Journal of Pharmaceutical Research International

33(20B): 94-106, 2021; Article no.JPRI.66772
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Role of Immunohistochemistry Markers (p53 and CEA), in Study of Colorectal Tumours

Sapam Chingkhei Lakpa[1], R. Vinoth Kumar[1] and Mary Lilly[1*]

[1]Department of Pathology, Sree Balaji Medical College & Hospital, Chennai – 600044, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i20B31362
Editor(s):
(1) Prof. John Yahya I. Elshimali, UCLA School of Medicine & Charles R. Drew University of Medicine and Science, USA.
Reviewers:
(1) Shazia Saaqib, King Edward Medical College, Pakistan.
(2) Sergio Britto Garcia, University of São Paulo, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/66772

Original Research Article

Received 25 January 2021
Accepted 31 March 2021
Published 06 April 2021

ABSTRACT

Colorectal cancer is the third most common cancer in men and the second in women globally. There is a marked variation in the incidence of colorectal carcinoma worldwide, where western countries having high rate compared to others. p53 tumour suppressor gene is one of the most intensively studied tumour markers in the colorectal tumours. Two markers were used, p53 (oncoprotein p53) and CEA (carcinoembryonic antigen) in the study. The 102 cases of paraffin-embedded samples were processed for the immunohistochemistry examination. After the analysis of the selected patients regarding the antibodies distribution, statistical analysis was performed. The current study showed that there was a statistically significant correlation existing between p53 and CEA in each tumour type irrespective of its histological grades. The immunohistochemistry (IHC) was performed on 4-µm thick sections from 10% formalin- fixed paraffin-embedded tissue blocks.

Keywords: Colorectal cancer; oncoprotein p53; carcinoembryonic antigen; tumour markers.

**Corresponding author: E-mail: marylilly.s@bharathuniv.ac.in;*

1. INTRODUCTION

Colorectal cancer is the third most common cancer in men (746,000 cases, 10.0% of the total) and the second in women (614,000 cases, 9.2% of the total) worldwide [1-3]. Almost 55% of the cases occur in more developed regions. There is wide geographical variation in incidence across the world and the geographical patterns are very similar in men and women: incidence rates vary ten-fold in both sexes worldwide [4,5], the highest estimated rates being in Australia/New Zealand (ASR 44.8 and 32.2 per 100,000 in men and women respectively), and the lowest in Western Africa (4.5 and 3.8 per 100,000). Mortality is comparatively higher (52%) in low developed countries than the developed countries (694,000 deaths, 8.5% of the total); this reflects a poorer survival in those regions. There is less variability in mortality rates worldwide (six-fold in men, four-fold in women), with the highest estimated mortality rates in both sexes in Central and Eastern Europe (20.3 per 100,000 for men, 11.7 per 100,000 for women), and the lowest in Western Africa (3.5 and 3.0, respectively) [6].

Risk factors for development of colorectal carcinoma are old age, History of colorectal adenoma Inflammatory intestinal conditions, Inherited syndromes (familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, which is also known as lynch syndrome, gardner syndrome, old field syndrome, Turcott syndrome, Zanca syndrome, Peutz-Jeghers syndrome, juvenile polyposis syndrome}, family history of colorectal carcinoma, radiation therapy for cancer [7-9]. People taking high calorie diet along with sedentary life style habits are more prone to develop colorectal carcinoma [10,11]. Diabetes and obesity increases the risk of developing colorectal carcinoma. Statistical studies show that smoking, alcohol and animal fat consumption are the major predisposing factors [12-15]. Majority of Colorectal carcinomas remain asymptomatic for years. They most often present with fatigue and weakness as these bulky lesions bleed readily and cause anaemia [16].

In this prospective study of intestinal tumours, incidence with respect to age, sex, site, and histomorphological features of various tumours in large intestine were studied. The present study also evaluates the role of tumour suppressor gene protein p53 and cell surface glycoprotein CEA in intestinal neoplasms and its prognostic value in colorectal adenocarcinomas.

2. MATERIALS AND METHODS

The prospective study conducted in Department of Pathology, Sree Balaji Medical College and Hospital, Chennai, during the period of October 2015 to September 2017. Based on inclusion and exclusion criteria, the samples were screened for the study. A total of 104 samples were included in the studies which were received from General Surgery and Medical Gastroenterology as resected specimens and mucosal biopsies. Proper consents were taken from patients whose sample was included in the study. Patients' particulars, brief clinical history and clinical examination findings were recorded.

2.1 Inclusion Criteria

All cases proved to be adenoma or adenocarcinoma of colorectal region by histopathology irrespective of age and both of the sexes were included for the study.

2.2 Exclusion Criteria

All non-neoplastic lesions such as inflammatory polyps and cases with incomplete clinical data were excluded from the study.

2.3 Method of Data Collection

All the cases enrolled in the study as specified by the above criteria were assessed. Of the 104 cases, 100 cases were found to be suitable for the study. Clinical history and colonoscopy findings were tabulated. Adequate samples were taken from the growths in these specimens.

The tissues so obtained were processed and sections were cut at 4 microns. Hematoxylin and eosin staining of the sections were done and histopathological [17] findings were studied. Histopathological images were taken using Canon DSLR 700D with AmScope microscope adapter mounted on Olympus CH20i. Fiji (Image-J), an open source software, was used to analyse the images captured by the camera.

The adenocarcinomas found have been categorized into well differentiated, moderately differentiated and poorly differentiated based on the amount of glandular architecture. The number of cases in each category was

tabulated. The staging of the malignancy was done according to the TNM staging system and the results were tabulated.

The data were statistically analysed using Microsoft Excel 2016 and STATA ver. 14 on Mac OSX Sierra. The significance of the results was assessed by determining the probability factor 'p-Value' using descriptive tests such as the Pearson Chi squared test, student t-test, Pearson's pairwise correlation test and Kendall's tau test.

P≤0.05= Significant
P<0.01= Highly significant
P>0.05= Not significant

3. RESULTS

3.1 Relation of Regions Involved and Types of Colorectal Tumours

From the 100 cases studied, the most affected region of large intestine is rectum (50%) followed by sigmoid colon (23%) then caecum (12%). In contrast, the mucinous carcinoma showed caecum with higher number of frequency (71.43% of mucinous carcinoma). The Fig. 1 show distribution of different types of tumours in particular regions of large intestine, in the case studied. The statistical analysis using Pearson's Chi squared test showed p- Value of 0.000 which less than 0.01, indicating the statistics for site distribution of particular tumour type is highly significant.

3.2 p53 Scoring

For scoring p53 expression, all the p53 stain slides were given a score from Immunoreactive scoring (IRS) system devised, in which the intensity of the staining and the percentage of stained were multiplied. the score is interpreted

In the study, benign and pre-malignant cases show negative and weak expression, and malignant cases show stronger expression of p53. The proportion of expression of p53 in benign and malignant cases are shown in Fig. 3. The study also shows that there is strong relation between the expression of p53 and the lesion type (benign and malignant) with p-Value of 0.000, measured using Pearson's Chi squared test.

In overall tumour types, the p53 is found to be expressed to a greater extent in case of adenocarcinoma as shown (P= 0.000). Mucinous carcinoma also shows strong expression of p53.

The staging of the resected tumor cases is done using 'AJCC Colon and Rectum Staging System' (7^{th} edition). Staging is done only in resected specimens only, to eliminate the possibility of error due to lack of full clinical details particularly metastasis, the stage III and stage IV have been put in one single category.

The Fig. 5 show the expression of CEA in different grades of adenocarcinoma, and Fig. 6 show the expression of CEA in adenoma cases with different degree of dysplasia. It is evident that CEA is 100% expressed in grade-I (well differentiated), and reduced in grade-II (moderately differentiated) when compared to grade-I. CEA expression is more in higher grades of dysplasia compared to low grade. There is poor evidence of relation between CEA expression and histological grades of adenocarcinoma, and with the grades of dysplasia in adenoma. The p-Value are found to be statistically not significant in histological grading of adenocarcinoma (P = 0.440) and adenoma (P = 0.619).

In overall tumor types, the CEA is found to be expressed to a greater extent in case of adenocarcinoma as shown (P= 0.000). Mucinous carcinoma also shows strong expression of CEA.

3.3 Comparing p53 and CEA Expression

From the 100 cases studied, a two-way comparison table of p53 and CEA expressions shows correlation which is statistically significant with p-Value of 0.002. The Fig. 8 shows means of p53 expression and CEA expression in stacked (100 percent) bar graph. The figure shows proportion of p53 and CEA expressions in each tumor type irrespective of its histological grades.

4. DISCUSSION

Colorectal cancer is the third most common cancer in men and the second in women globally. There is a marked variation in the incidence of colorectal carcinoma worldwide,

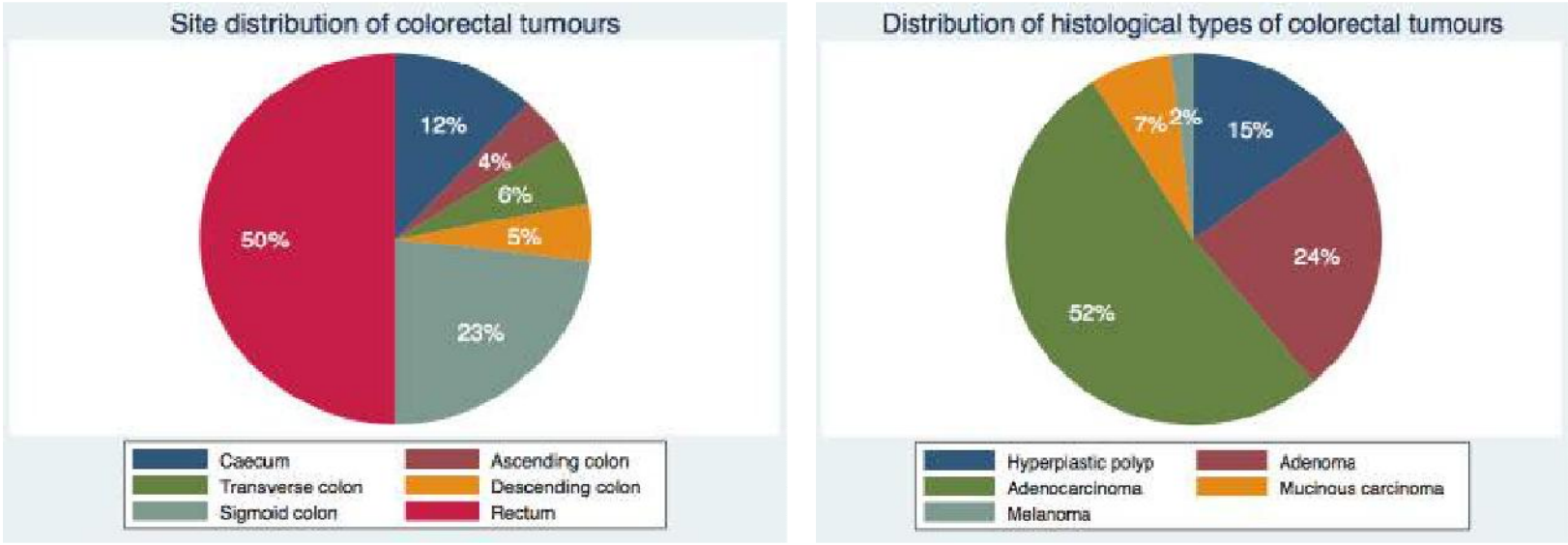

Fig. 1. Pie charts showing (left) distribution of cases in different regions of large intestine and (right) proportion of tumour types in the case studied

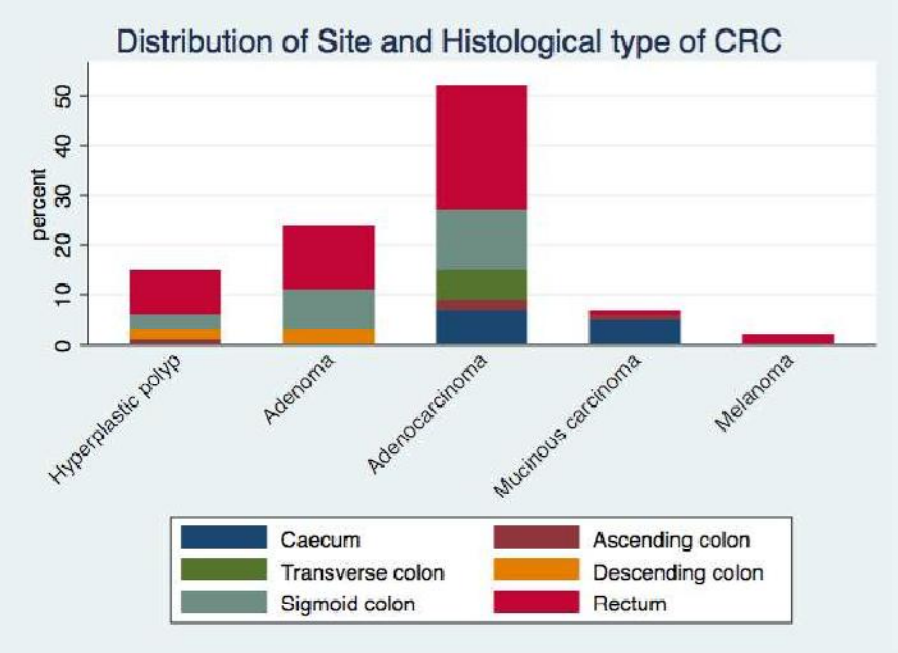

Fig. 2. Distribution of site and histological type of CRC

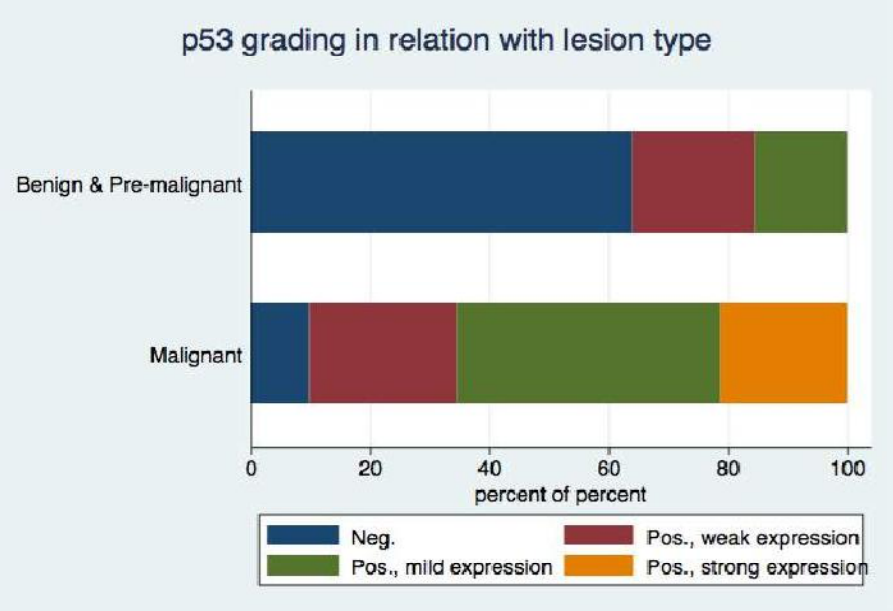

Fig. 3. Stacked horizontal bar graph showing proportion of p53 expression in benign & pre-malignant and malignant cases

where western countries having high rate compared to others. Almost 55% of the cases occur in more developed regions. In India, colon cancer ranks 8th and rectal cancer ranks 9th among men. For women, colon cancer ranks 9th. The incidence of both small and large bowel cancers are low in India [18]. The incidence of colorectal cancer in India is 3.6% when compared worldwide (10%) [6].

Carcinomas of the large bowel present in a range of macroscopic appearances. These vary

somewhat with the anatomical site of origin. Conventionally a number of distinct macroscopic forms of large bowel cancer have been recognized as polyploidy, exophytic or fungating, ulcerating, stenosing, and diffusely infiltrating. 5-year relative survival ranges from greater than 90% in patients with stage I disease to slightly greater than 10% in patients with stage IV disease. Screening has been shown to reduce colorectal cancer incidence and mortality, but organized screening programs are still to be implemented in most countries [19-23].

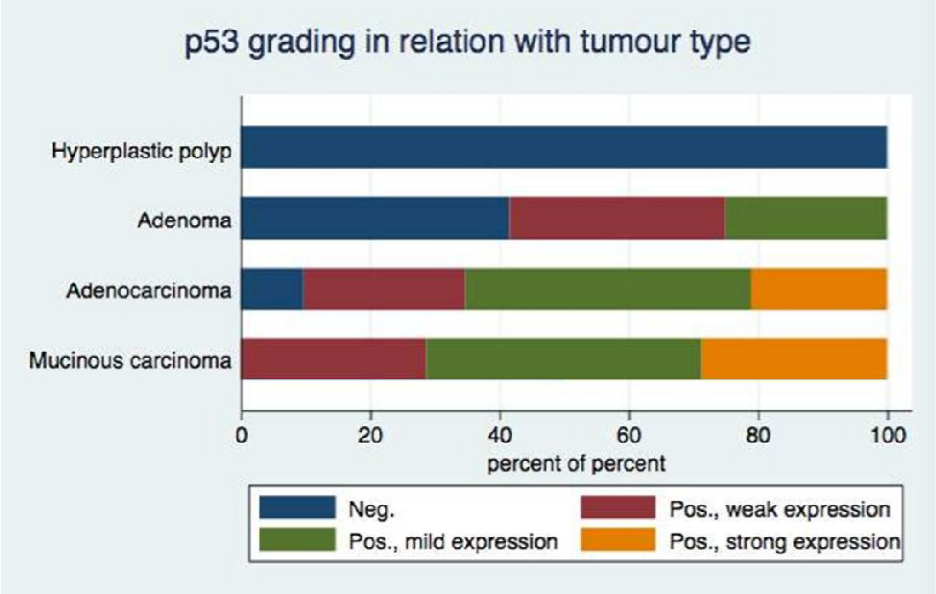

Fig. 4. Stacked horizontal bar graph showing proportion of p53 expression in different tumour types

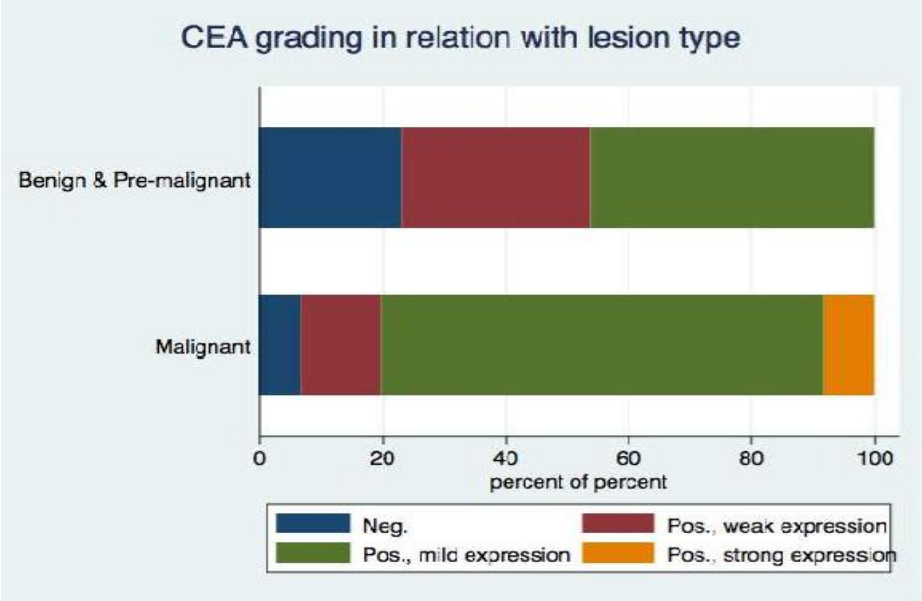

Fig. 5. Stacked horizontal bar graph showing proportion of CEA expression in benign & pre-malignant, and malignant cases

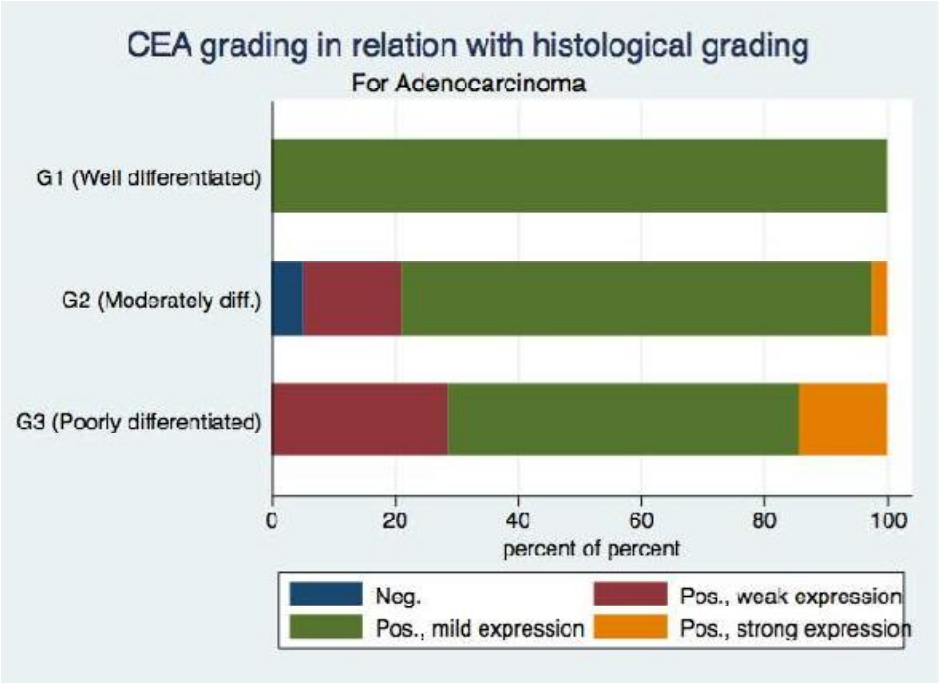

Fig. 6. Stacked horizontal bar graph showing proportion of CEA expression in different histological grades of adenocarcinoma cases

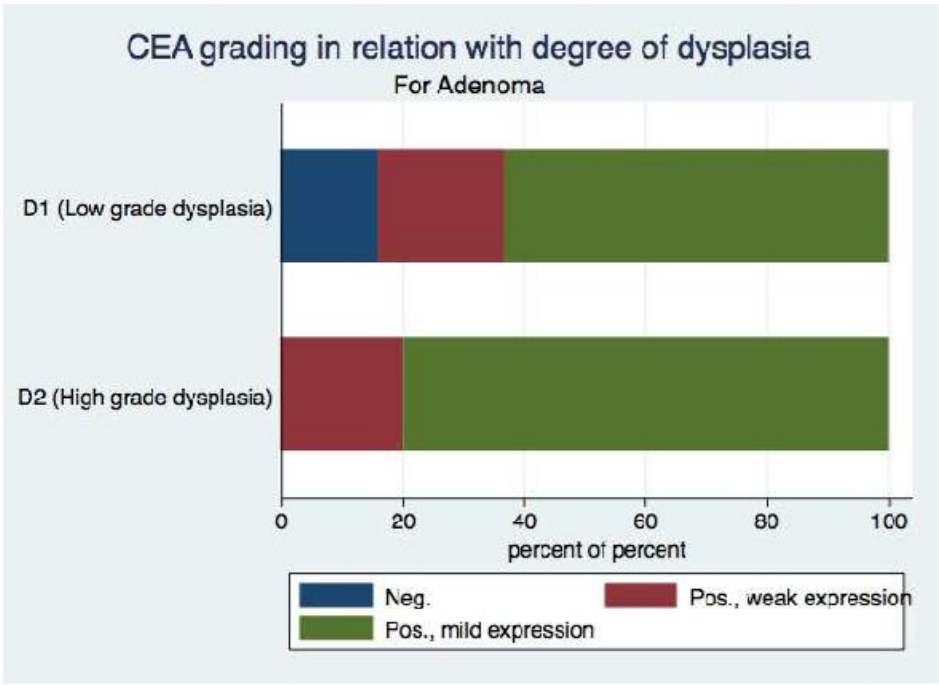

Fig. 7a. Stacked horizontal bar graph showing proportion of CEA expression in different degree of dysplasia of adenoma cases

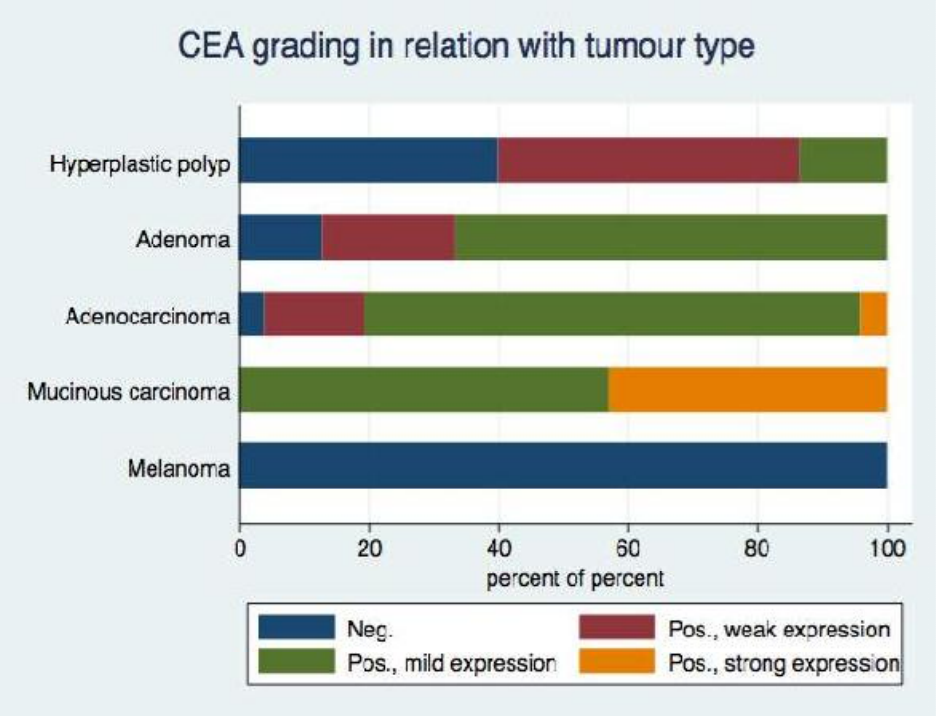

Fig. 7b. Stacked horizontal bar graph showing proportion of CEA expression in different tumor types

There are a large number of factors that play a direct role in driving the polyp to CRC sequence, including, but not limited to, gene mutations, epigenetic alterations, and local inflammatory changes [22]. Patients with any type of polyposis syndrome are at increased risk for the development of large bowel carcinoma. In FAP and Gardner syndrome the incidence is almost always 100%. FAP is an autosomal dominant mode disorder that affects one in 13,000 births [23]. The most compelling feature is the onset and progression of hundreds to thousands of small adenomatous polyps throughout the colon. Such polyps typically trace their emergence to the second decade of life, but have been noted to occur until age 40.

In this study, age group ranges from 22-79 years with mean age of 59.41 years. The mean ages of male and female are 58.72±11.76 and 60.53±11.62 years respectively. Male show more frequency of occurrence than female in colorectal carcinomas.

In our study of 100 colorectal tumors 61 cases are malignant, remaining 32 cases are benign. Of the malignant cases, adenocarcinoma represents 85.2% (n=52), mucinous carcinoma 11.5% (n=7) and melanoma 3.3% (n=2). Similar findings are observed in a study conducted by Fatimah Biade Abdulkareem et al. [24] where 87.1% were adenocarcinoma (comprising of well differentiated adenocarcinoma 55.5%, moderately differentiated carcinoma 21% and poorly differentiated adenocarcinoma 10.7%) and 8.1% of mucinous carcinoma.

p53 tumor suppressor gene is one of the most intensively studied tumor markers in the colorectal tumors [25,26]. In this study 100 cases of colorectal tumors are selected for the study of p53 expression by immunohistochemistry in various grades and types which included 7 cases of mucinous carcinomas. When compared to the findings of other studies, there are some variation. These differences may be due to the use of different scoring system and inter-observer variability. In this study, the p53 shows strong expression in 11 (21%) cases from 52 cases of adenocarcinoma. The criteria devised by Agnieszka Halon et al. [27] for categorising the cases as overexpression of a marker, is based on a modified IRS system [22]. Using this criterion, it is found that in the present study 65.4% of cases of adenocarcinoma show p53 overexpression.

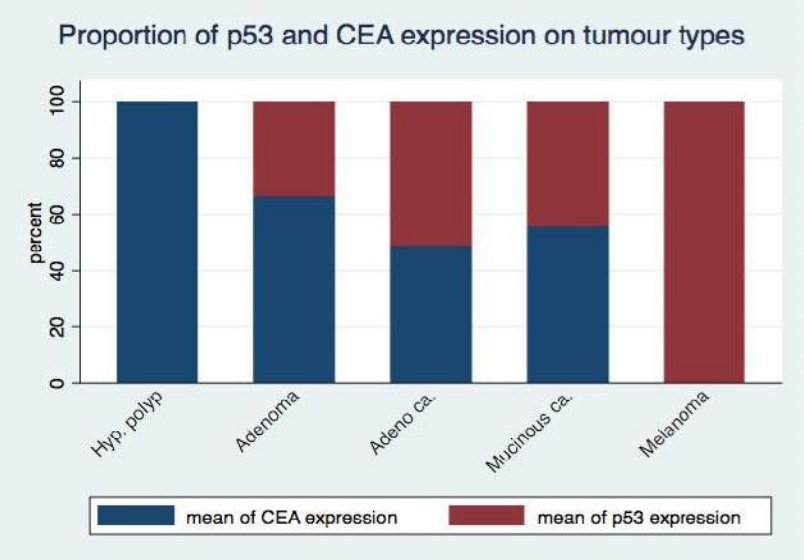

Fig. 8. Stacked bar graph showing proportion of CEA expression and p53 expression in different tumor types

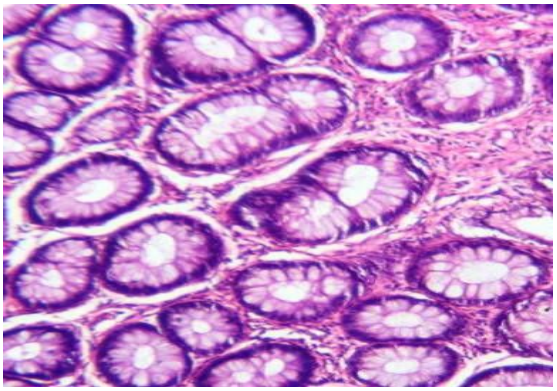

Fig. 9. H&E section showing hyperplastic polyp in low power magnification

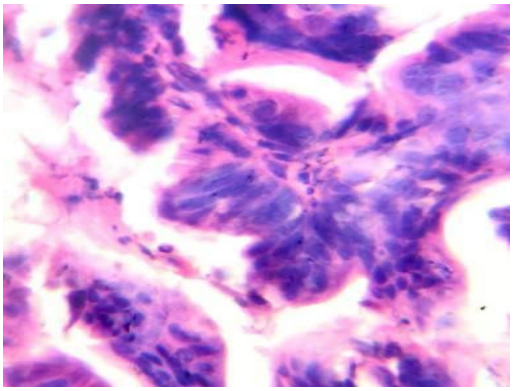

Fig. 10. H&E section showing serrated polyp in high power magnification

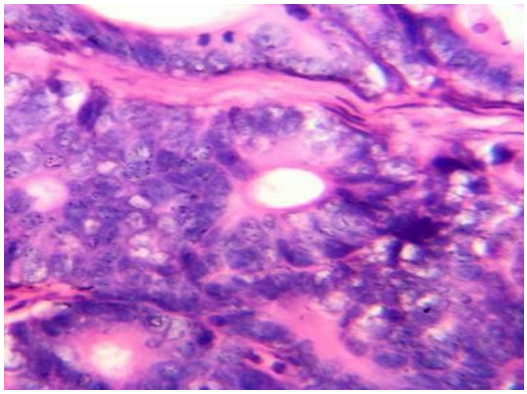

Fig. 11. H&E section showing adenocarcinoma in high power magnification

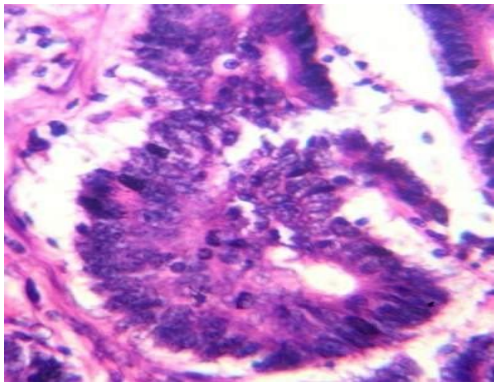

Fig. 12. H&E section showing adenocarcinoma in high power magnification

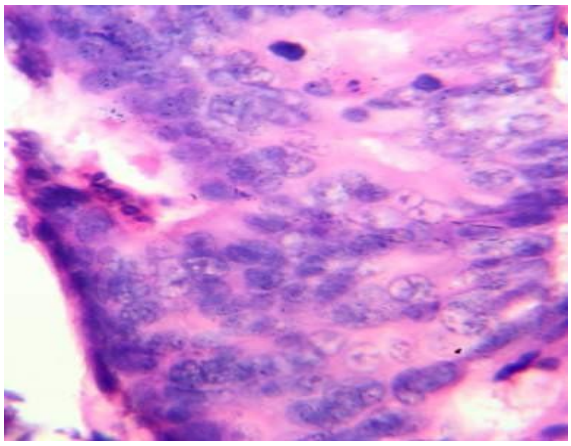

Fig. 13. H&E section showing adenocarcinoma in high power magnification

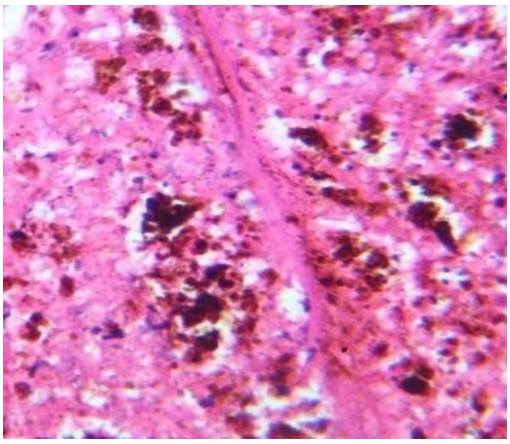

Fig. 14. H&E section showing malignant melanoma in high power magnification

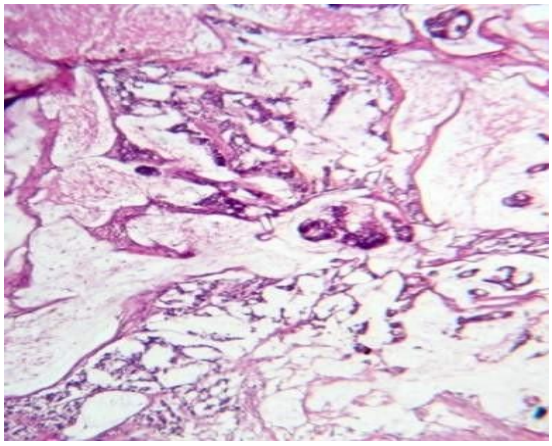

Fig. 15. H&E section showing mucinous carcinoma in low power magnification

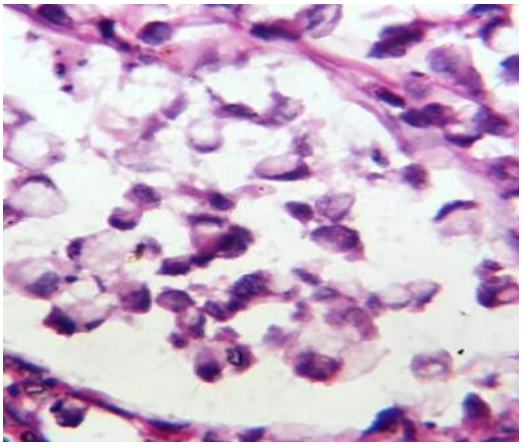

Fig. 16. H&E section showing mucinous carcinoma in high power magnification

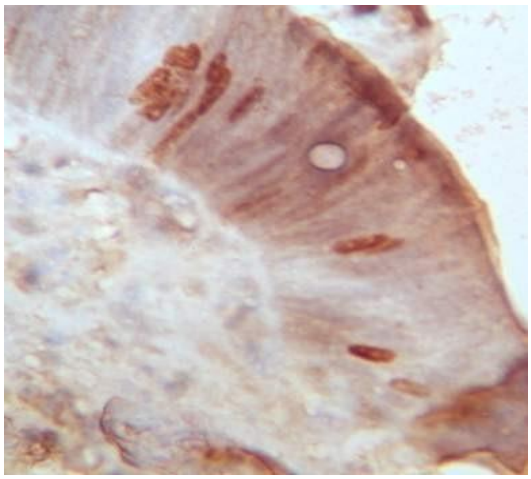

Fig. 17. Section showing p53 score of 1 (1x1) which is considered negative

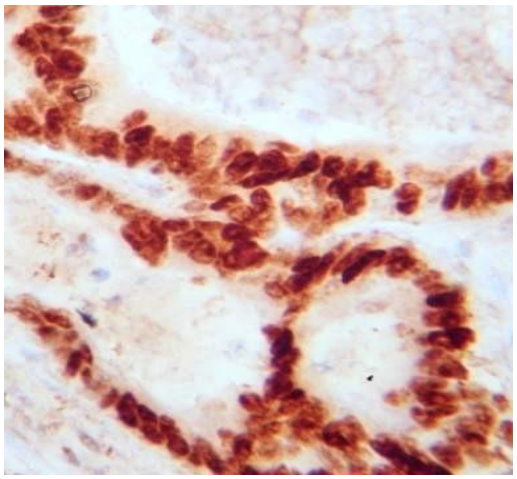

Fig. 18. Section from adenoma showing p53 score of 4 (2x2)

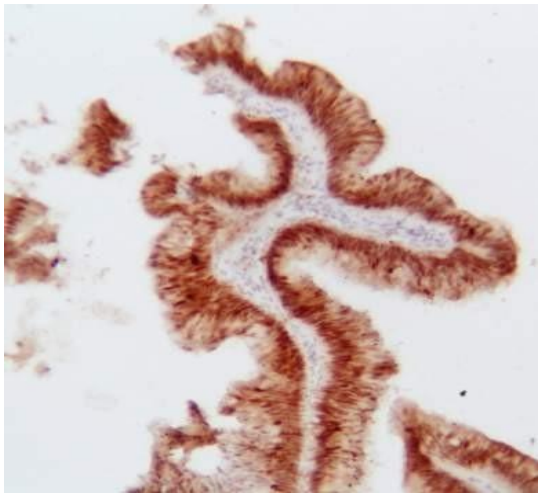

Fig. 19. Section from adenoma showing p53 score of 2 (2x1)

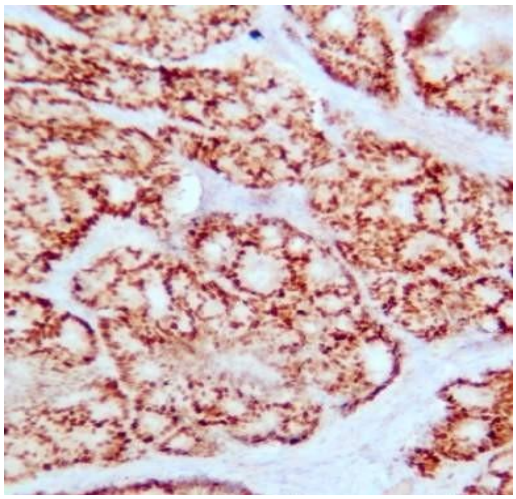

Fig. 20. Section from adenocarcinoma showing p53 score of 6 (3x2)

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. [28] 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 the present study, p53 protein overexpression is seen in relatively high percentage of patients, which indicates that p53 mutation plays an important role in development of CRC.

CEA is a cell surface glycoprotein normally expressed in foetal tissue and transcriptionally silent in adults. CEA functions as an intercellular adhesion molecule and plays an important role during development. However, CEA overexpression is associated with a variety of cancers of epithelial origin. Monoclonal or polyclonal antibodies may identify several antigens belonging to glycoproteins of CEA family. Inflamed or necrotic tissues react with CEA antibodies, making up a specific pattern. The new variants of monoclonal CEA antibodies react only with epitopes of “true” CEA 80–100% of colorectal cancers make a strong and diffuse positive staining [29,6]. In this study 100 cases of colorectal tumors are selected for the study of CEA expression by immunohisto-chemistry in various grades and types.

When compared to the findings of other studies, there are some variation. These differences may be due to the use of different scoring system and inter-observer variability. In the present study, 96.2% of cases of adenocarcinoma show CEA positivity. Since its sensitivity is high, it may be useful in differentiating tumor tissue from normal tissue. it was found that 98.8% of tumor tissues stained positive for CEA and differential CEA expression within normal/tumor pairs was appreciable, providing evidence that CEA is a reliable marker for differentiating between normal and tumor tissue [29,30]. It was found that the tissue expression for CEA in CRC was 94%. Monika Cerna et al (2006) did a quantitative estimation of CEA in the tumour tissue, it was observed that almost all tissue sample stained positive for CEA. They also found that there were differences in staining of CEA between tumor types and there was no relationship to staging or clinical development [6,16,30].

The present study shows that CEA have poor association with great and stage of the colorectal carcinoma (P=0.1000). And the study also shows that CEA is negative for melanoma which is in accordance with the study done by Selby WL et al (1992) where they found malignant melanoma showed no reactivity to monoclonal CEA. Though there was a clear evidence of association between the EA and colorectal cancer, the present study suffered by the limited number of cases. Thus this study also warranted for further level analysis to optimizing the role of CEA in cancers.

5. CONCLUSION

This is a prospective study undertaken in the Department of Pathology over a period of 2 years, from October 2015 to September 2017. Resected specimens and mucosal biopsies of large intestinal tumors were included for the study. The number of samples included for the study is 100, of which biopsy specimens were 54 (54%) and resected specimens were 46 (46%). In this study age, sex and site of the lesion were recorded. After H&E staining of the sections obtained from the specimen, histomorphological grading of colorectal tumors was done. Immunohistochemistry using p53 and CEA antibodies were done to assess the role of their expressions in various types of colorectal tumors. Results were tabulated and analyzed.

In colorectal tumors p53 expression increases with the histomorphological grades. P53 protein overexpression has been associated with a worst overall survival after cancer diagnosis. In the present study, 65.4% of cases of adenocarcinoma show p53 overexpression. The p53 protein overexpression is seen in relatively high percentage of patients, which indicates that p53 mutation plays an important role in development of CRC. In colorectal tumors p53 expression increases as the stage progresses and also there is significant correlation between high p53 levels and stage of colorectal carcinomas. It is evident that p53 can be used as a prognostic marker to assess aggressiveness of the colorectal tumors.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

ACKNOWLEDGEMENTS

The encouragement and support from Bharath University, Chennai is gratefully acknowledged. For provided the laboratory facilities to carry out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Young B, Woodford P, O'Dowd G. Wheater's Functional Histology E-Book: A Text and Colour Atlas. Elsevier Health Sciences; 2013.
2. Bosman FT, Carneiro F, Hruban RH, Theise ND. WHO classification of tumours of the digestive system. 4th Edition. International Agency for Research on Cancer, Lyon; 2010.
3. Nandakumar A. Three-year report of population based cancer registries 2012-2014. NCDIR-NCRP, Bengaluru; 2016.
4. Hamilton SR, Aaltonen LA. Pathology and Genetics of Tumours of the Digestive System. IARC Press, Lyon; 2000.
5. Wei EK, Giovannucci E, Wu K, Rosner B, Fuchs CS, Willett WC, Colditz GA. Comparison of risk factors for colon and rectal cancer. Int J Cancer. 2004;108:433–442.
6. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. Globocan Cancer Fact Sheets: Colorectal Cancers. In: Globocan; 2012.
7. Chalya PL, Mchembe MD, Mabula JB, Rambau PF, Jaka H, Koy M, Mkongo E, Masalu N. Clinicopathological patterns and challenges of management of colorectal cancer in a resource-limited setting: A Tanzanian experience. World J Surg Oncol. 2013;11:88
8. Hadi Ni, Shakoor KA, Waseem B. Is mucin content a prognostic indicator in colorectal carcinoma? J Surg Pakistan. 2009;14:1.
9. Fazeli MS, Adel MG, Lebaschi AH. Colorectal carcinoma: A retrospective, descriptive study of age, gender, subsite, stage, and differentiation in Iran from 1995 to 2001 as observed in Tehran University. Dis Colon Rectum. 2007;50: 990–995.
10. Gao R-N, Neutel CI, Wai E. Gender differences in colorectal cancer incidence, mortality, hospitalizations and surgical procedures in Canada. J Public Health (Oxf). 2008;30:194– 201.
11. Ahnen DJ, Feigl P, Quan G, Fenoglio-Preiser C, Lovato LC, Bunn PA, et al. Ki-ras mutation and p53 overexpression predict the clinical behavior of colorectal cancer: A southwest oncology group study. Cancer Res. 1998;58:1149–1158.
12. Bisgaard ML, Fenger K, Bülow S, Niebuhr E, Mohr J. Familial adenomatous polyposis (FAP): Frequency, penetrance and

mutation rate. Hum Mutat. 1994;3:121–125.
13. Kaserer K, Schmaus J, Bethge U, Migschitz B, Fasching S, Walch A, et al. Staining patterns of p53 immunohisto-chemistry and their biological significance in colorectal cancer. J Pathol 2000;190: 450–456.
14. Siegel RL, Ward EM, Jemal A. Trends in colorectal cancer incidence rates in the United States by tumor location and stage, 1992-2008. Cancer Epidemiol Biomarkers Prev. 2012;21:411–416.
15. Chengwu X, Chen VW, Steele B, Ruiz B, Fulton J, Liu L, Carozza SE, Greenlee R. Subsite-specific incidence rate and stage of disease in colorectal cancer by race, gender, and age group in the United States, 1992-1997. Cancer. 2001;92: 2547–2554.
16. Robbins SL, Cotran RS, Kumar V, Abbas AK, Fausto N. Robbins and Cotran pathologic basis of disease, 8th Editio. Elsevier Saunders, Philadelphia, Pa; 2010.
17. Liang, John, et al. Utility of p16INK4a, CEA, Ki67, P53 and ER/PR in the differential diagnosis of benign, premalignant, and malignant glandular lesions of the uterine cervix and their relationship with Silverberg scoring system for endocervical glandular lesions. International Journal of Gynecological Pathology. 2007;26(1):71-75.
18. Dekker E, Van Gulik T. Colorectal cancer: what the clinician wants to know. Cancer Imaging; 2005.
DOI: 10.1102/1470- 7330.2005.0036
19. Mohandas KM, Desai DC. Epidemiology of digestive tract cancers in India. V. Large and small bowel. Indian J Gastroenterol 1999;18:118–121.
20. Brenner H, Kloor M, Pox CP. Colorectal cancer. In: Lancet. 2014;1490–1502.
21. Murphy G, Devesa SS, Cross AJ, Inskip PD, McGlynn KA, Cook MB. Sex disparities in colorectal cancer incidence by anatomic subsite, race and age. Int J Cancer. 2011;128:1668–1675.
22. Weisburger JH. Causes relevant mechanisms, and prevention of large bowel cancer. In: Semin. Oncol. Elsevier, 1991;316–336.
23. Grady WM, Markowitz SD. The molecular pathogenesis of colorectal cancer and its potential application to colorectal cancer screening. Dig Dis Sci. 2015;60: 762–772.
24. Abdulkareem FB, Abudu EK, Awolola NA, et al. Colorectal carcinoma in Lagos and Sagamu, Southwest Nigeria: A histopathological review. World J Gastroenterol. 2008;14:6531–6535.
25. Huang CC, Cheng YW, Chen MC, Lin YS, Chou MC, Lee H. Different p53 mutation patterns in colorectal tumors from smokers and nonsmokers. Environ Mol Mutagen. 2006;47:527–532.
26. Zhang H. Evaluation of four antibodies in detecting p53 protein for predicting clinicopathological and prognostic significance in colorectal adenocarcinoma. Clin Cancer Res. 1999;5:4126–4132.
27. Halon A, Donizy P, Biecek P, Rudno-Rudzinska J, Kielan W, Matkowski R. HER-2 Expression in Immunohisto-chemistry Has No Prognostic Significance in Gastric Cancer Patients. Sci World J. 2012;1–6.
28. Walker J, Quirke P. Prognosis and response to therapy in colorectal cancer. Eur J Cancer. 2002;38:880–886.
29. RD O, Noffsinger A. Neoplastic diseases of the small and large intestines. Silverberg's Princ. Pract. Surg. Pathol. Cytopathol; 2006.
30. Langman J, Sadler TW, Sadler-Redmond SL, Tosney K, Byrne J, Imseis H, Langman J. Langman's medical embryology, 13th Editi. Wolters Kluwer Health, Philadelphia; 2015.

© 2021 Lakpa et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/66772