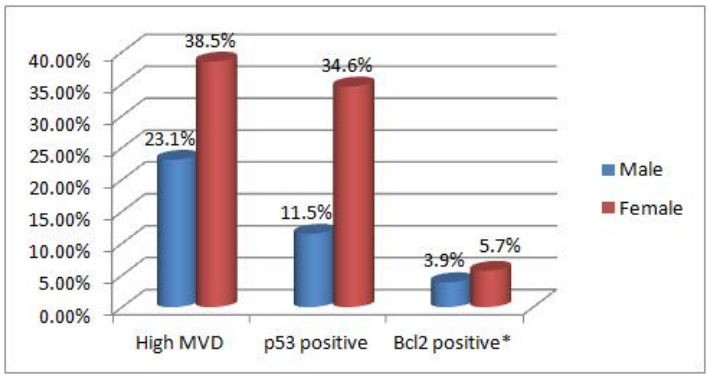

Figure 7. Association of high microvessel density (MVD), p53 and BCl_2 with gender. p= {MVD: 0.857, p53: 0.06, Bcl_2: 0.99}, X^2 used, * Fisher Exact test used,

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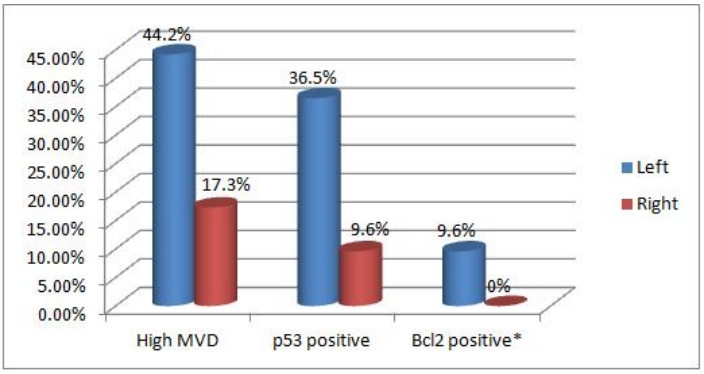

Figure 8. Association of high microvessel density (MVD), p53 and BCl_2 with tumor side. p= {MVD: 0.647, p53: 0.28, Bcl_2: 0.253}, X^2 used, * Fisher Exact test used.

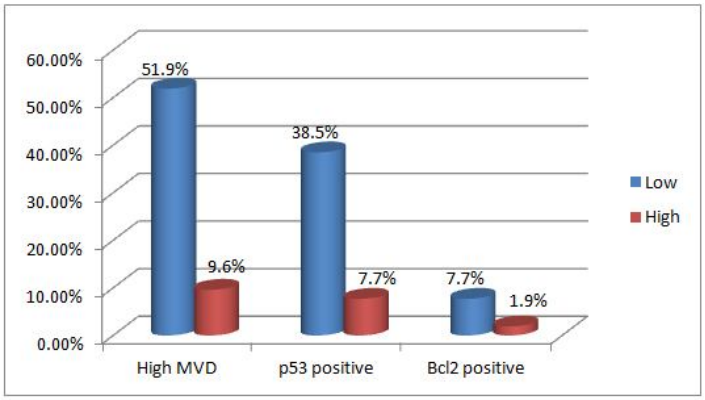

Figure 9. Association of high microvessel density (MVD), p53 and BCl_2 with tumor grade. p= {MVD: 0.628, p53: 0.94, Bcl_2: 0.99}, Fisher Exact test used.

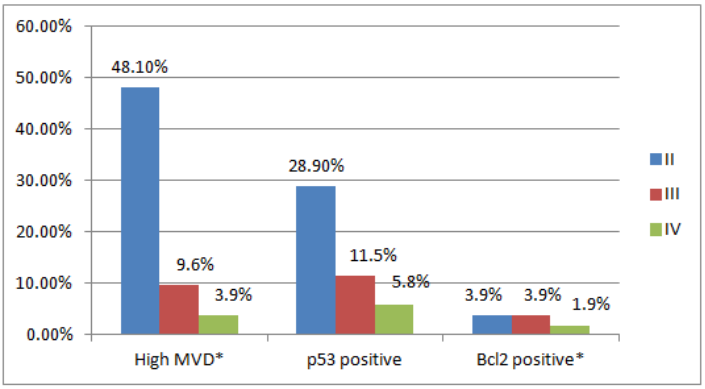

Figure 10. Association of high microvessel density (MVD) and expression of p53 and BCl_2 with tumor stage. p= {MVD: 0.274, p53: 0.202, Bcl_2: 0.274}, X^2 used, * Fisher Exact test used

On the other hand, both p53 expression and active angiogenesis were found to be increased in CRC with no significant difference was observed between the two parameters (p = 0.169); both were significantly higher than Bcl_2 expression (p-value < 0.001).

4 DISCUSSION

In the present study, p53 nuclear expression (46.2%) and active angiogenesis (61.5%) fall within the rate ranges reported in the literature while Bcl_2 expression (9.6%) was among the lowest reports (Table 2).

Table 2. FREQUENCY RATES OF P53, BCL_2 AND MICROVESSEL DENSITY (MVD), LITERATURE REVIEW.

Series	Cancer location	Sample No.	P53 %	Bcl_2%	MVD%
Lin et al, 2011 (China)[1]	Colon	53	72	94	-
Petrisor et al, 2008 (Romania)[2]	Colon	30	66	46.6	-
Zlatian, 2010 (Romania)[3]	Colon	50	86	12	-
Qasim et al, 2012 (Iraq)[4*]	Colon	33	78.8	48.5	-
Rambau et al, 2008 (Uganda)[5]	Colon	109	56	-	-
Yuan et al, 2002 (Taiwan)[6]	Lung	65	39.7	-	43.1
Yoo et al, 2005 (Korea)[7]	Lung	147	72	17	72
Zhao et al, 2005 (China)[8]	Colon	93	40	53	-
Contu PC et al, 2006 (Brazil)[9]	Colon	132	-	29.5	-
Koukourakis et al, 2005 (Greece)[11]	Colon	130	-	-	48.4
Giatromanolaki et al, 1999 (Greece)[16]	Colon	106	42	31	37
Fondevila et al, 2004 (Spain)[17]	Stomach	156	45.5	-	52.6
Zlobec et al, 2006 (Canada)[23]	Colon	87	37	<10	-
Sharifi et al, 2009 (Iran)[24]	Colon	62	-	-	51.6
Imail et al, 2007 (Egypt)[26]	Colon	104	33.7	26	
Saleh et al, 2000 (USA)[27]	Colon	52	67.3	51.9	-
Afrem et al, 2012 (Romania)[28]	Colon	37	-	43.2	-
Lazari et al, 2008 (Romania)[30]	Stomach	61			38.7
Current (Iraq), 2012	**Colon**	**52**	**46.2**	**9.6**	**61.5**

* Study done in Baghdad/Iraq

The divergent results of these parameters, seen in the table, owe to the differences in sample sizes, could be actual differences or there is a heterogeneity within different tumor types and in different organs.[3,6,15,17,22,23,24] Nevertheless, our data and the literature results emphasize that at least a subset of colorectal carcinogenesis might be attributed to p53 mutation.[1-5,8,16,25] In contrast, studies on Bcl_2 status in CRC have presented controversial results with a variable interobserver reproducibility.[1-4,23] This may explain the wide range rates reported in the literature (9.6% to 94%). There is a need to conduct molecular studies to ascertain the type of genetic mutation and to

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find out if some cases could be due to epigenetic alteration.

An important observation in the present study was the significant difference observed between the expression Bcl_2 and p53 proteins. To our knowledge, this finding has not been verified in any of the known studies.[1,8,16,24,26,27] Evaluation of the role of other members of the Bcl protein family possibly clarifies the lack of association between Bcl_2 with p53 observed in our series.

We also demonstrated a significant difference between Bcl_2 expression and active angiogenesis. This is another important observation which was also reported by Yoo et al in patients with non small cell lung carcinoma and by Georgiou et al in CRC.[7,12]

On the other hand, both active angiogenesis and p53 expression were increased in our series with no significant difference was demonstrated between them. In parallel tissue sections from patients with CRC and gastric carcinoma done by Georgiou et al, Fondevila et al and Perrone et al, they found an association between p53 overexpression and MVD in the vascular hot spots.[12,17,18] In contrast, Giatromanolaki et al were unable to confirm any correlation between p53 gene status and MVD in CRC.[16] This implies that intratumoral neoangiogenesis might be regulated by different molecular pathways that do or do not involve p53 alteration.[12,15,22]

Furthermore, we failed to demonstrate any significant association between any of p53, Bcl_2 and active angiogenesis with patient's age, gender and tumor site despite a trend showing a decrease among right sided tumors. The originally lower proportion of right sided tumors (26.9%) in our series may contribute to this variation. However, this finding was also observed by Contu et al in Brazilian patients and by Sharifi et al in Iranian patients with CRC.[9,24] It has been argued that the pathogenesis of left and right sided colonic cancers is different. In sporadic CRC, mutation of the p53 gene is found to be a common finding in left colon tumors compared with the right.[25] This can infer that many cases of CRC in our setting are sporadic and the expression of p53 in 46.2% in the current study tends to support this view.

In the same line, we observed low p53, active angiogenesis and Bcl_2 with worsening histological grade and advanced tumor stage although the differences didn't reach the level of significance. This finding was previously demonstrated by Zhao et al on Chinese patients, Contu et al and Carneiro et al on Brazilian patients, Sharifi et al on Iranian patients and Afrem et al on Romanian patients with CRC.[8,9,24,28,29] In contrast, Giatromanolaki et al and Lazari et al in their study on patients with CRC and gastric carcinoma respectively, demonstrated a significant correlation of high MVD with advanced stages.[16,30] This can infer that the grading and staging systems applied may represent potential biases and may account for these substantial differences.[11,15,22] Another plausible explanation is that p53, Bcl_2 and angiogenesis in CRC are possibly not influenced by the degree of tumor dedifferentiation and metastasis.[11,15,16,22,30]

5 CONCLUSIONS

Colorectal carcinoma is associated with increased p53 expression and angiogenesis compared with Bcl_2 expression although their potential use in the clinical set of CRC appears to be of limited value.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

1) M.S. Lin, W.C. Chen, J.X. Huang, H.J. Gao, B.F. Zhang, J. Fang J, Q. Zhou, and Y. Hu. Tissue microarrays in Chinese human rec-

International Journal of Advancements in Research & Technology, Volume 2, Issue 3, March 2013
ISSN 2278-7763

tal cancer: study of expressions of the tumor-associated genes. Hepato-gastroenterology. 2011; 58:1937-42.

2) O. Petrisor, S.E. Giusca, M. Sajin, G. Dobrescu, and I.D. Caruntu. Ki-67, p53 and Bcl2 analysis in colonic versus rectal adenocarcinoma. RJME. 2008, 49(2):163–71.

3) O.M. Zlatian. Study of tumor heterogeneity in colorectal cancer based on protein expression and gene mutation. PhD thesis. University of Medicine and pharmacy of Craiova. Romania. 27p.

4) B. Qasim, H. Ali, and A. Hussein. Immunohistochemical expression of p53 and Bcl2 in colorectal adenomas and carcinomas using automated cellular imaging system. IJP. 2012;7(4):215-23.

5) P.F. Rambau, M. Odida, and H. Wabinga. p53 expression in colorectal carcinoma in relation to histopathological features in Ugandan patients. African Health Sciences. 2008;8(4):234-8.

6) B.A. Yuan, C.J. Yu, K.T. Luh, S.H. Kuo, Y.C. Lee, and P.C. Yang. Aberrant p53 expression correlates with expression of vascular endothelial growth factor mRNA and Interleukin-8 mRNA and aeoangiogenesis in non–small cell lung cancer. J Clin Oncol. 2002;20:900-10.

7) J. Yoo, J.H. Jung, H.J. Choi, S.J. Kang, and C.S. Kang. Expression of Bcl2, p53 and VEGF in non-small cell lung carcinomas; their relation with microvessel density and prognosis. Korean J Pathol. 2005;39(2):74-80.

8) D. Zhao, X. Ding, J. Peng, Y. Zheng, and S. Zhang. Prognostic significance of Bcl2 and p53 expression in colorectal carcinoma. JZUS. 2005;6(12):1163-9.

9) P.C. Contu, S.S. Contu, and L.F. Moreira. Bcl-2 expression in rectal cancer. Arq. Gastroenterol. 2006;43(4):284-7.

10) J. Rosai. Gastrointestinal tract in: Ackerman's Surgical Pathology. 9th ed. St Louis: Mosby. 2004;p.750-858.

11) M.I. Koukourakis, A. Giatromanolaki, E. Sivridis, K.C. Gatter, and A.L. Harris. Inclusion of vasculature-related variables in the Dukes staging system of colon cancer. Clin Cancer Res. 2005;11:8653-60.

12) L. Georgiou, G. Minopoulos, N. Lirantzopoulos, A. Fiska-Demetrio, E. Maltezos, and E. Sivridis. Angiogenesis and p53 at the invading tumor edge: prognostic markers for colorectal cancer beyond stage. Journal of Surgical Research. 2006;131(1):118-23.

13) G.D. Guetz, B. Uzzan, P. Nicolas, M. Cucherat, I.F. Morere, and R. Benamouzig. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. BJC. 2006;94(12):1823-32.

14) S. Gurzu, J. Jung, L. Azamfirei, T. Mezei, A.M. Cimpean, and Z. Szentirmay. The angiogenesis in colorectal carcinomas with and without lymph node metastases. RJME. 2008;49(2):149-52.

15) G. Galizia, F. Ferraraccio, E. Lieto, M. Orditura, P. Castellano, and V. Imperatore. Prognostic value of p27, p53, and vascular endothelial growth factor in Dukes A and B colon cancer patients undergoing potentially curative surgery. Dis Colon Rectum. 2004;47:1904-14.

16) A. Giatromanolaki, G.P. Stathopoulos, E. Tsiompanou, C. Papadimitriou, V. Georgoulias, and K.C. Gatter. Combined role of tumor angiogenesis, Bcl2, and p53 expression in the prognosis of patients with colorectal carcinoma. Cancer. 1999;86(8):1421-30.

17) C. Fondevila, J.P. Metges, J. Fuster, J.J. Grau, A. Palacı, and A. Castells. p53 and VEGF expression are independent predictors of tumor recurrence and survival following curative resection of gastric cancer. BJC. 2004;90:206-15.

18) G. Perrone, B. Vincenzi, D. Santini, A. Verza, G. Tonini, and A. Vetrani. Correlation of p53 and Bcl2 expression with vascular endothelial growth factor (VEGF), microvessel density (MVD) and clinico-pathological features in colon cancer. Cancer Lett. 2004;208(2):227-34.

19) I.S. Pity. Histopathological and Immunohistochemical Approach for Characterization of Undifferentiated Malignant Tumors. JABHS. 2011;12(2):49-57

20) I.S. Pity, and A.M. Baizeed. Identification of Helicobacter Pylori in Gastric Biopsies of Patients with Chronic Gastritis, Histopathological, Immunohistochemical Study. Duhok Med J. 2011;5(1):69-77.

21) N. Weidner, J.P. Semple, and W.R. Welch, and J. Folkman. Tumor angiogenesis and metastasis: correlation in invasive breast carcinoma. N Engl J Med. 1991;324(1):1-8.

22) S. Cascinu, F. Graziano, V. Catalano, S. Barni, P. Giordani, A.M. Baldelli. Vascular endothelial growth factor and p53 expressions in liver and abdominal metastases from colon cancer. Tumor Biol. 2003;24:77-81.

23) I. Zlobec, R. Steele, R.P. Michel, C.C. Compton, A. Lugli, and J.R. Jass. Scoring of p53, VEGF, Bcl2 and APAF-1 immunohisto-

International Journal of Advancements in Research & Technology, Volume 2, Issue 3, March 2013
ISSN 2278-7763

chemistry and interobserver reliability in colorectal cancer. Modern Pathology. 2006;19:1236-42.

24) N. Sharifi, K. Sharifi, H. Ayatollahi, M.T. Shakeri, M.H. Sadeghian, and J.B. Azari. Evaluation of angiogenesis in colorectal carcinoma by CD34 immunohistochemistry method and its correlation with clinicopathologic parameters. Acta Medica Iranica. 2009;47(3):161-4.

25) V. Bazan, M. Migliavacca, C. Tubiolo, M. Macaluso, I. Zanna, and S. Corsale. Have p53 gene mutations and Protein expression a different biological significance in colorectal cancer? Journal of Cellular Physiology. 2002;191:237-46.

26) H.M. Ismail, M. El-Baradie, M. Moneer, O. Khorshid, and A. Touny. Clinico-pathological and prognostic significance of p53, Bcl_2 and Her-2/neu protein markers in colorectal cancer using tissue microarray. Journal of the Egyptian Nat. Cancer Inst. 2007;19(1):3-14.

27) H.A. Saleh, H. Jackson, and M. Banerjee. Immunohistochemical expression of Bcl_2 and p53 oncoproteins: correlation with Ki67 proliferation index and prognostic histopathologic parameters in colorectal neoplasia. Appl Immunohistochem Mol Morphol. 2000;8(3):175-82.

28) G. Afrem, S.S. Mogonata, F.A. Secureanu, A. Olaru, C. Neamtu, and B.D. Totolici. Study of molecular prognostic factors Bcl_2 and EGFR in rectal mucinous carcinomas. RJME. 2012;53(2):277-85.

29) F.P. Carneiro, L.N. Ramalho, S. Britto-Garcia, A. Ribeiro-Silva, and S. Zucoloto. Immunohistochemical expression of p16, p53, and p63 in colorectal adenomas and adenocarcinomas. Diseases of the Colon and Rectum. 2006;49(5):588-94.

30) D. Lazari, S. Taban, M. Raica, I. Sporea, M. Cornanu, and A. Goldis. Immunohistochemical evaluation of the tumor neoangiogenesis as a prognostic factor for gastric cancers. RJME. 2008, 49(2):137-48.