Prognostic Value of p53 Expression in Intestinal Neoplasms - a Study in a Tertiary Health Care Centre

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Abstract: *Colorectal cancer is one of the most common tumours of the world with the varying distribution. Though small intestine is an uncommon site for tumour, it accounts for significant number of gastro-intestinal malignancies. Pathological assessment of the resected specimen is mandatory for assessing the postoperative outcome and the rationale for adjuvant therapy. Among the various prognostic factors, Mutation of the p53 tumour suppressor gene, plays an important role in the progression of colorectal carcinoma and so it represents a clinically useful marker of prognosis. The present study evaluates the role of p53 in intestinal neoplasms by assessing its level of expression and to correlate the level of p53 expression with the stage of the colorectal adenocarcinomas so that its prognostic value in colorectal adenocarcinomas can be calculated.*
Keywords :*Intestinal malignancies, colorectal adenocarcinoma, guardian genome, p53 expression, prognostic marker*

I. Introduction

Colorectal cancer ranks second among the most common tumours of the world according to world cancer report 2000[1]. There is worldwide variation in the distribution of intestinal neoplasm, which appear largely due to exogenous factors rather than genetic. Cancer of the colon is not a very common disease in our country and the incidence is much lower than in the western world. The incidence in India is about 7 / 100000[2].

Curiously the small intestine is an uncommon site for tumour, despite its great length and vast pool of dividing cells. Small bowel tumours account for 1-2% of all gastrointestinal neoplasms.[3]

Surgical resection is the primary treatment modality for colorectal carcinoma and the pathological assessment of the resection specimen provides data that is essential for patient management including the estimation of postoperative outcome and the rationale for adjuvant therapy. The essential elements of the pathological assessment of colorectal carcinoma resection specimen include pathological determination of TNM stage, tumour type, histological grade, status of resection margin and vascular invasion [4].

The Tp53 gene located in chromosome 17p13.1 is the single most common target for genetic alteration in human tumours. As it plays an important role in cell cycle control and in the induction of apoptosis, it has been described as "the guardian of the genome", "the guardian angel gene", and "the master watchman" referring to its role in conserving stability by preventing genome mutation. This p53 tumour suppressor gene is mutated in approximately 70-80% of colorectal carcinoma. Mutation or loss of p53 usually occurs at the time of transition from adenoma to carcinoma in the adenoma-carcinoma sequence [5]. The frequency of p53 abnormalities increases with the progression of the lesion. Mutation of the p53 tumour suppressor gene is thought to play an important role in the progression of colorectal carcinoma and might therefore represent a clinically useful marker of prognosis.

The present study evaluates the role of tumour suppressor gene protein p53 in intestinal neoplasms by assessing its level of expression of p53 in intestinal neoplasms and to correlate the level of p53 expression with the stage of the colorectal adenocarcinomas so that its prognostic value in colorectal adenocarcinomas can be calculated.

II. Materials And Methods

Out of 11891 cases reported totally at Thanjavur Medical College, 111 cases of intestinal neoplasms diagnosed during the period of January 2010 to June 2012 were included in this retrospective study. All the cases of intestinal malignancies irrespective of the age, sex, type, stage and grade were included and the specimens were fixed in 10% buffered neutral formalin and were subjected to routine histopathological examination. Sections of 3-5μ thickness were made and routine staining with hematoxycilin and eosin was done.

A total of 30 cases of colorectal adenocarcinomas and 7 cases of small intestinal neoplasms were selected for studying the p53 expression. Formalin fixed paraffin embedded 4μ thick tissue sections were stained with antibody directed against p53. Staining was performed according to the standard immunohistochemical staining protocol. The percentages of p53 positive tumour cells were calculated by

counting the number of brown stained tumour nuclei among the total number of cells in the most highly stained area in the selected microscopic fields at 400X magnification.

According to George E. Theodoropoulos et al p53 positivity was calculated [6]. If ≥10% or more of malignant nuclei were stained, the slide was scored as positive. While if fewer than 10% of the nuclei were stained, the slide was scored as negative. For small intestinal malignancy, all p53 positive nuclei were counted and the following semi-quantitative method was used: [±] – negative or equivocal (<5% positive cells), [+] – weak\moderate (5-50% positivity) and [++] – intensely positive (>50% positivity).[7] The statistical analysis was performed to determine whether p53 protein expression was correlated with the stage of the colorectal adenocarcinomas. Statistical analysis was carried out with the aid of Chi-square test (Java script package). P value of less than 0.05 was taken to indicate statistical significance.

III. Observation And Results

1.1 Incidence:

TABLE – 1: Incidence of Intestinal Neoplasms in this Study

Period	Total No. of specimens	Total neoplasms	Intestinal neoplasms	Incidence (%)
Jan 2010 – June 2012	11891	4286	111	2.6

Out of the 4286 neoplasms diagnosed totally, during the period of study (January 2010 – June 2012), there were 111 neoplasms arising in the intestine. Thus the annual incidence of intestinal neoplasms in our study was 2.6% (TABLE 1). Of 111 neoplasms, large intestinal neoplasm, with 100 cases, contributes significantly higher incidence with 90% (TABLE 2).

TABLE – 2: Incidence of Small and Large Intestine Neoplasms in this Study

Total No. of neoplasms	Small intestine		Large intestine	
	No. of cases	%	No. of cases	%
111	11	10	100	90

3.2 Analysis of p53 Expression:

TABLE – 3: p53 Expression in Colorectal Adenocarcinoma in relation to histological type
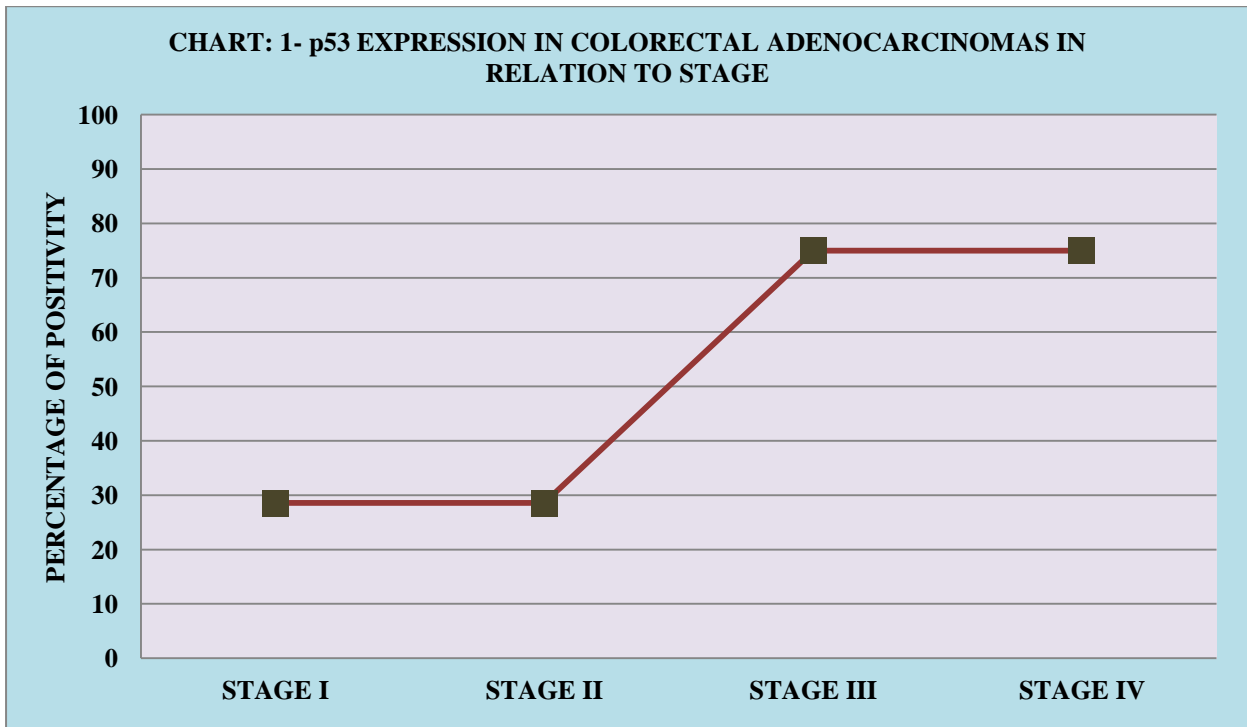
ADENOCARCINOMA	p53				TOTAL
	+	%	-	%	
CONVENTIONAL ADENOCARCINOMA	17	65	9	35	26
MUCINOUS ADENOCARCINOMA	1	25	3	75	4
TOTAL	18	60	12	40	30

In this study 53.3% of the colorectal adenocarcinomas showed p53 positivity. p53 over expression was more frequent in non-mucinous adenocarcinomas than in mucinous adenocarcinomas (TABLE- 3).

TABLE – 4: p53 Expression in Colorectal Adenocarcinomas in Relation to Stage

STAGE	p53		TOTAL	POSITIVE CASES %
	+	-		
I	3	4	7	42.86
II	3	4	7	42.86
III	9	3	12	75
IV	3	1	4	75
TOTAL	18	12	30	

For the purpose of statistical analysis stages I and II were considered as low stage tumours and stages III and IV were considered as high stage. The proportion of positively reacting colorectal tumours increases as the stage progresses. Statistical analysis using Chi- square test was done which revealed a P value of <0.05 (X^2 = 4.74) which is statistically significant. Hence p53 expression is significantly correlated with the stage of tumour in colorectal adenocarcinoma (TABLE- 4) (CHART- 1).

CHART: 1- p53 EXPRESSION IN COLORECTAL ADENOCARCINOMAS IN RELATION TO STAGE

TABLE – 5 : p53 Expression in Small Intestinal Tumours

TUMOURS	p53				TOTAL
	+	%	-	%	
ADENOCARCINOMA	2	67	1	33	3
LYMPHOMA	1	50	1	50	2

In this study 60% of the small intestinal tumours revealed p53 positivity. 67% of the adenocarcinomas and 50% of the lymphomas showed immune reactivity for p53 protein (TABLE-5).

DIAGRAMS: Well differentiated colorectal adenocarcinoma expressing p53 (score 0-4)

Figure 1: p 53- score 0
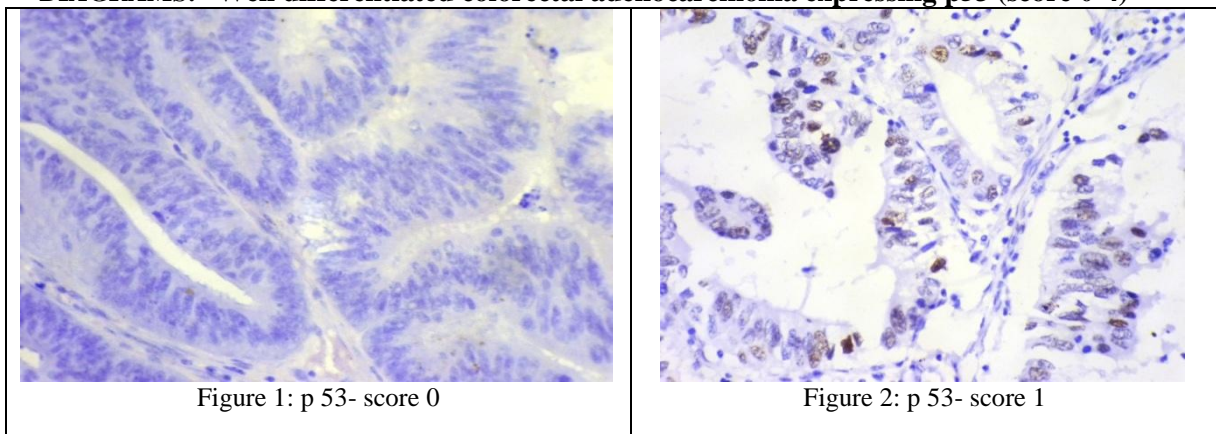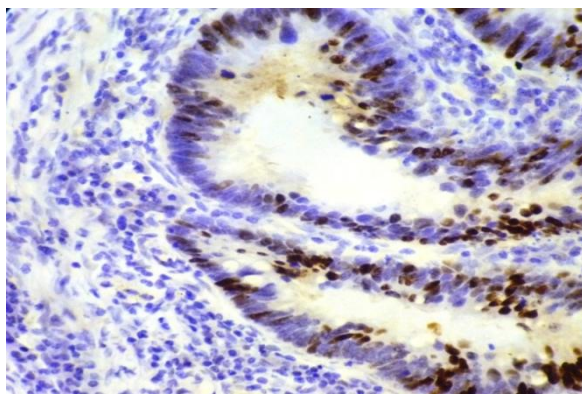
Figure 2: p 53- score 1
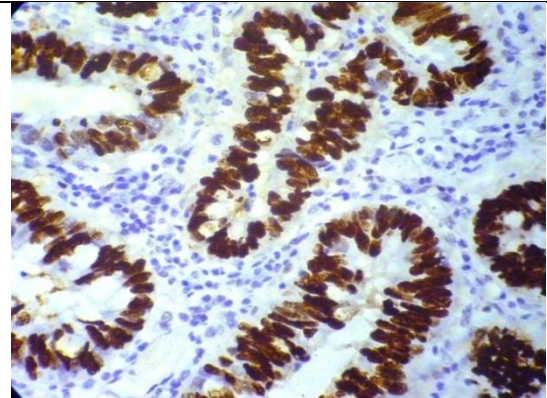
Figure 3: p 53- score 2

Figure 4: p 53- score 3

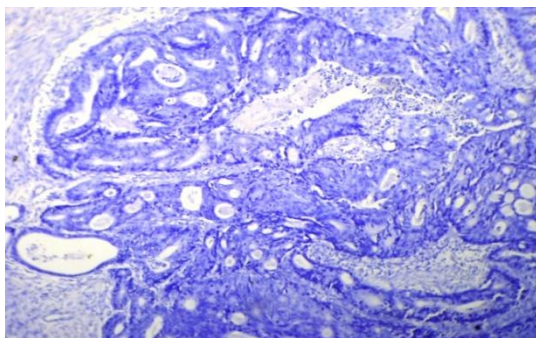**Moderately differentiated colorectal adenocarcinoma expressing p53 (score 0-4)**

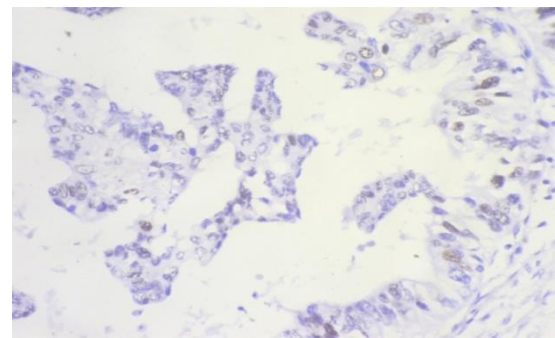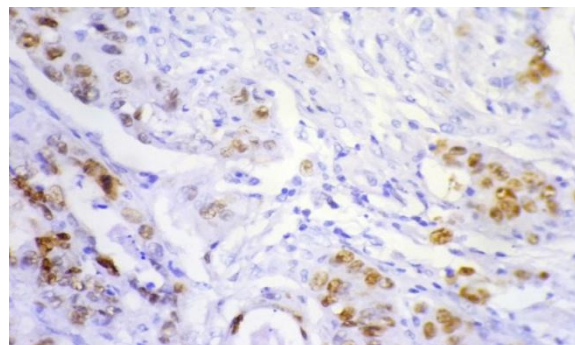Figure 5: p 53- score 0

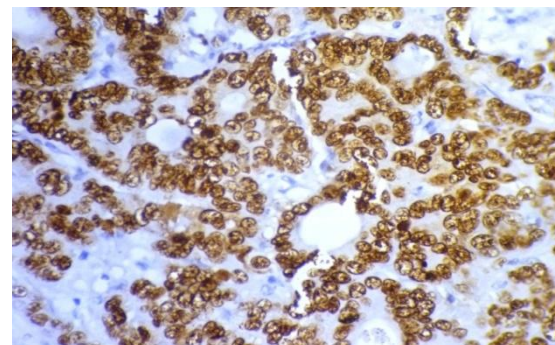Figure 6: p 53- score 1

Figure 7: p 53- score 2

Figure 8: p 53- score 3

Poorly differentiated colorectal adenocarcinoma expressing p 53(score 0-4)
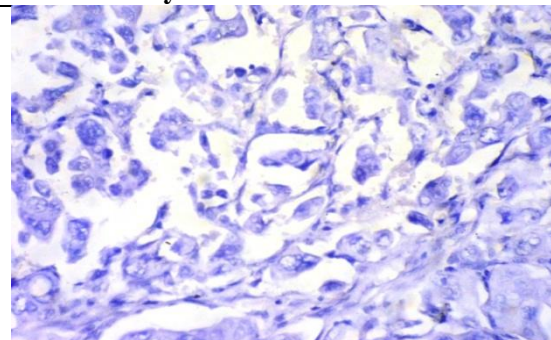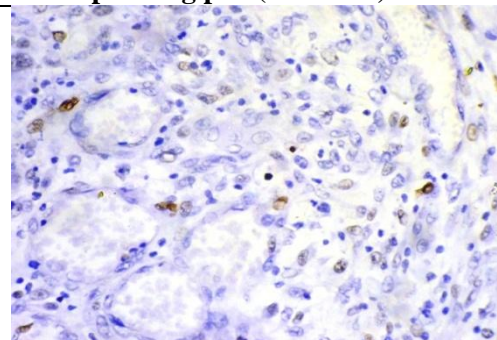

Figure 9: p 53- score 0
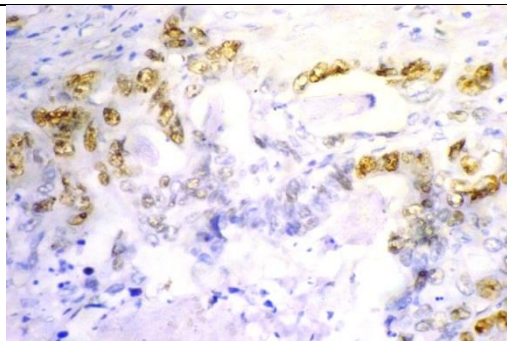

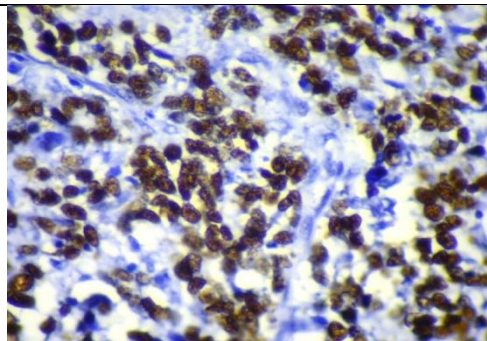Figure 10: p 53- score 1

Figure 11: p 53- score 2

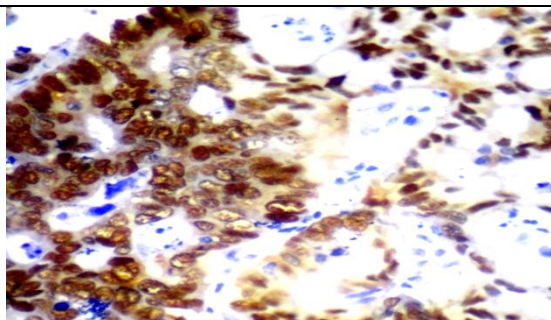Figure 12: p 53- score 3

Small Intestinal Malignancies

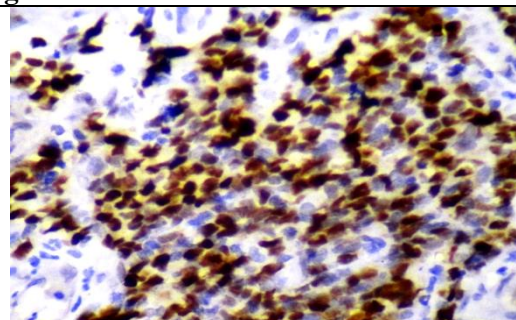Figure 13: small intestinal adenocarcinoma-p 53 positive

Figure 14: small intestinal lymphoma- p 53positive

IV. Discussion

4.1 p53 Immunoexpression in colorectal tumours:

p53 tumour suppressor gene is one of the most intensively studied tumour markers in the colorectal tumours. In this study 30 cases of colorectal carcinoma (colectomy specimens) were selected for the study of p53 expression by immunohistochemistry in various stages which included 4 mucinous adenocarcinomas. For the purpose of statistical analysis stages I and II were categorized as low stage and stages III and IV were categorized as high stage. According to George E Theodoropoulous et al[6] p53 immunostaining was done and the results were tabulated and analysed.

In the present study p53 expression was more frequent in non-mucinous adenocarcinomas (57.7% Vs 25%). (TABLE-3) This is in accordance with the study by Yuan-Tzu-Lan et al[8] who found that 39% of non-mucinous adenocarcinomas and 29% of mucinous adenocarcinomas showed p53 positivity and suggesting two different pathways, chromosomal instability and microsatellite instability of colorectal carcinogenesis respectively. T Starzynska et al[9] studied the relation of p53 expression in colorectal carcinoma with histological, clinical, prognostic features using follow-up data and concluded that p53 expression occurred as a late event and was associated significantly associated with advanced stage of disease, early relapse and death. J Walker et al[10] in their study found that the stage is the most accurate prognostic factor for survival. They concluded that p53 overexpression in colorectal carcinoma correlated with poor prognosis. In this study the proportion of p53 nuclear protein expression increases as the stage progresses. This association was proved to be statistically significant by using chi square test ($P < 0.05$). This is in accordance with the study done by T Starzynska et al,[9] George E Theodoropoulous et al.[6] Several previously published series have supported the deleterious effect of p53 over expression.[11,12]

Scott N et al[13] analysed 52 colorectal carcinomas in their study and found no correlation with p53 overexpression and several factors related to prognosis.

Campo et al (1991)[14] also, did not find relationship between p53 expression and degree of differentiation and stage of the tumour, concluded that p53 is not a good prognostic indicator in large bowel malignancy. The discrepancies in the prognostic role of p53 in colorectal adenocarcinomas might be a result of different kind of materials, methods and antibodies used or differences in the number of tumours examined.

p53 expression increases as the stage progresses, stage is the established prognostic factor in colorectal carcinoma, and also there is significant correlation between high p53 levels and stage of the colorectal carcinomas, so it can be used as a prognostic marker to assess the invasiveness and metastatic potential of the colorectal tu

TABLE – 6

S.NO	STUDIES	p53 EXPRESSION (%)
1.	Mohammad – Reza et al[15]	59
2.	T Starzynska et al[9]	46
3.	Scott N et al[13]	42
4.	O Petrisor et al[16]	66
5.	George E Theodoropoulous et al[6]	63.4
6.	Present study	53.3

In our study 53.3% of colorectal adenocarcinomas showed p53 nuclear protein overexpression. These differences may be due to the use of different scoring system and inter-observer variability. (TABLE- 6)

4.2 p53 Immunoexpression – Small Intestine:

p53 expression was studied in 5 small intestinal tumours of which 3 cases were adenocarcinoma and 2 cases were lymphomas. p53 expression was quantified according to DA Spandidios et al[7] and results were tabulated and analysed. In the present study p53 expression is seen in 60% of small intestinal tumours. Of 3 cases of adenocarcinoma 2 cases (67%) were p53 positive and 1 out of 2 cases (50%) of lymphoma were positive for p53. Intense positivity were seen in lymphomas (>50% of cells). This is in accordance with the study done by DA Spandidos et al.[7]

DA Spandidos et al[7] found immunoreactivity in 46% of small intestinal tumours. Ge C, He S, Tian Y[17] reported nuclear positivity for p53 in 75% of small intestinal tumours. Tadashi Terada [18] found 51.21% of p53 positivity in small intestinal tumours. M Svrcek et al[19] found p53 positivity in 51.85% of small intestinal adenocarcinoma. Krugmann et al[20] reported p53 overexpression in 75% of small intestinal lymphomas. These differences may be due to the use of different scoring system and inter-observer variability.

D A Spandidos et al[7] concluded that p53 gene may be involved in the pathogenesis of small intestinal tumours. Tadashi Terada,[18] in his study, found that p53 protein overexpression was present in most of the primary small intestinal carcinomas. Ge C et al[17] in their study showed that the degree of p53 protein expression was significantly correlating with the grade of the tumour cell differentiation, invasion, metastasis and prognosis. According to Krugmann et al[20] and Ming Qing et al[21] p53 overexpression in small intestinal lymphomas is a critical factor in the transformation of low grade MALT lymphoma and the overexpression also indicate the aggressive behavior of high grade tumours.

p53 immunoexpression was seen in both small intestinal adenocarcinoma and lymphoma. Intense positivity was observed in small intestinal lymphoma. This shows the involvement of p53 gene in the pathogenesis of small intestinal tumours. Due to low sample size, the prognostic value of p53 could not be determined.

V. Conclusion

In colorectal tumours p53 expression increases as the stage progresses and also there is significant correlation between high p53 levels and stage of colorectal carcinomas. So it can be used as a prognostic marker to assess the invasiveness and metastatic potential of the colorectal tumours.

There is a statistically significant correlation between p53 protein expression and stage of the colorectal cancer and hence p53 immunoreactivity is an important independent prognostic marker in patients with colorectal carcinoma. P53 protein overexpression has been associated with a worst overall survival after cancer diagnosis. Considering the acceptable reliability and feasibility of detection of p53 by immunohistochemistry, this marker may be expected to serve as a new genetic marker for predicting recurrence and response to chemotherapy in patients with colorectal cancer.

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