p53 expression in colorectal carcinoma in relation to histopathological features in Ugandan patients

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Abstract

Background: It has been shown that colorectal carcinoma is increasing in incidence in African countries. This could be due to change in life style. Molecular pathogenesis of colorectal cancer commonly involves mutation in *p53* gene which leads to expression of *p53* protein in tumor cells. Expression of *p53* protein has been associated with poor clinical outcome and reduced survival in patients.

Objective: This was a retrospective laboratory based study carried out in the Department of Pathology Makerere University, Kampala, Uganda. The aim of the study was to evaluate the expression of p53 protein in colorectal carcinoma in Ugandan patients, specifically its association with histological types, degree of differentiation, sites of the tumor and demographic characteristics of the patients.

Methods: Immunohistochemistry was carried out on 109 patient's paraffin embedded tissue blocks of colorectal carcinoma diagnosed in the Pathology Department, Faculty of Medicine Makerere University Kampala during the period 1995 to 2005. The indirect immunoperoxidase method using monoclonal antibody p53 DO-7 and Envision + Dual link system-HRP to detect p53 expression was used. Haematoxylin and eosin stain was used for evaluation of histological types and degree of differentiation of the tumors. Topography of the tumors and demographic data were obtained from accompanying histological request forms.

Results: Out of 109 patient's tissue blocks that were studied, 61 cases (56%) expressed *p53* protein in the nucleus of malignant cells. Right sided colonic tumors were commoner (53.2%) than left sided colonic tumors (46.8%). *p53* protein was expressed more in left sided colonic tumors with a significant difference (p<0.05), it was also expressed more in well differentiated tumors and non mucinous adenocarcinomas but with no significant difference (p>0.05). *p53* expression was not affected by age or sex.

Conclusion: Frequency of *p53* protein expression in Ugandan patients did not differ from that reported in the other parts of the world. It was expressed more in the left sided colonic tumors and this could support the hypothesis that right and left colonic tumors could have different pathogenesis and probably also responsible for difference in prognosis in these two topographic sites.

Key words: *p53*, colorectal carcinoma, Immunohistochemistry.

Introduction

There is a marked variation in the incidence of colorectal carcinoma worldwide ranking the third common cancer with Western countries having high rate compared to Africa. The incidence (per 100,000 populations) in North America is 40.6 in male and 30.6 in female where in Northern Europe is 34.7 in males and 25.2 in females. In Asian countries for example South Eastern Asia the incidence is 12.6 in males and 10 in females where in Sub Saharan Africa it is 5.7 in male and 4.6 in female per 100,000 populations[1]. In Uganda it has risen from 3 to 6.8 in females, and from 2.7 to 6.6 per 100,000 in males over the period 1960-1997[2]. Topography of the tumor is also different with low risk areas having the caecum as the commonest site of the tumor[2], whereas in high risk areas the tumor commonly affects the left side. These differences are thought to be due to interaction of environmental factors and several genetic alterations. It has been shown that *p53 gene* mutation is frequent phenomena occurring in about half of the cases of colorectal carcinoma[3, 4]. The product of *p53 gene* is 53kd protein (wild type) which is involved in cell cycle regulation. Mutation of *p53 gene* gives rise to abnormal protein with a long half life rendering it to be easily detected by immunohistochemistry. The mutant form of the *p53 gene* appears to act as dominant oncogene, but the wild type *p53* normally acts as recessive tumor suppressor gene[5]. It has been reported that in colorectal carcinoma *p53 gene* mutation causes conversion of late adenomas to carcinoma in adenoma carcinoma sequence[6]

Detection of *p53* protein in malignant cells has also been associated with poor clinical outcome and reduced survival in several tumor types[7, 8]. This study was therefore designed to assess whether p53 expression do exist in the Ugandan patients, and if the magnitude is the same as compared to that of the other parts of the

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world. This could provide a clue in the pathogenesis of colorectal carcinoma in low risk populations.

Materials and methods

109 patients were recruited for the study from the years 1995-2004 where their paraffin embedded tissue blocks were retrieved from the repository of the Department of Pathology, Faculty of Medicine Makerere University. During this time there was 150 cases of colorectal carcinoma investigated at Mulago hospital, the ones with incomplete clinical data, demography and tissue samples which was not adequate were ruled out so remaining with 109 cases. For the patients with more than one blocks all the blocks were examined and the one which represent the tumor best (with no necrosis, no much mesenchymal tissue) was selected for the study. Haematoxylin and Eosin sections were prepared to establish histological types and degree of differentiation of the tumor. Request forms accompanied these biopsies were retrieved and topography of the tumor and demographic data was obtained.

Tissue sections of 3ì m thickness were prepared and mounted on sialinized slides. Immunohistochemical staining was done following standard procedures as described by a manufacturer. Following incubation overnight at 56ºC, the sections were dewaxed and rehaydrated. Antigen retrieval was done by immersing the slides in Tris-Edta solution pH 9.0 and heated for 10 min at high power and then 15 min at low power in the domestic microwave rated 800W. Sections were then transferred to phosphate buffered saline (PBS) pH 7.6 after cooling. This buffer was used for washing in all steps. Endogenous peroxidase activity of the tissue was blocked with 3% hydrogen peroxide for 10 min (Dako Denmark K4065) and sections were washed three times. Sections were incubated with monoclonal antibody p53 DO-7 (Dako Denmark) for 60 min followed by wash in PBS.

Antibody binding was detected by using Envision [+] Dual link system-HRP (Dako Denmark) for 45 min, followed by 3 3'-Diaminobenzidine in chromogen diluted in the ratio of 1: 50 with DAB substrate buffer after wash. Sections were counterstained by Meyer's haematoxylin for 10 min and then mounted with DPX. A section of esophageal carcinoma known to stain positive for *p53* was included in each run as positive control and N- Universal negative control mouse (Dako) which does not recognize *p53* protein as negative control. A tumor was classified as *p53* positive when nuclear staining was observed in 5% or more of the cells counted in 10 high power fields[9, 10]. Right sided tumors were classified as those originating proximal to the splenic flexure and left side tumor as those located distal to or at this site including rectal tumors[9, 11].

SPSS for Windows 10.0 software was used to analyze the data and X^2 test was used to assess the association between *p53* expression and histopathological features and demographic characteristics of the patient. For statistical significance, the cut off point for p-value was set at 0.05.

Approval was sought from Department of Pathology and Makerere Faculty of medicine research and ethics committee. No names of patients were used; biopsy numbers were used to identify the specimens and searching demographic data from the request form.

Results

A total of 109 biopsy specimens were studied and of these 61 cases (56%) expressed *p53* in the nuclei of the malignant cells (95% confidence limits 46%-65%). Fifty one cases (56.8%) were males of whom (56.9%) expressed *p53*, and 58 (53.2%) were females of whom (55.2%) expressed *p53*. Patient's age ranged from 13-85 years with mean of 48.4 and standard deviation of 15.9. Most of the patients belonged to the local Ganda group while the remaining came from other tribal groups from different parts of Uganda. Expression of *p53* by age groups is shown in table 1 and the age group 51-60 years expressed *p53* more than the other age groups (75%) with no significant difference (p> 0.05). This trend remains the same even after stratifying the population in two age groups, young age (below 50 years) and old group (above 50 years). When *p53* was analyzed by the degree of differentiation of the tumor, 65.9% of well differentiated tumors expressed it, 54.3% in moderately differentiated and 45.5% in poorly differentiated tumors. The trend shows decrease in *p53* expression with worsening histological grade with no significant difference (p >0.05). This trend was also the same when p53 was analyzed by histological types of the tumor; whereas non mucinous adenocarcinoma expressed more *p53* (59%) compared to the mucinous adenocarcinoma (45.8%) and signet ring cell carcinoma 57.9% (p>0.05). Tumors of the left colon expressed *p53* (55.7%) more than the right side (44.3%) as shown in the table 2, with significant difference (p< 0.05).

Table 1: p53 protein expression in different age groups

Age group	p53 expression	
	Positive (n %)	Negative (n %)
11-20	7 (58.3%)	5 (41.7%)
21-30	9 (56.3%)	7 (43.8%)
31-40	7 (58.3%)	6 (46.2%)
41-50	7 (58.3%)	5 (41.7%)
51-60	12 (75.0%)	4 (25%)
61-70	3 (33.3%)	6 (66.7%)
71-80	11 (61.1%)	7 (38.9%)
81-90	5 (38.5%)	8 (61.5%)

$X^2 = 6.111$, $p = 0.527$

Table 2: p53 expression in right colon and left colon

Site of tumor	p53 expression	
	Positive (n %)	Negative (n %)
Right colon	27 (44.3%)	31 (64.6%)
Left colon	34 (55.7%)	17 (35.5%)

$X^2 = 4.455$ $p = 0.035$

Figure 1: Photomicrograph showing uniform nuclear staining for p53 protein in a well differentiated adenocarcinoma x100
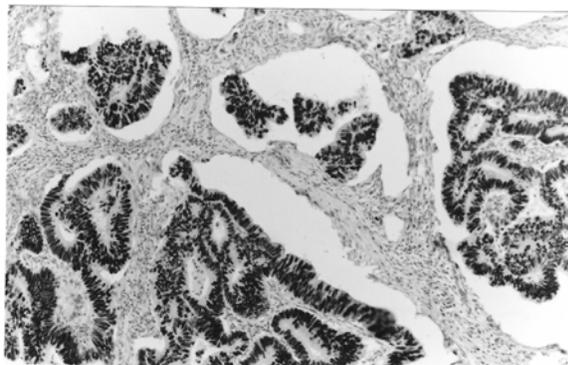
Discussion

In this study, *p53* protein expression was seen in 56% of colorectal carcinomas. This prevalence is similar to what have reported and ranged from 52.5% to 61.4% [11-17]. These figures are lower than that reported by Banu Lebe et al[18] but higher than those which were found in some studies which varied from 43% to 48% [10, 19, 20]. The over expression of *p53* seen in this study and other previous studies, support the hypothesis that *p53* gene mutations are important in colorectal carcinogenesis. This is also supported by results from a number of studies showing that *p53* expression is positively correlated with *p53* gene mutation[14, 20-22]. These findings suggest that a subset of colorectal carcinomas in both low and high incidence groups is due to *p53* gene mutation.

A study from Israel showed that the prevalence of *p53* in high risk, intermediate risk and low risk groups for colorectal cancer was similar[23]. This could be due to a common pathway in *p53* mutation in colorectal carcinogenesis. Two common agents, alcohol and tobacco smoke have been implicated as possible factors in *p53* alterations in the pathogenesis of colorectal cancer in both low risk and high risk populations[21, 24]. These two agents could also be important in Ugandan cases.

There have been reports about clinical and pathological differences between right and left side colorectal tumors[15, 17, 23, 25]. Our findings of a higher proportion of *p53* positive tumors on the left colon compared to the right colon tend to support these views. This implies that the pathogenesis of left and right sided colonic tumors are different, and possibly the reasons for differences in their prognosis. In sporadic colorectal carcinomas mutation of the *p53 gene* is common finding in left colon tumors but less in right colon tumors[10, 22]; this can infer that most of colorectal cancers in our setting are sporadic.

It is known that adenomatous polyposis syndrome predispose to the tumors occurring in the left colon while right colonic tumors are commonly associated with hereditary non polyposis colorectal cancer (HNPCC)[26, 27]. In our setting adenomatous

polyposis syndrome has rarely been reported. Environmental factors and particularly dietary are thought to play key roles in pathogenesis of colorectal cancer especially in the left colon[22, 26]. Change in the life style of Ugandans with introduction of fast foods could be one of the mechanisms for the pathogenesis of the left colon cancers. This could explain the increase in incidence of colorectal carcinoma which has been reported by Wabinga et al[28]. On the other hand, hereditary non polyposis colorectal cancer syndrome produces few adenomas with carcinoma occurring mainly in the right side. In this study there were more cases of the right side tumors which could be due to a high prevalence of carcinoma of the caecum in Ugandan[29]. Another suggestion is that hereditary non polyposis syndrome could be involved in the pathogenesis of colorectal cancer in our setting.

Several studies have shown that *p53* over expression is associated with poorer prognosis in patients with colorectal cancer [19, 21, 30, 31], while others has shown that *p53* expression has prognostic implication on left side tumors only with short survival[11, 15, 22]. With regard to grade Kapiteijn et al 2001[22] found that in poorly differentiated colorectal carcinomas, over expression of *p53* occurred in more than 70% of tumors compared to less than 30% in those with well or moderately well differentiated tumors. In this study, *p53* expression was more common in well differentiated tumors than in poorly differentiated tumors. Although the differences were not significant, the trend showed a decreased in p53 expression with worsening histological grade, suggesting a possible association. This could be due to the fact that there were more cases of well differentiated tumors in this study; this also can infer that expression of *p53* in Ugandan patients is not influenced by degree of tumor differentiation, and this can be linked by poor prognosis of this cancer in Ugandan patients as five year survival rate is very low (8.3%) compared to 54.2% for Black American patients[32].

Lack of *p53* nuclear staining does not always rule out absence of p53 mutation. *p53* staining is common in missense mutations which increase stability of the protein rendering it easily to be detected by immunohistochemisry. Mutations which produce deletion, truncation or no protein can not be detected by immunohistochemistry but only by molecular methods [10, 12, 14]. It is possible that in our setting as the grade of the tumor advance the mutations patterns are different and does not lead to expression of the *p53* protein, this require molecular studies to confirm. One study found that patients with mutations due to deletion had advanced disease and tumor stage[10], it is possible

that most of poorly differentiated tumors in our area has this type of mutations. In contrast to our findings and those of Kapiteijn et al 2001[22], Cameiro et al[33] found no relationship between *p53* expression and histological differentiation. These divergent results could be due to differences in the various populations which need to be further investigated.

In this study, *p53* expression was used as a marker of *p53 gene* mutation. Previous studies have shown that *p53* expression is an approximate measure of *p53* gene mutation since it can detect up to 75% of tumors with real gene mutation, while other studies have shown up to 94% agreement between p53 expression and mutation status of *p53* gene by molecular studies[22, 34]. For routine purposes, detection of *p53* mutation using immunohistochemistry is more feasible and cheaper than molecular analysis which is more expensive and difficult to apply when using archival material[12].

Conclusion

p53 expression in Ugandan patients appear to be similar to those from the other parts of the world where such studies has been done, it was expressed more on the left sided colonic tumors. This could support the hypothesis that right and left colonic tumors could have different pathogenesis and reasons for different prognosis in these tumors. There is need to conduct further studies to ascertain the types of genetic mutations and also to find out if some cases could be due to epigenetic factors.

References

1. Bernard W. Stewart, P.K., ed. World Cancer burden. 2003, IARC Press: Lyon. 16-17.
2. Parkin DM, Ferlay J. Hamdi-Cherif H, Sitas F, Thomas J.O, Wabinga H, Whelan S.L. Cancer in Africa. IARC Press Lyon 2003:277-280
3. Kressner U, Inganas M, Blicstand I, Påhlam L, Glimelius B, Lindmark G. Prognostic value of p53 genetic change in colorectal cancer. J Clin Oncol 1999; **17**: 593-599.
4. Mueller RF, Yung LD. Elements of medical genetics. 9 ed; Churchill :172
5. Curtis C. Harris, Hollstein M. Clinical implications of the p53 tumor suppressor gene. New Eng J of Med 1993; **329**: 1318-1327.

6. Fearon E.R, Vogelstan B. Genetic model for colorectal Tumorigenesis. Cell 1990; **61**: 759-767.

7. Dignam JJ, Ye Y, Colangelo L, Smith R, Mamouncs EP, Wie and HS, Wolmark N. Prognosis after rectal cancer in blacks and whites participating in adjuvant therapy. Clin Oncol 2003; **21**: 413-420.

8. Levine AJ, Perry ME, Chang A, Silver A, Dittmer D, Wu M, Welsh D. The role of p53 tumor suppressor genes in tumorigenesis. Br J Cancer 1994; **57**: 1-9.

9. Elsaleh H, Powell B, McCaul K, Grieu F, Grant, Joseph D, Iacopeta B. p53 Alteration and microsatellite instability have predictive value for survival benefit from chemotherapy in stage III colorectal carcinoma. Clin Cancer Res 2001; **7**: 1343-1349.

10. Bazan V, Migliavacca M, Tubiolo C *et al*. Have p53 gene mutations and Protein expression a different biological significance in colorectal cancer? Journal of Cellular Physiology 2002; **191**: 237-246.

11. Paluszkiewicz P, Berbeæ H, Pawlowska-Wakowicz B, Cybusk M, Paszkowsca A. p53 protein accumulation in colorectal cancer tissue has prognostic value only in left sided colon tumors. Cancer detection and prevention 2004; **28**: 252-259

12. Nassierowsken-Guttmejer A, Treeciale L, Nowacki MP, Ostrowsk J. p53 protein accumulation and p53 gene mutation in colorectal cancer. Path Oncol Res 2000; **6**: 275-279.

13. Cotran R, Kumar V, Collins T. Pathological basis of disease. 7 ed. W.B and Saunders; Philadelphia 2004: 864

14. Colomer A, Erill N, Verdm M *et al*. Lack of p53 nuclear immunostaining is not indicative of absence of TP53 gene mutation in colorectal adenocarcinomas. . Appl immunohistochemistry and Molecular Morphology 2003; **11**:130-137.

15. Rosati G, Chiacchio R, Reggiardo G, De Sanctis D, Manzione L. Thymidylate synthase expression, p53, bcl-2, Ki-67 amd p27 in colorectal cancer: Relationship with tumor recurrence and survival. Tumor Biol 2004; **25**:258-263.

16. Zhau X, Yu J, Chen H, Yu H, Luo H. Expression of cellular FLICE- inhibitory protein and its association with p53 mutation in colon cancer. World Gastroenterol 2005; **11**: 2482-2485.

17. Jong K, Gouw Annete SH, Peeters P *et al*. p53 mutation analysis of colorectal liver metastases. Relation to actual survival, angiogenic status and p53 overexpression. Clin Cancer Res 2005; **11**: 4067-4073.

18. Lebe B, Sarioðlu S, Sökmen S, Hülya E, Füzün M, Küpelioðlu A. The clinical significance of p53, p21, and p27 expression in rectal carcinoma. Appl Immunohistochem Mol Morphol 2005; **13**:38-44.

19. Bauzoorene H, Gervas P, Cerottini JP *et al*. p53 and k-ras as prognostic factor for Dukes' stage B colorectal cancer. European Journal of Cancer 2000; **36**:1008-1015.

20. Zhao D, Ding X, Peng J, Zheng Y, Zhang S. Prognostic significance of bcl-2 and p53 expression in colorectal carcinoma. Journal of Zhejiang University 2005; **6**:1163-1169.

21. Huang C, Cheng Y, Chen M Shyang Lin, Chou M, Lee H. Different p53 mutation patterns in colorectal tumors from smokers and nonsmokers. . Environmental and Molecular mutagenesis 2006; **47**: 527-532.

22. Kapiteiijn E, Liefers G, Los L *et al*. Mechanisms of oncogenesis in colon versus rectal cancer. J Pathol 2001; **195**: 171-178.

23. Darwish H, Trejo I, Shapira I *et al*. Fighting colorectal cancer: Molecular epidemiology differences among Ashkenazi and Sephardic Jews and Palestinians. . Annals of Oncology 2002; **13**:1497-1501.

24. Terry M, Neugut A, Mansukhani M, Waye J, Harpaz N, Hibshoosh H. Tobacco, alcohol and p53 overexpression in early colorectal neoplasia. BMC Cancer 2003; **3**: 29.

25. Smyth EF, Sharma A, Sivarajasingham N, Hartley J, Monson J. Prognostic implication of hMLH1 and p53 immunohistochemical status in Right-sided colon cancer. Dis Colon Rectum 2004; **47**: 2086-2092.

26. Bufill JA. Colorectal cancer: Evidence for distinct genetic categories based on proximal or distal tumor location. Ann Int Med 1990; **113**: 779-788.

27. Nagorni A, Kutic V, Milanovic J, Zivkovic V, Bjelakovic G. The Lynch Syndrome: Report on family "S". Arch Gastroenterohepatol 2002; **21**: 1-2.

28. Wabinga HR, Parkin DM, Wabwire-Maugen F, Nambooze S. Trends in cancer incidence in Kyadondo County, Uganda 1960-1997. Br J Cancer 2000; **83**: 1585-92.

29. Owor R. Carcinoma of colon and rectum in Ugandan Africans. East Afr Med J 1983; 60: 228-31.

30. Zhang H. Evaluation of four antibodies in detecting p53 protein for predicting clinicopatological and prognostic significance in colorectal adenocarcinoma. Clin Cancer Res 1999; **5**: 4126-4132.

31. Lashner Bret A, Bauer William M, Rybicki Lisa A, Goldblum John R. Abnormal p53 immunohistochemistry is associated with an increased colorectal cancer- related mortality in patients with ulcerative colitis. Amer J of gastroenterol 2003; **98**: 1423-1427.

32. Gondos A, Brenner H, Wabinga H, Parkin DM. Cancer survival in Kampala, Uganda. Br J. Cancer 2005; **92**: 1808-1812.

33. Cameiro Fabian P, Ramalho Leandra NZ., Britto-Garcia, Ribeiro-Silva A, Zucoloto S. Immunohistochemical Expression of p16, p53 and p63 in Colorectal Adenomas and Adenocarcinomas. Dis Colon Rectum 2006; **49**: 588-594.

34. Servomaa K, Kosma V-M, Hirvikoski P, Rytömaa T. p53 and K-ras mutations in carcinoma of the rectum among Finish women. J Clin Pathol: Mol Pathol 2000; **53**: 24-30.