Prognostic Relevance of SFRP1 Gene Promoter Methylation in Colorectal Carcinoma

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Abstract

Background: The development of colorectal carcinoma (CRC) involves many genetic and epigenetic alterations and methylation being an important epigenetic event has been described as a diagnostic and prognostic biomarker. Secreted Frizzled-Related Protein 1 (SFRP1) gene regulates diverse physiological processes via the Wnt signaling. Promoter hypermethylation of SFRP1 gene is an epigenetic regulation mechanism that downregulates SFRP1 protein level in the tumor, and happens to be one of the significant events in colorectal carcinogenesis. We studied the clinicopathological relationship of CRC including survival outcomes with SFRP1 gene promoter methylation. Methods: We evaluated promoter methylation status of SFRP1 gene by methylation-specific PCR (MS-PCR) in the tumor tissue in 54 cases of stage II-III CRC patients in north India. The MS-PCR result was further validated by bisulfite sequencing. Results: SFRP1 gene was methylated in 72.2% cases and un-methylated in 27.8%. We found, that SFRP1 gene methylation in tumor was associated with lymph node invasion (p=0.05). The mean overall survival was 22.318 months and 45.173 months respectively for patients with methylated and unmethylated SFRP1 gene (p= 0.010, log rank test), (HR = 17.313, 95% CI: 2.021-148.290 P=0.009). Conclusion: Study indicates that promoter methylation of SFRP1 gene is associated with lymph-node metastasis and poor mean overall survival and it can be a prognostic marker in CRC.

Keywords: Colorectal carcinoma- Methylation Specific PCR (MS-PCR)- Promoter hypermethylation,- SFRP1 gene

Introduction

Colorectal carcinoma (CRC) is one of the leading causes of mortality worldwide. It is the third most common cancer in males and second in females globally and recorded as second major cause of cancer-related deaths worldwide (Bray et al., 2018). In India CRC is fifth most common cancer in females and forth in males with incidence rate of 3.1% and 5.8% respectively (Bray et al., 2018). There is an increasing incidence of CRC in India. CRC is a heterogenous disease, influenced by genetic and epigenetic alterations and the heterogenicity is due to several pathways involved in CRC tumorigenesis (Colussi et al., 2013). CRC patients show a significant difference in prognosis and individual treatment responses even when presenting at same clinical stage. Multiple factors deregulate the expression of cancer related genes (like APC, KRAS, BRAF, TP53, SFRPs MLH1, MSH1) and promoter methylation mediated silencing is one of them (Armaghany et al., 2012; Thiel et al., 2013; Fearon and Vogelstein, 1990; Wheeler et al., 2000). Secreted Frizzled Related Protein1 (SFRP1) gene is known for its ability to negatively modulate the Wnt signaling cascade (Mii and Taira, 2011). SFRP1 gene codes for SFRP1 protein that works as an antagonist of Wnt protein and plays a significant role in the regulation of Wnt/βcatenin signaling pathway. β-catenin dependent canonical WNT signaling maintains crypt stem cell compartment in the intestine but overactivation of this pathway by genetic or epigenetic changes has been seen in colorectal carcinoma (Novellasdemunt et al., 2015). This Wnt/βcatenin pathway also plays important role in tumorigenesis of several other types of cancers like breast, ovarian, gastrointestinal cancer (Clevers and Hans, 2006; Huang et al., 2006; Zhan et al.,2006). In CRC, SFRP1 gene expression is found to be downregulated due to aberrant methylation in its promoter region and this promoter methylation is a common epigenetic alteration found in human cancers including colorectal carcinoma (Suzuki et al., 2004; Jones and Jomary, 2002). CpG islands are susceptible for methylation and since most of gene promoter regions are CpG island rich, it implies that promoter regions are most susceptible for hypermethylation and thus, promoter methylation leads transcriptional silencing of the gene (Nandakumar et al., 2011). If promoter hypermethylation occurs in tumor suppressor gene it may lead to tumorigenesis. Promoter hypermethylation mediated epigenetic silencing of SFRP1 gene is a major
cause of downregulation of SFRP1 protein level and leads overactivation of Wnt signaling in CRC. Methylation based molecular makers are successfully being used in routine as prognostic/predictive marker for better patient management in various cancer Eg. MGMT in Gliomas (Weller et al., 2010). Some studies have also described value of SFRP1 as prognostic/predictive biomarker in cancer (Leygo et al., 2017; Zheng et al., 2015). The aim of our study was to look for promoter hypermethylation of SFRP1 gene in CRC, and find its prognostic significance. It explores for association of promoter methylation of SFRP1 gene with clinicopathological features of CRC and patient survival.

Materials and Methods

Patients and tissue specimen

We enrolled 54 histopathologically confirmed cases of CRC, who underwent curative surgery in Departments of Surgical Gastroenterology and Surgical Oncology, Dr. R M L Institute of Medical Sciences Lucknow, UP, India. Of these 54 cases 28 (51.85%) case were stage II and 26 (48.15%) cases were stage III at the time of diagnosis (Lippincott-Raven et al., 1998). After histopathological examination (HPE), the FFPE tissue blocks were taken for molecular analysis. HPE (staging and grading) were done by standard procedure. Patient demographic and histopathological details and follow up were recorded. This study was approved by Institutional Ethics Committee (IEC no-8/15) of Dr. RMLIMS, Lucknow, and written informed consent was taken for all cases included in this study. The selection of tumor and non-tumor regions was done by examining Hematoxylin - Eosin (H and E) stained sections. Patient follow up and mean survival was noted up to the close of study observations (July 2018) or the death of the patient which was earlier.

Genomic DNA extraction

DNA extraction from FFPE tissues were done by using QIAamp FFPE tissue Kit, REF no. 56404 (Qiagen, Hilden, Germany) by following manufacturer’s protocol. DNA quality and quantity checked by spectrophotometrically. Purity and integrity checked by agarose gel electrophoresis in 0.8% agarose gel.

Bisulfite modification of DNA

The genomic DNA isolated from the CRC tumor and adjacent normal tissue were subjected to bisulfite methylation analysis. Bisulfite conversion of DNA was done by using Epitect Bisulfite kit (Cat No./ID: 59104 Qiagen, Hilden, Germany) by following manufacturer’s protocol designed for processing DNA isolated from FFPE tissue samples. Briefly, the 20 µl solution of DNA (500ng-2µg) mix with 35 µl of DNA protecting buffer and 85 µl bisulfite mix and incubated for conversion in thermo cycler at recommended temperature. After completion of bisulfite conversion reaction, 310 µl freshly prepared buffer BL containing 10 µg/ml carrier RNA (Carrier RNA increases binding of DNA to the spin-column membrane) added to sample then sample transferred to spin columns after that washing by wash buffer. Followed by desulphonation step performed by adding 500 µl de-sulfonation buffer BD to the spin columns and incubate for 15 min at room temperature. Then sample twice washed by wash buffer. Then final bisulfite converted DNA eluted in 20 µl elution buffer. Bisulfited converted DNA used for MS-PCR analysis within 24 hours.

Methylation specific PCR (MS-PCR)

Methylation specific PCR was set up according to the method described by Herman et al., (1996). 2.5 µl bisulfite converted DNA was amplified using methylation specific primers that specifically recognized either the unmethylated or methylated SFRP1 gene sequence after bisulfate conversion (Takada et al., 2004). Sequences of the primers for MS-PCR of the SFRP1 promoter region were commercially procured. The sequences for Methylated primer were Forward: 5’-TGATTTTCGGAGTTGTCGCCG-3’, Reverse: 5’-CCTACGATCGAAACGCGAGACGC-3’ (126bp); unmethylated primers, Forward-5’-TTTGTAGTTTTGTGGAGTTGTGTTGTG-3’, Reverse: 5’CTACACCTAAAATCAAAACACACAAACA-3’ (135bp). All PCR reactions were performed using AmpliTaq Gold PCR master mix PCR cycling conditions were as following: initial denaturation at 95°C for 10 min then 35 cycles consisting of three steps: 95°C for 10s, respective annealing temperature for 30s at 59°C, extension at 68°C for 10s followed by a final extension at 72°C for 10 min. The annealing temperature for amplification of methylated and un-methylated SFRP1 promoter region was 59°C, and 58°C respectively. Methylated and un-methylated bisulfite converted human control DNA procured from Qiagen, Hilden, Germany was used as positive control for methylation and unmethylation.

SFRP1 Promoter methylation sequencing analysis

Validation of MS-PCR results and methylation pattern of CpG in promoter region was done by bisulfite sequencing using the method defined by Susan et al., (1994). MS-PCR products from tumor and normal tissues were sequenced by using ABI sequencing platform Genetic analyzer 3500, and sequence analysis and alignment was done using Bioedit software and CLUSTALW online tool.

Statistical analysis

Statistical analyses was done by using SPSS software (version 20). Chi square test was used to analyze the statistical association between clinic-pathological data and methylation status of SFRP1. Kaplan Meier survival curve and Log-rank test were used for survival analysis. To evaluate the prognostic impact, all clinicopathologic variables were evaluated along with SFRP1 methylation status by using univariate cox proportional hazard model analysis. P-value <0.05 was considered as significant.

Results

Clinicopathological characteristics

The clinicopathological details are summarized in Table 1. The median age of patients at the time of
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SFRP1 gene promoter methylation status in tumor tissue was analyzed. In 39 out of 54 (72.2%) CRC cases SFRP1 gene was methylated while in 15/54 (27.8%) cases it was unmethylated. Whereas in only 13/54 (24%) cases it was methylated and in 41/54 (76%) cases it was unmethylated.

Diagnosis was 49 years (range 18-76 years). Of these 33 (61.1%) were male and 21 (38.9%) were female patients (M:F = 1.57:1). 28 (51.85%) cases were of CRC stage II, and 26 (48.15%) stage III. The tumor site was right colon in 18 (33.3%), left colon (excluding rectum) in 20 (37%) and rectum in 16 (29.6%) cases. Histologically, 47 (87%) tumors were infiltrating adenocarcinoma NOS, 7 (13%) were mucinous adenocarcinoma. The tumor grade was well differentiated in 32 (59.3%) cases, moderate differentiation in 13 (24%) and poor differentiation in 9 (16.6%) cases. Clinical follow up ranging from 12 to 56 months was available in these cases.

**SFRP1 gene promoter methylation in tumor tissue**

We analyzed SFRP1 gene promoter methylation status in tumor tissue and adjacent normal tissue. In 39 out of 54 (72.2%) CRC cases SFRP1 gene was methylated while in 15/54 (27.8%) cases it was unmethylated. Whereas in only 13/54 (24%) cases it was methylated and in 41/54 (76%) cases it was unmethylated.

**Figure 1.** Promoter Methylation Analysis of SFRP1 Gene in CRC Tumor Tissue by Methylation Specific Polymerase Chain Reaction (MS-PCR). MS-PCR amplified product run in 4.0% agarose gel. (a), Shows distinct band of methylated fragment in lane 2, 4, 6 and their intensity higher than respective unmethylated band lane 3, 5, 7 in 3 colorectal carcinoma tumor tissue. 20pb ladder in lane 1 and 50pb ladder in lane 8; (b), Shows the methylated DNA band in a CRC tumour tissue (lane 5) with control, positive control unmethylation (lane 2), positive control methylation (lane 3) and negative control NTC (lane 6) with 50 pb ladder (lane 1). Size of Methylated and Un-methylated fragment is 126bp and 135bp respectively. M (Methylation specific polymerase chain reaction), U (Un-methylation specific polymerase chain reaction), L (Ladder) NTC (Non Template Control, water used instead of DNA template).

**Figure 2.** SFRP1 gene promoter methylation analysis by bisulphite sequencing. (a), Chromatogram of methylated SFRP1 gene promoter – Bold blue C represents methylated cytosine that’s remained unchanged during bisulfite conversion due to its methylation and C represents un-methylated cytosine that converted to thymine represented T, and CG in round corner rectangle represents CpG sites; (b), Chromatogram in a case of un-methylated SFRP1 gene represented un-methylated cytosine that converted to thymine during bisulfite conversion. Arrow marked “a” is sequence is normal 126bp of DNA sequence of SFRP1 gene of Homo sapiens SFRP1 gene (NCBI Reference Sequence: NM_003012.4), “b” Bisulfite converted DNA sequence of methylated SFRP1 gene of CRC case “c” chromatogram of bisulfite sequencing.
2 out of 28 (7%) cases adjacent non tumor tissue showed methylated SFRP1 gene. Thus a significant difference in methylation status (P<0.0001) was present between tumor and non-tumor tissue (Figure 1). Methylated in relation to clinical stage was noted in 60.7% cases of stage II and 84.6% cases of stage III tumor. To ascertain the methylation status of CpG sites present within promoter region of SFRP1 gene, we performed Bisulfite Sequencing of the 126 bp DNA fragment of SFRP1 gene amplified by MS-PCR in representative cases. Bisulphite sequencing showed methylated Cytosine nucleotide in the CpG sites. This 126 bp DNA sequence, in the cases showing methylated SFRP1 in MS-PCR, contained 22 CpG cites within which most of the cytosine nucleotides were methylated (Figure 2).

**SFRP1 promoter methylation associated with lymph node invasion**

Promoter methylation status of SFRP1 gene was compared with patient’s clinicopathological characteristics such as age, gender, tumor location, lymph node involvement, tumor stage, and tumor grade (Table 1). Chi square test results show that lymph node metastasis was significantly associated with methylation status of SFRP1 gene in the tumor. Lymph node involvement (pN1-3) was noted in 84.6% cases with methylated SFRP1. Location of tumor was not associated with methylation status. A higher frequency of methylation was observed in patients over 60 years age, however this was not statistically significant.

**Table 1. Clinicopathological Characteristics of CRC Cases and Their Association with Methylation**

| Variable              | Categories          | No. of cases | Methylation status of SFRP1 gene promoter | p-value |
|-----------------------|---------------------|--------------|------------------------------------------|---------|
|                       |                     |              | Un-methylated n =15 (27.8%) | Methylated n=39 (72.2%) |
| Age group             | <50 year            | 27           | 9 (33.3)                                | 18 (66.7) | 0.54 |
|                       | >50 year            | 27           | 6 (22.2)                                | 21 (77.8) | 0.54 |
| Gender                | Male                | 33           | 8 (24.2%)                               | 25 (75.8%) | 0.54 |
|                       | Female              | 21           | 7 (33.3%)                               | 14 (66.7%) | 0.77 |
| Tumour Stage          | T2                  | 9            | 3 (33.3%)                               | 6 (66.7%) | 0.05 |
|                       | T3                  | 23           | 7 (30.4%)                               | 16 (69.9%) | 0.65 |
|                       | T4                  | 22           | 5 (22.7%)                               | 17 (77.3%) | 0.05 |
| Lymph-node involvement| pN0                 | 28           | 11 (39.3%)                              | 17 (60.7%) | 0.05 |
|                       | pN1-3               | 26           | 4 (15.4%)                               | 22 (84.6%) | 0.05 |
| Histological type     | Infiltrating adenocarcinoma NOS | 47 | 13 (27.7%) | 34 (72.3%) | 0.1 |
|                       | Mucinous adenocarcinoma | 7  | 2(28.6)% | 5 (71.4%) | 0.77 |
| Tumour grade          | Poorly differentiated| 9            | 3 (33.3%)                               | 6 (66.7%) | 0.83 |
|                       | Moderately differentiated | 13 | 4 (30.8%) | 9 (69.2%) | 0.97 |
|                       | Well differentiated  | 32           | 8 (25%)                                 | 24 (75%) | 0.747 |
| Tumour location       | Colon               | 38           | 10 (26.3)                               | 28 (73.7) | 0.747 |
|                       | Rectum              | 16           | 5 (31.2%)                               | 11 (68.8%) | 0.747 |

Table 1- Showing association of clinico-pathological parameters in relation to methylation status of SFRP1 gene using Chi square test (significant p-value <0.05).
SFRP1 gene promoter methylation in colorectal carcinoma

Based on the observed methylation status of SFRP1 gene in the tumor tissue, methylated and unmethylated groups were defined. Follow-up in 54 patients ranging from 12 month to 56 month (median follow-up 28 months) 18 CRC patients had died due to disease related event and advanced tumor stage and 12 patients lost to follow-up. The overall mean survival of unmethylated and methylated group was 45.173 months and 22.318 months respectively survival curve in (Figure 3a). The combined estimated OS of both the groups were 33.461 months. Unmethylated groups survival was significantly better as compare to methylated group (p= 0.010 by Log rank test) and poor survival associated with methylation of SFRP1. We also analyzed survival of CRC patients with reference of 8 different conventional pathological factors such as Age group, Gender, Tumor stage, Clinical stage, Lymph node status, Differentiation of tumor and Tumor subtype (Table 3). Kaplan-Meir survival analysis results shows survival is dependent on many factors but it was majorly influenced by lymph node status and methylation of SFRP1. If we talk about survival on the basis of clinical

### Table 2. Association between Clinicopathological Characteristics and Prognosis of the Disease by Using Univariate Cox Regression Analysis

| Variables                  | Categories            | No. of cases | No of Events (deaths) | HR      | 95% CI # (Lower-Upper) |
|----------------------------|-----------------------|--------------|-----------------------|---------|-----------------------|
| Age group                  | <50 year              | 27           | 11                    | 1       | Ref.                  |
|                            | >50 year              | 27           | 7                     | 0.672   | (0.183-2.475)         |
| Gender                     | Male                  | 33           | 13                    | 1       | Ref.                  |
|                            | Female                | 21           | 5                     | 0.308   | (0.089-1.063)         |
| Histological type          | Infiltrating adenocarcinoma NOS | 47 | 14                | 1       | Ref.                  |
|                            | Mucinous adenocarcinoma | 7   | 4                | 2.404   | (0.664-8.701)         |
| Tumor grade                | Poorly differentiated | 9            | 4                     | 1       | Ref.                  |
|                            | Moderately differentiated | 13 | 3                | 0.267   | (0.042-1.681)         |
|                            | Well differentiated    | 32           | 11                    | 0.333   | (0.082-1.356)         |
| Tumor stage                | pT2                   | 9            | 3                     | 1       | Ref.                  |
|                            | pT3                   | 23           | 6                     | 0.387   | (0.075-1.982)         |
|                            | pT4                   | 22           | 9                     | 0.755   | (0.144-3.955)         |
| Tumor location             | Colon                 | 38           | 11                    | 1       | Ref.                  |
|                            | Rectum                | 16           | 7                     | 1.15    | (0.391-3.385)         |
| Lymph node involvement     | pN0                   | 28           | 6                     | 1       | Ref.                  |
|                            | pN1-3                 | 26           | 12                    | 1.281   | (0.391-4.471)         |
| Methylation status of SFRP1 gene promoter | Un-methylation | 15 | 2 | 1 | Ref. |
|                            | Methylated            | 39           | 16                    | 17.313  | (2.021-148.290)       |

HR, (Hazard Ratio); #, Hazard Ratio (95% Confidence Interval); Ref., taken as reference

### Table 3. Kaplan-Meier Survival Analysis with References to Clinicopathological Characteristics.

| Variables                  | Categories            | No. of cases | No of Events (death) | Mean Survival in months | P value |
|----------------------------|-----------------------|--------------|----------------------|-------------------------|---------|
| Methylation status of SFRP1 | Un-methylation       | 15           | 2                    | 45.17                   | 0.01    |
|                            | methylation           | 39           | 16                   | 22.32                   |         |
| Age group                  | >50 year              | 27           | 7                    | 34.68                   | 0.472   |
|                            | <50 year              | 27           | 11                   | 28.66                   |         |
| Lymph-node involvement     | pN0                   | 28           | 6                    | 40.96                   | 0.135   |
|                            | pN1-3                 | 26           | 12                   | 26.88                   |         |
| Gender                     | Male                  | 33           | 13                   | 29.34                   | 0.146   |
|                            | Female                | 21           | 5                    | 29.75                   |         |
| Tumour grade               | Poorly differentiated | 7            | 3                    | 27.10                   | 0.68    |
|                            | Moderately differentiated | 14 | 4 | 29.33 |         |
|                            | Well differentiated   | 26           | 7                    | 32.78                   |         |
| Tumour stage               | pT2                   | 9            | 3                    | 26.99                   | 0.284   |
|                            | pT3                   | 23           | 6                    | 37.81                   |         |
|                            | pT4                   | 22           | 9                    | 18.95                   |         |
| Histological type          | Infiltrating adenocarcinoma NOS | 47 | 14                | 34.70                   | 0.142   |
|                            | Mucinous adenocarcinoma | 7   | 4                | 24.85                   |         |
| Location of tumour         | Colon                 | 38           | 11                   | 26.17                   | 0.931   |
|                            | Rectum                | 16           | 7                    | 34.06                   |         |
stage II and III the mean survival was 40.96 and 26.88 months respectively $p=0.135$ by log rank test (Figure 3b). To explore the contribution of these variables and evaluation of their influence as potential prognostic marker, all these variables were analyzed by univariate cox regression model analysis. In univariate analysis, only SFRP1 methylation status could be verified as an independent prognostic factor, (HR = 17.313, 95% CI: 2.021-148.290, and $P$-value = 0.009, Table 2). Univariate cox model suggesting, among these variables, SFRP1 methylation can serve as an independent prognostic indicator of poor survival in CRC.

Discussion

Wnt signaling plays important role in embryonic development where it determines the cell fate, cell proliferation and cell migration (Clevers and Hans, 2006; Zhan et al. 2016). In life, Wnt signaling also controls tissue regeneration in adult bone marrow, skin and intestine (Goessling et al., 2009). Wnt signaling maintains intestinal stem cells by proliferation and differentiation. It is also involved in carcinogenesis of various tumors including CRC (Mii and Taira, 2011; Zhan et al., 2016; Zhou et al., 2015; Clevers and Hans, 2006; Huang et al., 2006). SFRP1 gene is known for its ability to negatively modulate the Wnt/β-catenin signaling cascade. Promoter methylation downregulates the expression of SFRP1 gene in CRC (Jones and Jomary, 2002; Suzuki et al., 2004; Shih et al., 2006, Fukui et al., 2005) Silencing of SFRP1 gene, allows constitutive WNT signaling via binding to Wnt protein and inhibits its binding to Wnt-frizzled receptor, consequently altering the proliferation and differentiation of tumor cells. Limited studies have looked in to the association of SFRP1 methylation with clinicopathological characters and survival in CRC. Studies done on other tumors suggest that methylation of SFRP1 gene can serve as epigenetic diagnostic, prognostic and predictive marker in liver, gall bladder, upper gastrointestinal tract and lung cancers (Kim et al., 2016; Mo et al., 2018; Suzuki et al., 2002; Müller et al., 2004; Zou et al., 2005; Su et al., 2009) In the present study, we have studied promoter methylation status of SFRP1 gene in CRC patients and its association with various clinicopathological characteristics. We found that SFRP1 was frequently methylated in tumor tissue compared with adjacent non tumor tissues. The frequency of SFRP1 gene promoter methylation in our patients was 72.2%. Previous studies, have shown a frequency ranging from 52-95% in colorectal cancer (Rawson et al., 2011; Bartáš, 2017; Zhou et al., 2015; Meng et al., 2011; Dalilol et al., 2012; Salehi et al., 2012). In present study we noted a slightly higher incidence of hypermethylation of SFRP1 in male patients than females (75.8% vs. 66.7%), however this difference was not statistically significant ($p=0.54$). The frequency of methylation was not influenced by the histological subtype of tumor. Infiltrating adenocarcinoma NOS and mucinous adenocarcinoma showed SFRP1 gene promoter methylation frequency of 72.3% and 71.4% respectively. In 84% cases with lymph node metastasis, SFRP1 gene methylation was noted which was significant ($P=0.05$). Other clinicopathological characters such as age, gender, tumor, location, tumor stage, tumor type, grade of tumor did not show any significant association with methylation status of SFRP1 gene. Our data suggests that SFRP1 promoter methylation is an epigenetic prognostic marker for poor survival in stage II and III CRC. The patients in the methylated group had shorter mean overall survival (22.318 months) as compared to the un-methylated group (45.173 months). A possible reason for shorter overall survival with methylated SFRP1 gene in CRC could be that promoter methylation reduces expression of SFRP1 gene allowing constitutive WNT signaling that may help tumor cell to proliferate. Epigenetic inactivation of SFRF genes allowing constitutive WNT signaling in colorectal cancer has been previously described by Suzuki et al., (2004). SFRP1 gene has also been studied in other tumors such as Head and neck squamous cell carcinoma, breast cancer and found to indicate poor patients survival (Alsofyani et al., 2016, Veeck et al., 2008, Kang et al., 2014) An implication of this findings can also be explored for targeted therapy in CRC using recombinant SFRP1 (Cooper et al., 2012).

This study has some limitations such as smaller sample size and shorter duration of follow-up. However even then the findings obtained are important with prognostic significance. Similar studies with larger sample size and longer follow-up would be helpful to substantiate our findings.

In conclusion our study shows that promoter methylation of SFRP1 gene occurs frequently in Colorectal Carcinoma. This SFRP1 promoter methylation is significantly associated with lymph node invasion and poor survival outcome in stage II and III CRC patients and it appears to be a poor prognostic marker.

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