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Comparison of Stage IV Colonic Carcinoma in Right Side to Left Side by Prognostic Markers like her-2-neu, p^{53} and KRAS Mutation

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Abstracts
Backgrounds: *Colorectal carcinomas vary considerably throughout the world, being one of the leading cancer sites in the developed countries. Colorectal cancer (CRC) is the third most commonly diagnosed cancer globally, accounting for 10.0% of all new cancer cases. Here, difference in expression of p53, Her-2-neu, K-RAS and prognosis along with survival of stage IV both right and left sided colon cancers were evaluated.*
Methods and Materials: *One year retrospective study of stage IV carcinoma colon cases was studied in our institute; separated as right and left side colonic carcinoma. Immunohistochemistry of p53, Her-2-neu and KRAS mutation study by RT-PCR was done on their respective blocks and the results were compared; prognosis studied by follow up of the patients for 1 year.*
Results: *A total of 512 histopathologically confirmed cases of colon carcinoma were screened and paraffin block of 15 cases of stage IV colon carcinoma elaborately studied by routine Haematoxylin & Eosin stain. Followed by IHC (P^{53} and Her 2/Neu), RT $_{PCR}$ study (K ras mutation) were carried out. Among them 6 were right and rest 9 were left side colon carcinoma.*
Right sided cases constituted 3 adenocarcinoma NOS (p53, Her 2 neu positive) 2 mucin secreting adenocarcinoma (p53 positive her 2 neu negative) and 1 signet ring cell adenocarcinoma (p53 positive Her 2 neu negative). Left sided cases constituted 5 adenocarcinoma NOS(p53 positive Her 2 neu negative), 2 mucin secreting(p53 positive Her 2 neu negative) and 2 signet ring cell (p53 , Her 2 /neu negative). K ras mutation was positive in 67% right sided and 40%left sided colonic cancers. Following up, 83% of right sided cancer patients were dead and 17% living whereas in left sided cancers, 67% were living responding well to chemotherapy.
Conclusion: *Anti-epidermal growth factor receptor monoclonal antibody treatment (targeted therapy), with cetuximab and panituzumab is more effective in left side colonic carcinoma than right side.*
Keywords: *EGFR, Right sided, left sided, KRAS, P^{53}, Her 2 /neu.*

Introduction

Colorectal carcinomas vary considerably throughout the world, being one of the leading cancer sites in the developed countries. Colorectal cancer (CRC) is the third most commonly diagnosed cancer globally, accounting for 10.0% of all new cancer cases. An estimated 746,300 of new CRC cases and 614,300 colorectal carcinoma deaths occurred in 2012 worldwide. It is also the fourth common cause of cancer-related deaths in men and the third in women worldwide[1]. According to the tumor position, CRCs are usually classified into three types: Right-Sided Colon Cancer (RSCC), Left-Sided Colon Cancer (LSCC) and Rectal Cancer, and each type approximately accounts for 30%[2,3]. Colon cancers consist of RSCC and LSCC, divided at the splenic flexure. Rectal cancers are referred to lesions located within 12 cm from the anal verge. The issue whether these three types should be considered as a single entity or three distinct entities is still controversial[2] The Cancer Genome Atlas Network conducted a genome-scale analysis of 276 samples, analyzing exome sequence, DNA copy number, promoter methylation, mRNA and micro RNA expression, and concluded that colon and rectal cancers had similar patterns of genomic alteration, and gene mutations of APC, TP53, SMAD4, PIK3CA and KRAS10. So, whether colon and rectal cancers have different gene expression and prognosis is still in debate.[10]

The distinction between RSCC and LSCC has received increasing attention in recent years. Some suggested that they were two distinct categories of colon cancer[11]. Many publications reported that there were significant differences regarding epidemiology, clinical presentation, pathology, genetic mutations and survival between RSCC and LSCC11. RSCC had been reported to be older, more often female and more often poorly differentiated tumors, and have more advanced stages, increased tumor sizes and different molecular features[11–14]. Data regard- ing prognosis in RSCC versus LSCC are conflicting, and it remains a matter of great debate whether tumor loca- tion itself has a significant prognostic impact[12]. Most studies demonstrated a poorer survival in RSCC compared to LSCC[15–17]. In contrast, several scholars found no difference in overall survival between RSCC and LSCC after adjusting for various variables[11,18,19]. Warschkow et al.[12] carried out a study including 91,416 patients, and found that RSCC patients had worse overall survival compared to LSCC patients; but the prognosis of RSCC was better than LSCC after matching clinical features. In addition, whether molecular features differ between LSCC and RSCC remains unclear[20]. Kuramochi et al. [21] had detected mRNA expression levels of 14 signal transduction genes in 52 cases of CRC, but only identified significant differences in PTEN mRNA expression level.

There are three main types of (epi)genetic instability in CRC: (a) chromosomal instability (CIN) caused by KRAS mutations; (b) microsatellite instability (MSI) resulted from deficient DNA mismatch repair (MMR); (c) CpG island methylator phenotype (CIMP) epigenetic instability[3]. The mutational profiles (KRAS, MMR, CIMP) of LSCC and rectal cancer were similar, but were different from that of RSCC. This result was attributed to their differing origins: RSCC originated from midgut, while LCRC originated from hindgut[3]. BRAF was preferentially mutated in RSCC, and EGFR (epidermal growth factor receptor) was prevalently amplified in LCRC3. Class III beta-tubulin (β-Tubulin III) had been reported to express at the invasive margin of CRC, and its expression level was correlated with tumor differentiation and lymphatic metastasis[25]. The mutation incidence of p53 gene was reported to be as high as 42.4% in CRC[26]. P53 plays an important role in the transformation from colorectal normal mucosa to carcinoma through adenoma[27]. Several studies reported that gene mutation and protein expression of P53 differed significantly between RSCC and LSCRC [26, 28, 29] but others showed that no significant association

was identified between p53 protein expression and tumor site [30].

Molecular genetic mechanisms are diverse, and recent data suggest two main pathways: a mutational pathway, which involves inactivation of tumour suppressor genes such as APC; and microsatellite instability which occurs in hereditary nonpolyposis colon cancer (HNPCC). Colorectal carcinomas that occur proximal (right) or distal (left) to the splenic flexure exhibit differences in their embryologic development, blood supply, macroscopic pathology, and clinicopathological parameters. The right colon arises from the embryonic midgut and is perfused by the superior mesenteric artery, whereas the left colon originates from the hindgut and is served by the inferior mesenteric artery. Right-sided colon cancers (RSCCs) are typically bulky, exophytic, polypoid lesions that project into the lumen and cause significant anemia, whereas left-sided colorectal cancers are typically infiltrating, constricting lesions that encircle the lumen, often leading to obstruction. Poorly differentiated adenocarcinoma or signet-ring cell carcinoma and mucinous adenocarcinoma are more frequently seen in the right colon than in the left colon. RSCCs typically present at a more advanced stage, and the patients with RSCCs have a significantly worse survival than patients with LSCRCs and the tumour markers vary significantly between left and right side right and left side colon carcinoma.

Recent studies have revealed distinguishable genomic patterns between LSCRC and RSCC [31, 32]. LSCRCs exhibit higher p53 gene mutation and cyclooxygenase-2 expression rates with more common chromosomal in- stability[33–36]. RSCCs are generally diploid, and they exhibit higher rates of microsatellite instability (MSI) and higher expression of cytoplasmic c-erbB2 and epi-dermal growth factor receptors (EGFRs)[37]. Recent whole genome analysis has shown that RSCCs are more likely to be hypermethylated as well as to have elevated mutation rates compared with LSCRCs[31]. Some studies have reported significantly more activating mutations in codon 12/13 of Kirsten rat sarcoma viral oncogene homolog (KRAS) in RSCCs than in LSCRCs [38,39]. However, other studies have reported that there is no substantial difference in the KRAS mutations between RSCCs and LSCRCs [32,40]. LSCRCs exhibit a significant association between KRAS activation and distant organ metastasis, whereas RSCCs do not. Mutation of KRAS was found to be associated with a significantly poorer prognosis in patients with LSCRCs, but not in those with RSCCs[41]. Cetuximab is a chimeric IgG1 monoclonal antibody that binds to the extracellular domain of EGFR; also, it blocks ligand-induced receptor signaling and induces immune-mediated antitumor mechanisms, such as antibody-dependent cell-mediated cytotoxicity [42, 43].

In the light of the aforementioned considerations, it is urgent for us to explore the possible differences of gene expression level and prognosis among right and left sided colonic carcinoma. Knowledge of the molecular differences would help us to improve the diagnosis and treatment strategy in clinical practice. Therefore we undertook this study to assess the difference in expression of p53, her-2-neu and K-RAS mutation and prognosis of right and left sided colonic carcinoma.

Materials and Methods

This retrospective study was undertaken from September 2016 to September 2017 in the department of pathology of a tertiary care teaching hospital. Both endoscopic and total colectomy cases of stage 4 colonic carcinomas (most commonly with omental metastasis) was collected. Clinicopathological data was collected from the medical records of the patients. The cases were divided into right sided and left sided colonic cancers based on the site of the tumour. Tumours of ascending colon, and caecum were categorized as right sided colonic cancers and tumours of descending colon, tranverse colon and sigmoid colon were categorized as left sided

colonic carcinoma. Further the tumour were also grouped into 3 morphological subtypes: a.Adenocarcinoma NOS b.Signet ring cell adenocarcinoma c.Mucin secreting adenocarcinoma.

Immunohistochemistry

Immunohistochemistry was performed on relevant section of the tissue using anti her2neu antibody and anti p53 antibody. Both positive and negative controls were put with every batch of IHC for proper evaluation.

Principle

Immunohistochemistry is a method based on antigen-antibody reactions for identifying substances in tissue, which can be made visible microscopically using a suitable label.

The primary antibody binds to specific tissue antigens. The biotinylated secondary antibody is directed to the primary antibody. The streptavidin/ horse radish peroxidase complex is then applied. Streptavidin then binds to the biotin on the secondary antibody and HRP acts as an indicator enzyme. On addition of DAB substrate, free oxygen radicals are released which oxidize DAB to a brown colored precipitate. The precipitate gets deposited on the antigen site and can be detected by microscopy.

K-RAS Mutational Study

The K-RAS mutational study is not practiced in our institution; so the respective blocks of the patients were sent outside for the study. And the mutational analysis was done on exons 2,3 and 4 by the real time polymerase chain reaction (RT-PCR).

The result of the mutational study was compared between the right and the left sided colonic carcinomas.

Follow Up

The patients were treated with chemotherapeutic agents cetuximab and panitumumab and were followed up for one year. The percentage of the response of the patients to the chemoptherapy were noted and prognosis of the right and the left sided colonic carcinoma were compared.

Results

The total number of cases collected of stage 4 colonic carcinoma was 15.Among them 6 were right sided and 9 were left sided. Among the right sided cases 3 were adenocarcinoma NOS, 2 were mucin secreting adenocarcinoma and 1 was signet ring cell adenocarcinoma. Among the left sided colonic cancers, 5 were adenocarcinoma NOS,2 were mucin secreting adenocarcinoma and 2 were signet ring cell adenocarcinoma (fig 1)

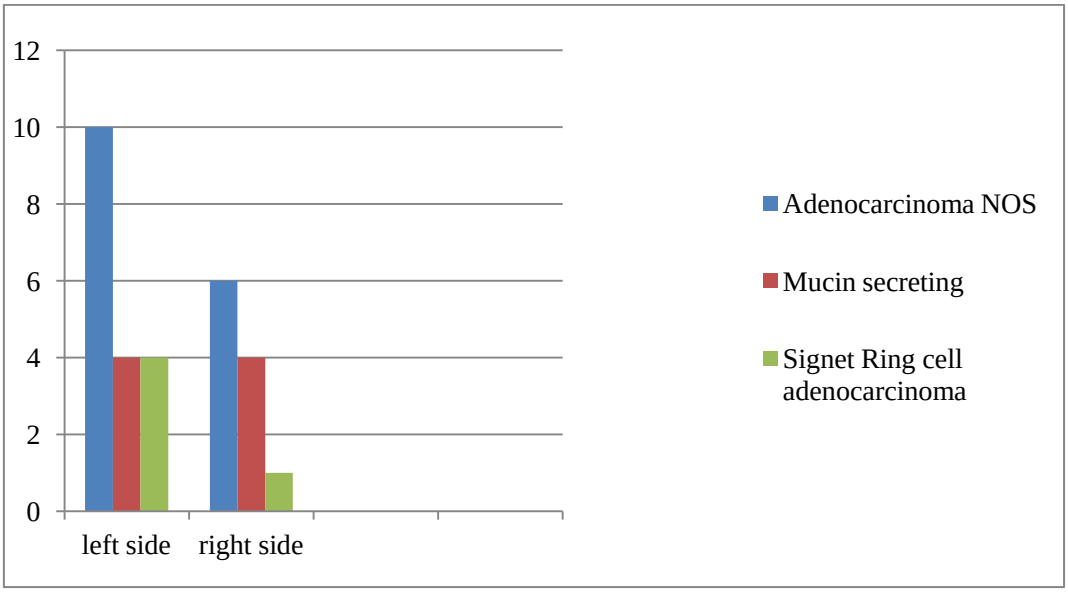


Fig 1- Histogram showing distribution of different histomorphological types of both side colon carcinoma.

On immunohistochemical study, right sided cases *adenocarcinoma NOS* (fig 2a) were p53 (fig 2b) & her 2 neu positive, *mucin secreting adenocarcinoma* were p53 positive & her 2 neu negative and *signet ring cell adenocarcinoma* was p53 positive her 2 neu negative. Left sided *adenocarcinoma NOS* were p53 positive & her 2 neunegative, *mucin secreting adenocarcinoma* were p53 positive & her 2 neu negative and *signet ring cell adenocarcinoma (fig 3a)*were p53 & her 2 neu negative (fig3b).

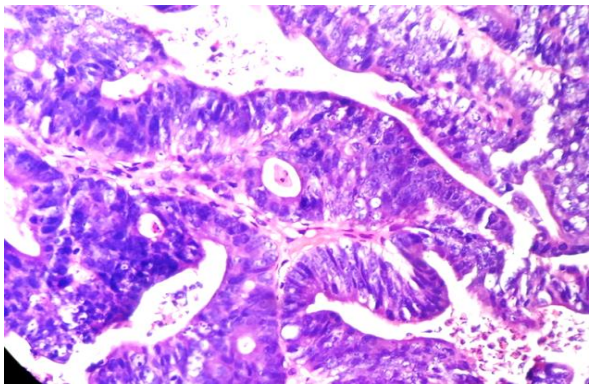


Fig 2a Photomicrograph showing *Adenocarcinoma NOS type*,right side colon.(H & E,400x).

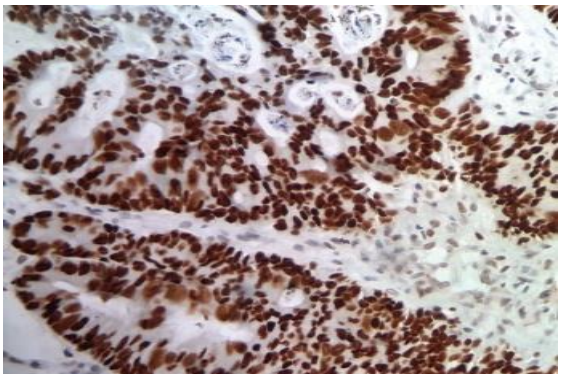


Fig 2b–photomicrograph showing p53 positivity in *Adenocarcinoma,NOS type*,right side colon

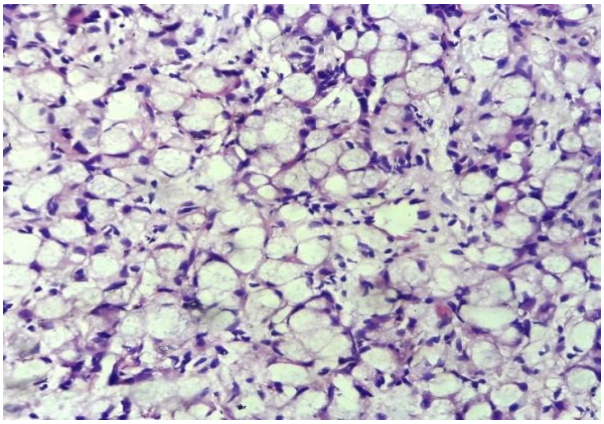


Fig 3a –Photomicrograph showing *signet ring cell carcinoma*, left side colon (H & E, 400x).

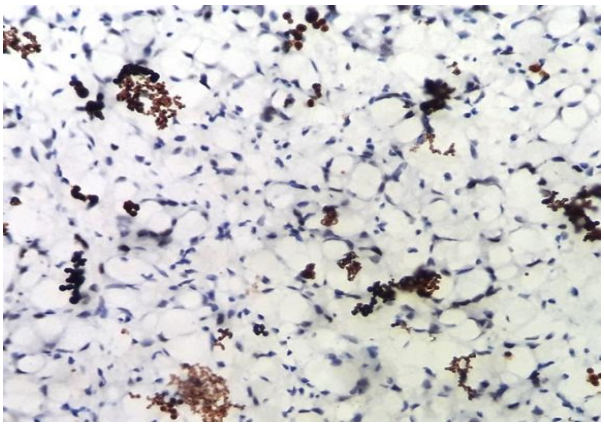


Fig 3b-Photomicrograph showing her-2 negativity in *signet ring cell carcinoma*, left side colon

On K-RAS mutational study, 4 out of 6(67%) of the right sided colonic cancers had a positive K-RAS mutation while 4 out of 9(40%) of the left sided colonic cancers were K-RAS positive. (fig 4)

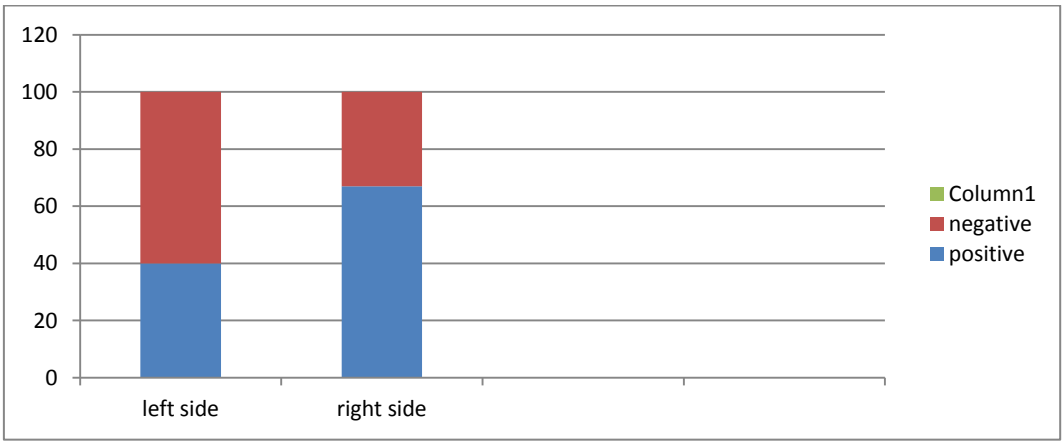
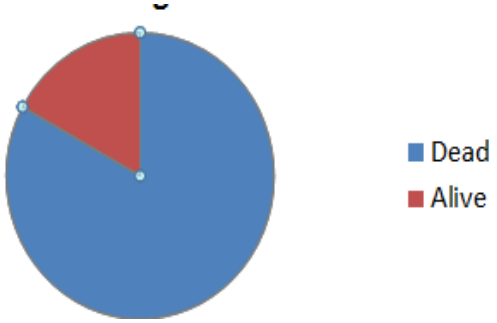
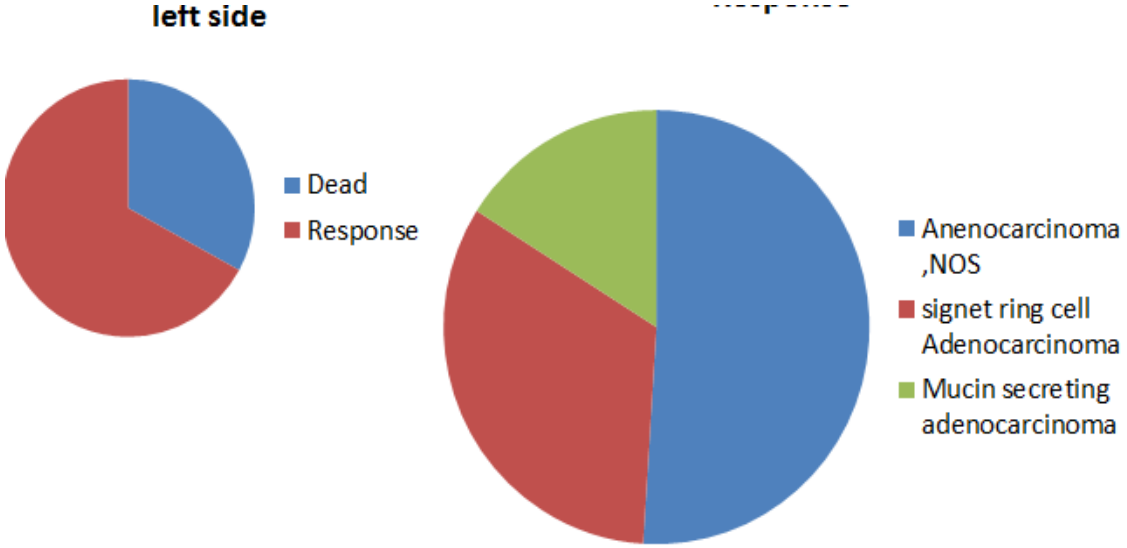


Fig 4-Histogram showing comparision of both sided K-RAS mutation

On follow up, Right sided colon carcinoma.5 deaths out of 6 cases.(83%).The living case case was of mucin secreting adenocarcinoma (fig 5).



Left sided colon carcinomas, Out of 9 cases 6 patients responded to chemotherapy well (67%). Among the 6 alive cases,3 were of adenocarcinoma NOS,2 were of signet ring cell type and 1 of mucin secreting(fig 6).



Discussion

Adenocarcinoma NOS shows strong p53 positivity on both sides and her-2/neu positivity in right side. Adenocarcinoma signet ring cell type shows p53 positivity in right side, negative in left side and her-2/neu negative in both. Adenocarcinoma mucin secreting type shows p53 positivity in both sides and her-2/neu negative in both sides.

Prognosis of right sided adenocarcinoma NOS is worse than left side (due to her2neu positivity). Prognosis of right sided signet ring cell carcinoma is worse than left side (due to p53 positivity in right side). Prognosis of mucin secreting adenocarcinoma is same for both sides because both sides show p53 positive her-2/neu negative.

Her-2-positivity is a bad prognostic sign for Right sided adenocarcinoma over left.

P53 positivity is a bad prognostic sign of right sided signet ring cell adenocarcinoma over left.

There is no difference in prognosis of the right and left sided carcinomas of mucin secreting type.

Also on follow up, the prognosis of right sided colon cancer was worse than the left side because of positive K-RAS mutation.

Due to the larger bowel lumen, RSCC usually becomes symptomatic later than LSCC, which in turn leads to later diagnosis, larger tumor size and advanced tumor stage [44,45]. Secondly, RSCC is located far away from the anal verge, so it is more difficult to be discovered by digit rectal examination and sigmoidoscopy. Hugen et al.[46] reported that the frequency of mucinous and signet-ring cell tumors was higher in RSCC (45%) than in that in LCRC (20%). It had been hypothesized that there were significant

differences in molecular features between RSCC and LSCC, which might serve as the cause of clinicopathological differences[47]. RSCC was reported to have a higher frequency of KRAS mutation than LSCC (57.3% vs 40.4%; P < 0.0001)[48], and a higher incidence of BRAF mutation with 18.4–22.4% in RSCC and 1.3–7.8% in LCRC42. But RSCC had also been reported to be associated with more mutant KRAS and more wild-type BRAF tumors. But no significant difference was found in KRAS and BRAF mutation in our study. Similarly, except for the TOPIIα immunostaining index, no significant difference was found in protein expression levels of MLH1, MSH2, MSH6, PMS2, β-tubulin III, P53, Ki67 and TopIIα between RSCC and LSCC. Our results were consistent with that of Zhu et al.20 and Cancer Genome Atlas Network[50]. Zhu et al. compared gene expression profiling of RSCC and LSCC using the Human Genome Array gene chip in 100 cases of patients, but only 11 genes were identified to be differentially expressed between RSCC and LSCC20. Cancer Genome Atlas Network conducted a genome-scale analysis of 276 samples, analyzing exome sequence, DNA copy number, promoter methylation, mRNA and micro RNA expression, and concluded colon and rectal cancers had similar patterns of genomic alteration[49]

Conclusion

In conclusion, RSCC patients have larger tumor size, poor differentiation, advanced TNM stage and poor survival, compared with LCRC patients.

Prognosis of right sided colonic carcinoma was worse than the left side due to:

KRAS mutation &P53 and her/2 positivity

These factors indicate the oncogenicity due to microsatellite instability which is commoner in right side.

Anti-epidermal growth factor receptor (EGFR) monoclonal antibody treatment, including that with cetuximab and panitumumab is more effective in left side colonic carcinoma than right side.

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