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Original Research Article

A study on the investigation of the biomarker potential of miRNA-21 expression in colorectal tumor samples and serum

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ABSTRACT

Background: miRNAs have been highlighted as diagnostic, prognostic, and predictive biomarkers because of the high tissue specificity, stability and altered expression in tumor development. miRNA analysis could offer a less invasive and more cost-effective alternative to supplement existing screening approaches.
Methods: All colorectal cancer patients were subjected to serum miR21 expression and from biopsy by RT PCR.
Results: Higher stage tumors had higher expression of miR21 in both serum and biopsy. Higher grade tumors had higher expression of miR21 in both serum and biopsy.
Conclusions: From our study, we conclude that, Higher miRNA 21 expression in serum and biopsy were associated with acute presentations and advanced stages of colorectal malignancies. Serum miRNA 21 expression patterns can be found even in early-stage malignancies of colorectal tumors, hence serving as a useful noninvasive biomarker. miRNAs have been highlighted as diagnostic, prognostic, and predictive biomarkers because of the high tissue specificity, stability, and altered expression in tumor development. Thus, miRNA21 analysis could offer a less-invasive and more cost-effective alternative to supplement existing screening approaches.

Keywords: Biomarker, Colorectal maligmancy, miRNA-21

INTRODUCTION

Colorectal tumors are one of the most common gastro intestinal malignancies.[1] Diagnosis and clinical staging has evolved over the years. Genetic level biomarkers have been emerging for all the tumors over years. One such genetic level marker is micro RNA. MicroRNAs (miRNAs) are endogenously expressed short, noncoding, single- stranded RNAs of 18-25 nucleotides in length. In general, miRNAs are transcribed as a cluster called the pri-miRNA complex, which is cleaved in the nucleus to form the pre- miRNA, this is then translocated to the cytoplasm to undergo final maturation into a functional miRNA.[2] miRNAs can modulate gene expression at the post-transcriptional level by targeting mRNAs for translational repression or degradation. Many studies have demonstrated that miRNAs play key roles in the regulation of; cellular growth, differentiation, proliferation and apoptosis.[3-5] According to recent reports, several significant targets of miR-21 associated with malignancy have been experimentally validated, such as PTEN, programmed cell death 4 (PDCD4). Reversion-inducing cysteine-rich protein with Kazal motifs (RECK), Forkhead box O1 (FOXO1), RhoB, Cdc25a. These targets may exert different effects on tumor genesis. Among them, the PTEN protein was

reported to be frequently silenced in CRC, and it has been shown to suppress tumor formation by inhibiting the PI3K/AKT pathway. Therefore, miR-21 might act as an important oncogene by regulating PTEN as one of its several target genes and thus may be a useful biomarker for the diagnosis and treatment in CRC.[6,7] The first study of miR expression in colorectal tumor tissue compared to normal colonic tissue was reported in 2003 by Michael et al. In this study, miR-200c was first isolated in normal colonic tissue and miR-143 and -145 were found to be downregulated in tumor tissue compared to normal colon tissue. Recently miR-21 has been evolving as a biomarker in colorectal tumors and its expression has been linked with poor survival.[8] Hence miRNAs have been highlighted as diagnostic, prognostic and predictive biomarkers because of the high tissue specificity, stability and altered expression in tumor development.[9] Thus miRNA analysis could offer a less invasive and more cost-effective alternative to supplement existing screening approaches. Our study is to evaluate biomarker candidacy of serum mi-RNA 21 for colorectal cancer and to correlate the mi-RNA 21 expression in tumor with clinical stage and presentation of the tumor.

METHODS

Study design, location and duration

Prospective observational study was performed at department of surgical gastroenterology at DSMCH from July 2018 and March 2022'

Inclusion and exclusion criteria

All colorectal cancer patients aged 18-75 years of age irrespective of the staging were included. Patients not consenting for the study were excluded.

Data collection methods

All patients who are giving consent and full filling inclusion criteria will undergo a colonoscopy and biopsy after satisfactory bowel preparation. Tissue samples will be taken from the tumor during colonoscopy or from surgical cut section, preserved in RNA later solution at -80 degrees and sent for miRNA- 21 analysis. 5 ml of blood will be collected in EDTA tube, plasma separated and stored at -80 degrees and sent for analysis. RNA isolation was performed with miRNeasy Serum/Plasma/tissue Kit (Cat No: 217184, Qiagen). The kit is suitable for purifying total RNA, including small RNAs, from small volumes (up to 200 µl) of serum, plasma, orother body fluids.

Sample size calculation

Sample size was calculated using the formula mentioned below;

$$N = Z\,2 * (p) * (1 - p)/C2$$

Where: Z=Z value (e.g. 1.96 for 95% confidence level), p=percentage picking a choice, expressed as decimal (0.7 used for sample size needed),C=confidence interval (10%), expressed as decimal (e.g., 0.10 = ±10), Taking confidence level=95%, Confident interval=10% (Margin of error), Population=2000; sample size was calculated to be 92.

RESULTS

Neo adjuvant treatment (chemotherapy/radiotherapy) were given for 11 (12.0%) patients in the entire study group.

Table 1: Distribution of patients according to pre-op chemotherapy/radiotherapy.

Pre-op chemotherapy/radiotherapy	N	%
Given	11	12.0
Not given	81	88.0

Table 2: miRNA 21 expression in serum vs. mean haemoglobin.

miRNA 21 expression in serum	Haemoglobin (g/dl)
Low	10.58
Moderate	10.27
High	8.76

Table 3: miRNA 21 expression in biopsy vs. mean haemoglobin.

miRNA 21 expression in biopsy	Haemoglobin (g/dl)
Low	10.82
Moderate	10.18
High	8.89

Table 4: miRNA 21 expression in serum vs. mean CEA level.

miRNA 21 expression in serum	Mean CEA level (ng/ml)
Low	7.54
Moderate	14.21
High	14.72

Table 5: miRNA 21 expression in biopsy vs. mean CEA level.

miRNA 21 expression in biopsy	Mean CEA level (ng/ml)
Low	7.86
Moderate	14.47
High	14.12

miRNA 21 expression in serum and biopsy were correlated with hemoglobin CEA, grade, stage. As

miRNA21 expression in serum increases, hemoglobin levels decreased. The same trend of increased expression of miRNA21 in biopsy were associated with reduced haemoglobin values. As miRNA 21 expression increased in serum, CEA levels increased, though marginally in the moderate and high expressions.

Table 6: miRNA 21 expression in serum vs. grade.

miRNA 21 expression in serum	Grade 1	Grade 2	Grade 3
Low	11	11	2
Moderate	8	29	2
High	1	14	14

Table 7: miRNA 21 expression in biopsy vs. grade.

miRNA 21 expression in biopsy	Grade 1	Grade 2	Grade 3
Low	19	7	2
Moderate	1	32	1
High	0	14	16

In biopsy, moderate and high expressions had increased CEA levels. Higher expression of miRNA 21 in serum correlates with higher grade of tumors. Grade 1 tumors have low miRNA expression in biopsy, grade 3 tumors have higher miRNA expression and Grade 2 tumors have predominant moderate miRNA 21 expression levels.

Table 8: miRNA 21 expression in serum vs. stage of tumor.

Stage of tumor	miRNA 21 expression in serum		
	Low	Moderate	High
Stage 1	6	2	0
Stage 2a	13	4	0
Stage 2c	0	3	0
Stage 3a	1	6	0
Stage 3b	3	22	5
Stage 3c	2	2	14
Stage 4a	0	1	3
Stage 4c	0	0	5

Table 9: miRNA 21 expression in biopsy vs. stage of tumor.

Stage of tumor	miRNA 21 expression in biopsy		
	Low	Moderate	High
Stage 1	5	5	0
Stage 2a	9	9	0
Stage 2c	0	2	0
Stage 3a	7	1	0
Stage 3b	6	18	6
Stage 3c	2	2	14
Stage 4a	0	0	2
Stage 4c	0	0	4

Stage 1 and stage 2a tumors have lower expression, Stage 2c to Stage 3b tumors have moderate expression and Stage 3c and stage 4 tumors have higher expression. Stage 1 to stage 3a tumors have equivocal low and moderate expression, Stage 3b tumors have moderate expression and Stage 3c to stage 4c tumors have higher expression, so higher expression of miRNA 21 in biopsy correlates with higher stage.

DISCUSSION

The p values for miRNA expression in serum and biopsy with the following parameters; Hemoglobin, Grade, CEA and stage were calculated and significance were established (Table 10).

No significance could be established for miRNA and CEA. Even though significance could not be established the pattern of the graphs displayed before suggested that moderate and higher miRNA expressions may have higher CEA values. No significance could be established between hemoglobin and miRNA 21 serum expression levels. There was significance in miRNA21 levels in biopsy with hemoglobin, hence it can be concluded that as miRNA 21 expression values increase patient's hemoglobin values fall, which practically implies advanced nature of the tumor or a complication of the advanced tumor. Significance was established between miRNA 21 expression in both serum and biopsy with grade and stage of the tumors. In our study miRNA21 expression in serum and biopsy were measured for all patients. Even early-stage tumors had expression of miRNA21 in serum, which means the level of miRNA21 can predict the stage of the disease as well as act as a biomarker. So apart from measurement of CEA levels, miRNA 21 expression levels can be measured in all patients to ascertain the diagnosis and predict the prognosis, and even be recommended as a screening investigation in high-risk patients like HNPCC, FAP. According to Xia et al high expression of miR-21 might predict poor prognosis in patients with colorectal cancer. The other factors did not indicate anysignificant prognostic impact of higher expression of miR-21. These factors included age, gender, tumor location, and the CEA levels.[10] The same results are observed in our study. According to Toiyama et al 2013, High miR-21 expression in serum and tissue was statistically significantly associated with tumor size, distant metastasis, and poor survival. Serum miR-21 was reported as an independent prognostic marker for CRC.[11] According to O Slaby et al study on Altered expression of miR-21, miR-31, miR-143 and miR-145 in colorectal tumors, Expression levels of analyzed miRNAs significantly differed among tumors and adjacent non-tumor tissues: miR-21 (p=0.0001) and miR-31 (p=0.0006) were upregulated, and miR-143 (p=0.011) and miR-145 (p=0.003) were downregulated in tumors. For the first time, a high expression of miR-21 was associated with lymph node positivity (p=0.025) and the development of distant metastases (p=0.009) in CRC patients. Thus, expression of miR-21 correlated with CRC clinical stage (p=0.032).[12]

Table 10: P values of different parameters.

Parameters	P value serum	Significance	P value biopsy	Significance
Hemoglobin	0.146	Not significant	0.021	Significant
CEA	0.521	Not significant	0.321	Not significant
Grade	0.002	Significant	0.001	Significant
Stage	0.0012	Significant	0.003	Significant

Limitations

Limitation of current study was, In our study staging and miR21 association was established. Since our study is an observational study poor survival and therapeutic outcomes could not be studied.

CONCLUSION

Current study concluded that higher miRNA 21 expression in serum and biopsy were associated with acute presentations and advanced stages of colorectal malignancies. Serum miRNA 21 expression patterns can be found even in early-stage malignancies of colorectal tumors, hence serving as a useful noninvasive biomarker. miRNAs have been highlighted as diagnostic, prognostic, and predictive biomarkers because of the high tissue specificity, stability, and altered expression in tumor development. Thus, miRNA21 analysis could offer a less-invasive and more cost-effective alternative to supplement existing screening approaches.

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