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An Immunohistochemical Study of P53 and Bcl2 in Synchronous Colorectal Adenomas and Adenocarcinomas – A Study from South India

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Abstract: *Adenomatous polyps are the commonest types of polyps of the large intestine. The malignant potential of these polyps has been studied by Vogelstein et al, who proposed the classic adenoma-carcinoma sequence. Other mechanisms such as microsatellite instability and hypermethylation have also been proposed in recent times in the pathogenesis of colorectal carcinomas.Aim: To study (1)the incidence and morphology of colorectal adenomas in a large hospital-based population (2)the incidence of non-contiguous colorectal adenocarcinomas occurring synchronously with colorectal adenomas (3) the expression of p53 and bcl-2 in polyps occurring synchronously with and without cancers. Materials and methods: All colorectal adenomatous polyps were included in the study. The incidence and morphology of colorectal adenocarcinomas occurring synchronously with the adenomatous polyps were analysed.Immunohistochemical expression of p53 and bcl-2 were studied in 16 cases of polyps occurring with non-contiguous cancers and matched control group of polyps. Conclusion: 120 colorectal adenomatous polyps were studied, of which 16 cases showed co-existent non-contiguous adenocarcinomas. P53 & bcl2 were found to be higher in proportion in the "polyp with co-existent cancer" group than the "polyps alone" group. However, the difference in expression between the 2 groups were not statistically significant.P53 and bcl2, though are involved in malignant transformation, cannot be used as predictive markers for development of malignancy in adenomatous polyps. Study of more molecular markers may be necessary to explain colorectal carcinogenesis in adenomatous polyps.*

Key words: *adenomatous polyp, colorectal cancer, adenoma-carcinoma sequence, p53, bcl2.*

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I. Introduction

An adenoma is a circumscribed benign epithelial neoplasm with a potential for malignant change. 2 autopsy studies have shown that adenomas of large intestine are more commonly seen in populations at risk for colorectal carcinomas[1,2].They are generally evenly distributed along the length of large intestine. However, the incidence of carcinomas differ in their site distribution as they are more common in rectum and distal colon. This is due to higher conversion rate of adenoma to carcinoma in the rectum[2,3].

Adenomatous polyps of the large intestine are considered as precursor lesions for the development of colorectal carcinoma. However, at a given point of time, not all adenomatous polyps are found to be transformed to malignancy. The pathogenesis of colorectal carcinomas has been studied based on the many syndromes of colorectal cancers and different mechanisms proposed. The original study on this aspect was done by Vogelstein et al[4], who studied4 genetic alterations (K-ras, APC, p53 and del 18). Their study showed that alterations in APC, Kras and p53 were the principle events in the initiation, transformation and progression to adenocarcinoma.Torlakovic E et al[5] studied serrated colorectal polyps and identified a distinct group of serrated polyps with abnormal proliferation and decreased expression of hMHL1 and hMSH2. The role of mismatch repair deficiency has been elucidated from cases of HNPCC or Lynch syndrome. In addition, CpG island methylation pathway in microsatellite stable cases have also been found as a mechanism of cancer.

2 genes were taken into consideration for this study, namely p53 and bcl2. P53 is the commonest gene to be altered in human cancers and has been found to be mutated in the late stage of malignant transformation. Bcl2, on the other hand, has been found in some studies to be mutated early in the process.

II. Materials and Methods

This study was conducted at the department of pathology at Madras Medical College hospital, Chennai, India. After obtaining approval of the institutional ethics committee, all patients diagnosed with colorectal adenomatous polyps were included in the study. The study material included all the lesions identified as polyps

or polypoid lesions in the large intestine, which were 452 in number. Endoscopic biopsies (polypectomies) and intestinal resection specimens were both included.Lesions described as nodules or ulcerated proliferating masses were excluded to avoid confusion.A total of 120 specimens met the above criteria and were included in the study.

The specimens were collected along with relevant clinical details including age, sex, clinical presentation and family history of polyposis or GI cancers. The specimens were fixed using 10% neutral buffered formalin and processed as for routine histopathological studies using H & E stain. Cases with both colorectal adenomas and cancers present in the same patient were selected for Immunohistochemistry. A control group was selected by taking patients with isolated colorectal adenomas without any evidence of malignancy. The control population was matched with the case group for age, sex, site and type of adenomatous polyp. 16 cases and 16 matched controls were selected and immunohistochemical studies were done on 5-micron sections of paraffin blocks in which the antigen retrieval was done using microwave. p53 and bcl2 antibodies were used (details of antibodies given in table 1) and their expression identified using DAB chromogen.

Table 1. Details of antibodies used.

Antigen	Vendor	Species	Dilution
P53	BIOGENEX	Mouse	Ready to use
Bcl2	BIOGENEX	Rabbit	Ready to use

p53 and bcl2 were considered positive if more than 10% of representative epithelium (adenomatous epithelium) showed positivity. The lymphocytes in lamina propria served as internal control for bcl2. Statistical correlation was done using Fisher exact test.

III. Results

The adenomatous polyps detected in the study were 120 in number out of 452 gastrointestinal polyps (26.5%) and were distributed as follows: 64 cases(53%) were tubular adenomas, 38 cases(31.7%) were tubulovillous adenomas, 16 cases(13.3%) were villous adenomas and 2 cases were flat adenoma (1.7%).The subsite distribution of these lesions have been published by the authors elsewhere[19]. 5 patients were found to have adenomatous polyposis coli (more than 100 polyps in colon) and 16 cases of adenomatous polyps were found synchronously with a co-existing non-contiguous colorectal malignancy. 5 other cases of polyps displaying malignant change (contiguous cancer) were noted. The details are shown in tables 2-4.

TABLE 2: ADENOMATOUS POLYPS OF LARGE INTESTINE

Polyp	No Malignancy	Malignant Features Seen	With Non-Contiguous Cancer	Total (Percent)
TA	54	1	5	64(53.33%)
TVA	25	3	8	38(31.67%)
VA	11	1	3	16(13.33%)
FA	2			2(1.67%)
Total	92(76.7%)	5(4.2%)	16(13.3%)	120

TA – Tubular Adenoma;TVA – Tubulovillous Adenoma; VA – Villous Adenoma; FA – Flat Adenoma

TABLE 3: ADENOMATOUS POLYPOSIS COLI

	Age	Sex	Predominant type of polyp	Malignant change
1.	30	Female	Villous adenoma	Present
2.	35	Female	Tubulovillous adenoma	Present
3.	39	Male	Tubular adenoma	Absent
4.	42	Male	Tubular adenoma	Absent
5.	65	Male	Tubular adenoma	Absent

TABLE 4: DETAILS ABOUT POLYPS WITH CO-EXISTING COLONIC CANCERS

ADENOMA	ADENOCARCINOMA			TOTAL
	Well Differentiated	Moderately Differentiated	Poorly Differentiated	
Tubular	2	3	0	**5**
Tubulovillous	3	4	1	**8**
Villous	1	1	1	**3**
Total	**6(37.5%)**	**8(50%)**	**2(12.5%)**	**16**

The details of the immunohistochemical expression of p53 and bcl2 in the 3 groups are shown in tables 5&6. As can be seen from the tables, the expression of p53 and bcl2 are higher in the polyps with co-existent cancers than the comparative groups. Statistical analysis showed that the association between p53 expression or bcl2 expression with the occurrence of malignancy is not statistically significant at $p<0.05$. ($p=0.72$ for p53 and $p=0.22$ for bcl2)

TABLE 5: COMPARISON OF P53 EXPRESSION IN COMPARATIVE POLYPS, POLYPS WITH SYNCHRONOUS CANCERS AND IN CANCERS.

	P53 positive	P53 negative	Total
COMPARATIVE POLYPS (polyps without co-existent cancers)	7	9	16
POLYPS WITH SYNCHRONOUS CANCER	9	7	16
CANCERS	11	5	16

TABLE 6: COMPARISON OF BCL2 EXPRESSION IN COMPARATIVE POLYPS, POLYPS WITH SYNCHRONOUS CANCERS AND IN CANCERS.

	bcl2 positive	bcl2 negative	Total
COMPARATIVE POLYPS (polyps without co-existent cancers)	10	6	16
POLYPS WITH NEARBY CANCER	14	2	16
CANCERS	6	10	16

IV. Discussion

The incidence of colorectal cancers in India is relatively low as compared to the West, but on the increasing trend. In a study by Sinhaet al[6], incidence in the United States was found to be 40.6(men) and 30.7(women) per lakh population whereas the incidence rate in India is 4.7(men) and 3.2(women) per lakh population. Mohandas KM et al[7] studied the incidence of colonic cancer in 8 population registries in Indiaand found the incidence to vary from0.7 to 3.7 (men) and 0.4 to 3(women) per lakh population. They found that the incidence rates for large intestinal cancer in rural India is approximately half of that in urban India. Rectal cancer was also found to occur more commonly in young Indians.

In our study, 16 cases (13.3%) of adenomatous polyps were found with non-contiguous colorectal cancers. The published literature on study of synchronous occurrence of polyps and cancers in Indian population is very scarce. The incidence rate appears to be lesser than other studies from the West[8-11]. However, the occurrence of adenomas has been found more commonly in synchronous (multiple) cancers of the colon, compared to single cancers, in the studies from the West. The presence of associated adenomas or adenoma remnants is considered as an independent risk factor for occurrence of synchronous cancer[12].

Various individual studies of p53 and bcl2 expression in colorectal polyps and cancers have shown consistent results with p53 and more variable results with bcl2. Shanmugam et al[13]have found that expression of p53 and bcl-2 progressively increased from normal-appearing epithelium to adenomas to carcinomas. They concluded that the presence of p53 in the adenomatous epithelium is an indicator of aggressive behavior of colonic lesions, and that these patients are more likely to develop aggressive invasive cancer.The progressive increase in p53 expression has been noted in other human studies and has been proved in animal models but may be difficult to prove directly in humans as most polyps detected during endoscopy are removed by polypectomy. However, the increased expression of p53 can serve as an indication for placing such patients under surveillance.

In a study by Bosari et al[14], bcl2 expression was not correlated with p53 expression and had no prognostic significance. The different expressions of bcl2 in the three groups in this study indicate possible interactions between bcl2 and other genes involved in carcinogenesis. bcl-2 expression is seen in various pre-malignant and malignant lesions. Hence, it is suggested that bcl-2 genetic alterations are seen early in the

pathway of carcinogenesis. bcl 2 expression is said to have a favorable prognosis in breast and lung carcinomas and unfavorable prognosis in prostate carcinoma[15]. bcl-2 expression in colorectal carcinomas is found to be associated with a better clinical course, especially in the absence of p53 expression, indicating that neoplasia caused by inhibition of apoptosis may cause less aggressive malignancies than those caused by other oncogenes like p53 and K-ras[14,16,17]. An inverse relationship has been noted between bcl-2 and p53 expression in many malignancies, suggesting that these proteins may interact through opposite mechanisms: inhibition of apoptosis (bcl-2), and promotion of apoptosis (p53)[17,18].

V. Conclusion

Synchronous occurrence of adenomatous polyps and colorectal carcinomas is not uncommon, with lesser incidence compared to the West.The expression of p53 and bcl2 appears in progressively higher proportions from adenomatous polyps to cancers. However, the association was not statistically significant and study of more samples may throw light on the subject. In addition, study of more molecular markers involved in other pathways of carcinogenesis in these cases may also help to delineate the mechanism of carcinogenesis in adenomatous polyps.

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