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STUDY OF SPECIFICITY, SENSITIVITY, EFFICIENCY & CLINICAL CORRELATION BETWEEN TIMP-1 AND MIF PROTEIN AS BIOCHEMICAL MARKERS IN COLORECTAL CANCER PATIENTS.

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ABSTRACT: Background: Colorectal cancer is the fifth most common cancer and the leading cause of cancer-related deaths in India. The incidence, mortality, and prevalence rates are consistently higher. Precautionary measures in preventing colorectal cancer still remain a challenging issue. **Aims and Objectives:** This study was undertaken to correlate the specificity, sensitivity & efficiency of TIMP-1 and MIF protein that are non-invasive and enable us to detect CRC quite early. Analysis of CEA, Ca19-9, Fecal Hemoglobin were done as diagnostic markers & TIMP-1, MIF protein, CRP, prolactin as prognostic and molecular markers. Total 120 patients were included in the study and categorized as stage I-IV with recurrences and without recurrences which were compared with healthy controls. **Results:** The study observed increased CEA, CA19-9, fecal Hb in various stages of colorectal cancer as compared to control. Prolactin, CRP, TIMP-1, MIF Protein show significant ($p<0.001$) rise in various stages. The elevated levels are increased concomitantly from stage 0 to IV suggesting the severity and distance spread of the disease.CEA, CA19-9, and fecal Hb have high specificity & prolactin, CRP, MIF, TIMP-1 protein have high sensitivity. TIMP-1 and MIF protein showed a significant correlation with other biochemical parameters. **Conclusions:** Increased sensitivity and specificity in CRC suggest that biomarkers are highly suitable for early tumor detection. Monitoring of these will be of great diagnostic importance, and this will be challenging in the clinical utility of the CRC patients and increase their survival rate.

INTRODUCTION: Colorectal cancer, also referred as cancer of colon and rectum, is one of the major causes of cancer death worldwide. It is estimated that 1.3 million cases of colorectal cancer worldwide were diagnosed in 2008. Colorectal cancer is third most commonest cancer in men with 663,000 cases and the second commonest cancer in women with 570,000 cases.

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This disease is most common in developed countries, particularly North America, Western Europe, Japan, and Australia. Globally the disease accounted for 608,000 deaths, 8% of all cancer deaths, making it the fourth commonest cause of cancer deaths [1, 2]. In India, Colorectal cancer is the fifth most common cancer and one of the leading causes of cancer-related deaths.

In India, the incidence of mortality and prevalence rates are consistently higher, further, the incidence is higher in males than females [3, 4]. The following study included the categorization and further analysis of colorectal cancer patients who appear to have an increased predisposition to colorectal cancer and/or colorectal polyps and do not possess

pathogenic mutations in one of the previously described colorectal cancer predisposition genes [5]. Identifying new genetic factors predisposing to colorectal cancer increases our ability to understand and maybe useful clinically in managing the pathological consequences [6]. The present study was undertaken to correlate the specificity, sensitivity & efficiency of TIMP-1 and MIF protein analysis that are non-invasive to enable us to detect CRC quite earlier. To diagnose colorectal cancer CEA, CA19-9 and fecal hemoglobin were analyzed [7]. CEA and CA19-9 are closely related to curative effects and the prognosis of advanced colorectal cancer. For clinical validation, genetic and prognostic markers like, TIMP-1, CRP, MIF and prolactin were analyzed. TIMP-1 (Tissue inhibitor of metalloproteinase) is natural inhibitor of matrix metalloproteinase, the enzymes involved in extracellular matrix maintenance and remodeling [8].

But TIMP-1 not only acts as an inhibitor of MMPs, which would be expected to result rather in an anticancerogenic effect, but it also has an MMP-independent role with a direct influence on cell growth apoptosis and angiogenesis [9, 10]. MIF factor originally identified as a product of activated lymphocytes, has been found to have multiple functions, including catalytic activity, lymphocyte immunity endocrine regulation, signal modulation and proinflammatory action. In addition to the pivotal effects of MIF on the immune system and inflammatory response, several reports have linked MIF to a fundamental process that controls cell proliferation, differentiation angiogenesis, tumor progression, and metastasis [11, 12]. We aimed to evaluate the specificity, sensitivity & efficiency of plasma TIMP-1 protein and MIF protein levels along with diagnostic and molecular markers in patients with colorectal carcinoma, with regard to possible early- prediction of recurrences of the disease, which may play a vital role in avoiding repeated colon surgeries and improving the survival rate of CRC patients.

MATERIAL AND METHODS:

Study Area: The present study was undertaken to correlate the "Specificity, sensitivity, efficiency & clinical correlation between TIMP-1 and MIF protein as biochemical markers in colorectal cancer patients. This study was conducted in Ashwini Rural medical college, hospital, and research center Solapur. Maharashtra, over 1 year after taking written consent from subjects. Ethical clearance was obtained from the institutional Ethical Committee. The DCGI Registration no. ECR/782/Inst/MH/2015/RR-18. The study included 120 early diagnosed patients with colorectal cancer. The patients were categorized as stage I-IV with recurrences and stage I-IV without recurrences and they compared with age and sex-matched healthy controls. Information on tumor size, lymph node status, lymphatic or vascular vessel invasion, mucinous cell type and tumor differentiation was retrieved from pathological records. Similarly, family history was obtained preoperatively through written questionnaires. Data on clinical stage, cancer recurrences, death, and cause of death was obtained from surgical and oncological hospital records.

Study Design: This is a case-control study.

Inclusion Criteria for Study Group Cases: Patients with known colorectal carcinoma were considered to be eligible for inclusion in the study.

Exclusion Criteria: To avoid false-positive results, care was taken to exclude patients with renal hepatobiliary disorders, systemic lupus erythematosus, lymph proliferative disorders, collagen disorders, and acquired immunodeficiency syndrome as well as malignancies other than colorectal cancer.

Methods: To diagnose colorectal cancer CEA, CA 19-9, and Fecal hemoglobin were measured. CEA (Carcinoembryonic Antigen) and CA 19-9 were measured by Biomerix kit using two steps immunoassay sandwich method with final fluorescent detection (ELFA) by using mini Vidas. And Fecal hemoglobin were measured by sigma kit using ELISA technique to-site "sandwich" technique with two selected antibodies that bind to different epitopes of human hemoglobin.

To analyze clinical validation genetic and prognostic markers like TIMP-1 and MIF protein were estimated by sigma kit using Enzyme-linked immunosorbent assay (ELISA) technique. CRP was measured using the Nyco card reader immunometric method and prolactin using the Biomerix kit immunoassay sandwich technique.

Statistical Analysis: The data collected was analyzed by student t-test and SPSS-17 software. The difference in mean values of various parameters was calculated and expressed in the p value. Specificity, sensitivity, and efficiency were analyzed for each parameter and were compared with control. Correlations between the parameters were evaluated in all study subjects and calculated by Pearson's method.

RESULT: Table 1 shows the elevated levels of diagnostic markers CEA CA 19-9 and Fecal Hb in patients of CRC as compared to controls ($p<0.001$). Similarly, there was significant rise in prognostic markers and genetic markers *i.e.* prolactin, CRP, MIF and TIMP-1 protein as compared to controls ($p<0.001$).

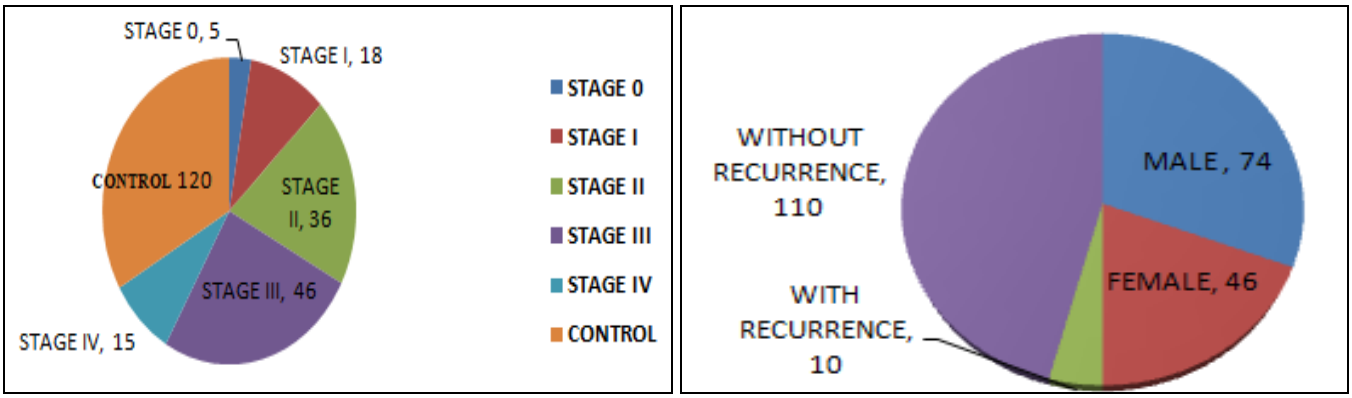

FIG. 1: PIE DIAGRAM: DISTRIBUTION OF HEALTHY CONTROL & STAGE WISE DISTRIBUTION OF COLORECTAL CANCER PATIENTS

TABLE 1: MEAN LEVEL OF ALL INCLUDED BIOCHEMICAL PARAMETERS OF COLORECTAL CANCER (CRC) AND CONTROL WITH THEIR SIGNIFICANT P-VALUE USING UNPAIRED T-TEST

Parameters	Case of CRC Mean ± SD	Control Mean ± SD	p value
CEA (ng/ml)	35.53 ± 12.27	2.2 ± 0.72	p<0.001
CA 19-9(ng/ml)	56.30 ± 7.31	22.68± 8.37	p<0.001
Fecal Hemoglobin(ng/ml)	65.08 ± 6.9	31.13±13.85	p<0.001
Prolactin	36.94 ± 3.94	20.27±5.92	p<0.001
CRP	24.08 ± 4.74	3.36±0.66	p<0.001
MIF protein	254.96 ± 86.35	8.14±4.11	p<0.001
TIMP-1 Protein	343.27± 63.07	132.31±19.37	p<0.001
Cholesterol (mg/dl)	278.75± 36.91	166.76±19.77	p<0.001
Triglyceride (mg/dl)	218.92± 56.78	151.16±21.38	p<0.01
Lipase (mg/dl)	38.37±11.61	30.30±9.85	p<0.05
Amylase (mg/dl)	115.78±32.97	73.27±21.69	p<0.01
Bilirubin (mg/dl)	1.99± 1.16	1.03±0.42	p<0.05
AST/ALT Ratio	2.39±0.30	0.96±0.12	p<0.01

p<0.001 - Highly significant p<0.01 - More significant p<0.05-Significant p>0.05- Not significant.

TABLE 2: ANALYSIS OF VARIANCE SHOWING THE VARIOUS PARAMETERS IN THE DIFFERENT STAGES OF COLORECTAL CANCER WITH THEIR P-VALUE

Parameters	CRC Cases stage-wise distribution					p value
	Stage 0	Stage I	Stage II	Stage III	Stage IV	
CEA (ng/ml)	18.52±1.29	20.38±1.63	27.05±2.28	44.54±5.01	52.12±6.12	p<0.001
CA 19-9(ng/ml)	41.62±1.41	45.45±2.34	55.40±3.90	60.05±3.74	64.84±3.11	p<0.001
Fecalhemoglobin (ng/ml)	54.36±1.35	56.71±3.06	63.51±3.65	66.61±3.05	77.75±3.59	p<0.001
Prolactin (ng/ml)	32.66±3.49	34.69±2.53	36.85±3.10	37.12±3.63	40.76±4.87	p<0.001
CRP(mg/l)	12.6±0.88	17.74±2.58	24.52±2.57	26.06±2.66	28.33±3.47	p<0.001
MIF protein (ng/ml)	105.1±12.07	162.12±24.45	205.29±33.37	299.60±37.55	398.67±34.35	p<0.001
TIMP-1 Protein (ng/ml)	224.44±19.47	262.26±23.11	318.92±18.06	371.32±17.81	452.51±29.11	p<0.001

Table 2 shows a highly significant (p<0.001) rise in diagnostic markers like CEA, CA19-9, and fecal Hb in various stages of colorectal cancer. Further, it is observed that these elevated levels are increased concomitantly from stage 0 to IV, suggesting the severity and the distance spread of the disease. The genetic and prognostic markers like prolactin, CRP, TIMP-1, and MIF Protein show a significant (p<0.001) rise in CRC patients of various stages.

TABLE 3: SENSITIVITY, SPECIFICITY AND EFFICIENCY OF DIFFERENT STUDY PARAMETERS

S. no.	Parameters	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Efficiency
1	CEA (ng/ml)	90%	93.33%	93.10%	90.31%	91.66%
2	CA 19-9(ng/ml)	82.5%	91.66%	90.82%	83.96%	87.08%
3	Fecal Hemoglobin (ng/ml)	81.66%	93.33%	92.45%	83.58%	87.5%
4	Prolactin	90%	91.66%	90%	91.66%	91.66%
5	CRP	95%	90.83%	91.2%	94.78%	92.91%
6	MIF protein (ng/ml)	97.5%	93.33%	93.6%	97.39%	95.41%
7	TIMP-1 Protein (ng/ml)	98.33%	94.16%	94.4%	98.28%	96.25%

Table 3 depicts that the diagnostic markers like CEA, CA19-9 & fecal Hb have high specificity and efficiency as compared to sensitivity. The prognostic and genetic markers like prolactin, CRP, MIF & TIMP-1 protein have high sensitivity compared to specificity and efficiency.

TABLE 4: MEAN LEVEL OF CRC WITH RECURRENCES PATIENTS AND CONTROL WITH THEIR SIGNIFICANT P VALUES BY USING UNPAIRED T-TEST

Parameters	Case of CRC with Recurrences Mean ± SD	Control Mean ±SD	p value
CEA (ng/ml)	52.82± 7.01	2.2 ± 0.72	p<0.001
CA 19-9(ng/ml)	54.64±4.80	22.68± 8.37	p<0.01
Fecal Hemoglobin(ng/ml)	62.78±13.31	31.13±13.85	p<0.01
Prolactin	26.29±3.08	20.27±5.92	p<0.05
CRP	26.58±3.33	3.36±0.66	p<0.001
MIF protein	261.72±37.61	8.14±4.11	p<0.001
TIMP-1 Protein	302.87±39.64	132.31±19.37	p<0.001

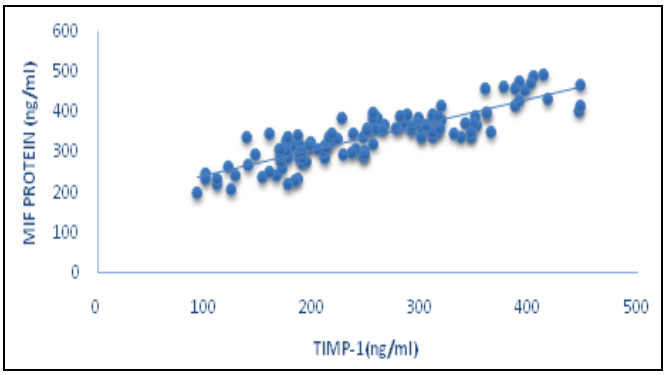

FIG. 2: CORRELATION BETWEEN MIF AND TIMP-1

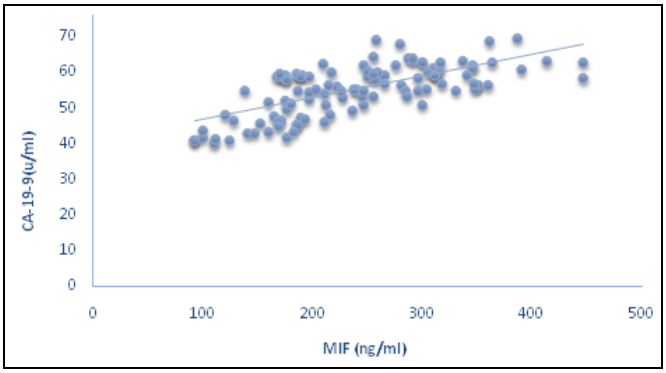

FIG. 3: CORRELATION BETWEEN MIF AND CA 19-9 IN PATIENTS

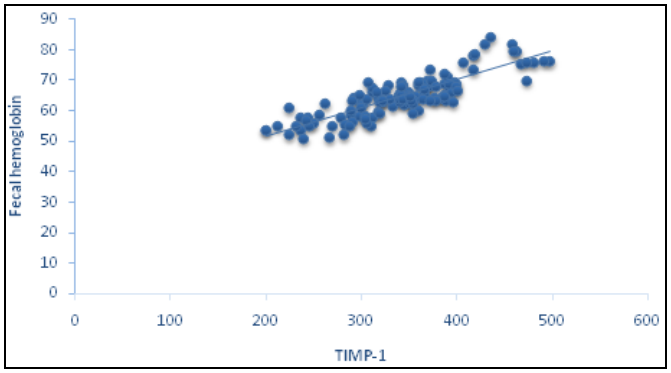

FIG. 4: CORRELATION BETWEEN FECAL TIMP-1 AND FECAL HEMOGLOBIN

DISCUSSION: Early diagnosis of CRC raises the success rate of cancer treatment significantly, and it is of utmost importance which parameters are to be investigated. Our study emphasizes parameters that predict to the diagnosis of CRC [13]. The present study predicts that the levels of CEA, CA 199 have significantly risen in CRC. When used together, they may serve as high specific tumor markers in CRC and may be proven of great diagnostic importance. It is observed that fecal hemoglobin can also serve as a screening test for diagnosis. The high specificity with increased positive predictive value may help screen and limit the number of false positives. TIMP-1 is a natural inhibitor of MMP and remodeling, it plays a vital role in anticancerogenic effect. Thus our study reveals that increased expression of TIMP-1 compared to normal counterparts may promote the accumulation of cancer-associated fibroblast within the colon cancer tissues. TIMP-1 may be connected with the degree of malignancy and survival rate of CRC patients [14]. Similarly, MIF is also involved in the fundamental process that controls cell proliferation, differentiation angiogenesis, tumor progression, and metastasis. Hence, the TIPM-1 and MIF protein assessment in the serum may be of great prognostic value [15].

TIMP-1 and MIF protein inhibit the ability of cancer cell to metastasize. In our study, we found a significant rise in these genetic markers compared to control. A further significant rise was observed when CRC patients were compared with recurrence patients. TIMP-1 and MIF protein appeared as highly sensitive markers in CRC in our findings. The high sensitivity revealed that excellent biomarkers in early detection of colorectal cancer, at the stage where it is localized and curable, will contribute substantially to reducing mortality of the disease [8, 17].

Further, we observed a significant increase in CRP and prolactin level with high sensitivity compared to controls. The elevated levels of CRP in colorectal cancer may be associated with tumor stage and recurrence, decreasing the survival rate [12, 16]. Circulating prolactin has been found to have a prognostic impact in colorectal cancer patients. Our study aimed to find out the importance of genetic markers in CRC. Our results and observation conclude that the evaluation of the markers like CEA, CA19-9, Fecal Hemoglobin has more specificity than sensitivity & efficiency. Whereas markers like CRP, MIF, TIMP-1 & prolactin are showing excellent sensitivity as compared to specificity. Hence, we conclude that monitoring these molecular markers in CRC patients will be of great diagnostic importance. This will be challenging in the clinical utility of the CRC patients and increase their survival rate. High sensitivity and specificity for colorectal cancer suggest that these biomarkers are highly suitable for early tumor detection and its better management.

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Ethics Approval and Consent to Participate: This study was approved by the Institutional Ethical Committee. The DCGI Registration no. ECR/782/Inst/MH/2015/RR-18 and each patient provided a written informed consent form to donate blood samples after diagnostic procedures.

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CONFLICTS OF INTEREST: The authors declared that they have no competing interests.

REFERENCE:

1. Ghuman S, Hemelrijck MV and Garmo H: Serum inflammatory markers and colorectal cancer risk and survival.British Journal of Cancer 2017; 116: 1358–1365.
2. Turano M, Delrio P and Daniela Rega: Promising Colorectal Cancer Biomarkers for Precision Prevention and Therapy. Cancers 2019; 11: 1932.
3. Lu Li and Xuhui Ma: Study on specificity of colon carcinoma-associated serum markers and establishment of SVM prediction model. Saudi Journal of Biological Sciences 2017; 24: 644–648.
4. Chunyan Meng1 and Xiaowei Yin: TIMP-1 is a novel serum biomarker for the diagnosis of colorectal cancer: A meta analysis. PLoS ONE 2018; 13(11): 020703.
5. Juan José Granados-Romero and Alan Isaac Valderrama-Treviño: Colorectal cancer: a review. International Journal of Research in Medical Sciences 2017; 5(11): 4667-4676.
6. B. Meyer and Chandrakanth Are: Current Status and Future Directions in Colorectal Cancer. Indian J Surg Oncol 2017; 017: 0711-9.
7. Varsha Kane and Mahesh C and Talpallikar: Study on risk factors for mortality and morbidity in patients undergoing

surgery for colorectal cancer. International Surgery Journal 2019; 6(8): 2766-2771.
8. Miana Gabriela Pop, Dana Monica Bartoș and Ana Maria Fiț: Thirty-day postoperative mortality in colon cancer surgery. A single-center analysis of 630 patients. International Jl of the Bioflux Society 2018; 10(2): 69-73.
9. Jun Jia, Pengfei Zhang and Miaomiao Gou: The Role of Serum CEA and CA19-9 in Efficacy Evaluations and Progression-Free Survival Predictions for Patients Treated with Cetuximab Combined with FOLFOX4 or FOLFIRI as a First-Line Treatment for Advanced Colorectal Cancer. Hindawi Disease Markers 2019; 6812045: 1- 8.
10. Shufang Ning, Wene Wei and Jilin Li: Clinical significance and diagnostic capacity of serum TK1, CEA, CA 19-9 and CA 72-4 levels in gastric and colorectal cancer patients. Journal of Cancer 2018; 9(3): 494-501.
11. Chunyan Meng, Xiaowei Yin and Jingting Liu: TIMP-1 is a novel serum biomarker for the diagnosis of colorectal cancer: A metaanalysis. PLoS ONE 2018; 13(11): 0207039.
12. Rei Mizuno, Kenji Kawada, Yoshiro Itatani and Ryotaro Ogawa: The Role of Tumor-Associated Neutrophils in Colorectal Cancer. Int J Mol Sci 2019; 20: 529.
13. Agnieszka Juchniewicz, Oksana Kowalczuk and Robert Milewsk: MMP-10, MMP-7, TIMP-1 and TIMP-2 mRNA expression in esophageal cancer. Biochimica Polonica 2017; 64(2): 295–299.
14. Thalia Pacheco-Fernández, Imelda Juárez-Avelar and Oscar Illescas: Macrophage Migration Inhibitory Factor Promotes the Interaction between the Tumor, Macrophages and T Cells to Regulate the Progression of Chemically Induced Colitis Associated Colorectal Cancer. Hindawi Mediators of Inflammation 2019; 2056085: 1-16.
15. Laura Soumoy, Nadège Kindt and Ghanem Ghanem: Role of Macrophage Migration Inhibitory Factor (MIF) in Melanoma. Cancers 2019; 11: 529.
16. Emily Berry, Stacie Miller and Mark Koch: Lower Abnormal Fecal Immunochemical Test Cut-Off Values Improve Detection of Colorectal Cancer in System-Level Screens. Clinical Gastroenterology and Hepatology 2020; 18(3): 647-653.
17. Das V, Kalita J and Pal M: Predictive and prognostic biomarkers in colorectal cancer: A systemic review of recent advances and challenges. Biomed Pharmacotherapy. 2017; 87: 8-19.

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