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RESEARCH ARTICLE

PROGNOSTIC SIGNIFICANCE OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) AND TISSUE INHIBITOR OF METALLOPROTEINASES-1(TIMP-1) IN COLORECTAL CANCER (CRC).

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ABSTRACT

Background: Colorectal cancer (CRC) is the third most common epithelial malignancy in the world. Colorectal cancer develops slowly from removable precancerous lesions, detection of lesion can reduce the incidence and mortality of this malignancy. Hence the present study was under taken to relate the TIMP-1 and MIF protein analysis that are non- invasive, which may enable us to detect colorectal cancer quite early. **Study design**: Case control study. **Aims and objectives:** The present study included total 120 patients diagnosed with colorectal cancer. They categorized as- Group I – included 10 patients with disease dissemination (stage I-IV with recurrences); Group II – 110 patients with non dissemination (stage I-IV without recurrences) and Group III – 120 age and sex matched healthy controls. To rule out the risk of colorectal cancer CEA, Ca 19-9 and fecal hemoglobin were analyzed. Genetic and prognostic markers like, TIMP-1 and MIF protein were analyzed. All statistical analysis was performed by using SPSS software and expressed as mean ± standard deviation, $p<0.005$ was considered as statistically significant. Correlations between the parameters were analyzed. **Results:** The concentration of CEA, Ca19-9, TIMP-1, MIF protein, level were significantly higher in patient group then in controls group ($p<0.005$). A significant correlation between CEA and CA19-9, TIMP-1 and MIF were observed. **Conclusions**: In this study the level of TIMP-1 and MIF expression exhibits an upper moderate diagnostic value in CRC, and this result could offer new approaches in the diagnosis, prognosis and monitoring risk factors in colorectal cancer.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common epithelial malignancy in the world. The incidence of colorectal cancer in India has increased over the past few decades, a fact which is perhaps related to the introduction of western foods.(16) The prognosis of colorectal cancer is closely related to cancer stage at the time of diagnosis, and approximately 30% of patients have distant metastasis when they are diagnosed. However, even when surgical treatment is performed during the early stages of colorectal cancer, approximately 30% of patients will develop recurrence and metastasis (Polatmd, 2014; Hugues Legendre, 2003). Presently the most used diagnostic approaches for CRC are the endoscopic procedures, such as colonoscopy, sigmoidoscopy and computed tomography (CT) colonography with high sensitivity and specificity for identifying polyps and cancer. However, the complexity of implementation, high cost, invasiveness, time consuming procedure, as well as requirements of repeating (3-5 years), have resulted in poor compliance rates (Varsha Kane, 2019; Garborg, 2013; Anderson, 2011) Some inexpensive and non-invasive methods, such as the fecal hemoglobin based screening, have also been developed, but with lower sensitivity and specificity. There is a great need to develop simpler, less invasive and accurate tests to improve the diagnosis of CRC (Mads Nikolaj Holten-Andersen, 2000; Thalia Pacheco-Fernández, 2019). The present study was undertaken to relate the prognostic significance of Macrophage Migration inhibitory factor (MIF) and Tissue inhibitor of metalloproteinases-1(TIMP-1) in colorectal cancer (CRC) that are non-invasive to enable us to detect CRC quite early. To diagnose colorectal cancer CEA, CA19-9 and fecal hemoglobin were analyzed.CEA and CA19-9 are closely related to curative effects and the prognosis of advanced colorectal cancer.

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For clinical validation genetic and prognostic markers like, TIMP-1, MIF and were analyzed. TIMP-1 (Tissue inhibitor of metalloproteinase) is natural inhibitor of matrix metalloproteinase, the enzymes involved in extracellular matrix maintenance and remodeling. 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 (Mads Nikolaj Holten-Andersen, 2004; Meng, 2008) 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 fundamental process that control cell proliferation, differentiation angiogenesis, tumor progression, and metastasis (Conroy, 2010; Li-Sheng Chen, 2013).

Our aim was to evaluate the relation of plasma TIMP-1 protein and MIF protein levels along with diagnostic 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 prolong the survival rate of CRC patients.

MATERIAL AND METHODS

Study area: The present study was undertaken to relate the Prognostic significance of Macrophage Migration inhibitory factor (MIF) and Tissue inhibitor of metalloproteinases-1(TIMP-1) in colorectal cancer (CRC)". This study was conducted in Ashwini Rural medical college, hospital and research centre Solapur. Maharashtra, over the period of 1 year after taking consent from subjects. Ethical clearance was obtained from the institutional Ethical Committee. In this study, we have evaluated a sample of 120 patients diagnosed with colorectal cancer. The patients were categorized as follows - Group I – included 10 patients with disease dissemination (stage I-IV with recurrences); Group II – included 110 patients with non dissemination (stage I-IV without recurrences) and Group III – included 120 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. Information about family history was obtained preoperatively through written questionnaires. Information on clinical stage, cancer recurrences, death and cause of death was obtained from surgical and oncological hospital records.

Study design: This is descriptive hospital based 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, lymphoproliferative disorders, collagen disorders, acquired immunodeficiency syndrome as well as malignancies other than colorectal cancer.

Collection and storage of sample: Desirable blood sample were collected aseptically. The serum and plasma was separated into clean and dry tubes and stored for determination of various biochemical parameters. Stool sample was collected for fecal hemoglobin determination.

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 two-site "sandwich" technique with two selected antibodies that bind to different epitopes of human hemoglobin.

To analyze clinical validation genetical and prognostic markers like TIMP-1 and MIF protein were analyzed, by sigma kit using Enzyme linked immunosorbent assay (ELISA) technique, and

Statistical analysis: The analysis of data was done by student t test and SPSS-17 software. The difference in mean values of various parameters were calculated and expressed in terms of p value. Correlations between the parameters were evaluated in all study subjects and calculated by Pearson's method.

RESULTS

The present study was undertaken to investigate the correlationship of various biochemical parameters and their effect in colorectal cancer patients. Table no.1shows a highly significant increased in 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. MIF and TIMP-1 protein as compared to controls ($p<0.001$). Table no. 2: Shows 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 genetical and prognostic markers like TIMP-1 and MIF Protein shows significant ($p<0.001$) rise in CRC patients of various stages. In the present study we included 25-35% of identified and diagnosed colorectal cancer patients who underwent operative surgical procedure and came with compliant of recurrence after 4 to 5 years. Table no. 3: Shows the diagnostic markers shows significant rise ($p<0.01$) in recurrence patients as compared to controls. Similarly prognostic and genetical markers also showed highly significant values ($p<0.001$) as compared to controls. Table 4: Shows that MIF and TIMP-1 proteins were positively correlated with other biochemical parameters.

DISCUSSION

Early diagnosis of CRC raises the successes rate of cancer treatment significantly and it is of upmost importance that which parameters are to be investigated. Our study emphasizes on parameters that predicts to diagnosis of CRC. In the present study we found that the levels of CEA, CA 199 have significantly increased in CRC and they may serve as high sensitivity tumor markers in CRC when used together.

Table no. 1, Showing various biochemical parameters in CRC and control patients.

Parameters	Case of CRC Mean ± SD	Control Mean ±SD	p value
1.CEA (ng/ml)	35.53 ± 12.27	2.2 ± 0.72	p<0.001***
2.CA 19-9(u/ml)	56.30 ± 7.31	22.68± 8.37	p<0.001***
3.Fecal Hemoglobin(ngHb/ml)	65.08 ± 6.9	31.13±13.85	p<0.001***
4.MIF protein(ng/ml)	254.96 ± 86.35	8.14±4.11	p<0.001***
5.TIMP-1 Protein(ng/ml)	343.27± 63.07	132.31±19.37	p<0.001***

$p<0.001^{***}$- Highly significant $p<0.01^{**}$- More significant $p<0.05^{*}$- Significant p>0.05- not significant

Table no.2. Demonstrates the various parameters in different stages of colorectal cancer with their p value

Parameters	CRC Cases stage wise distribution					p value
	STAGE0	STAGEI	STAGE II	STAGE III	STAGE IV	
CEA	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	41.62±1.41	45.45±2.34	55.40±3.90	60.05±3.74	64.84±3.11	$p<0.001^{***}$
Fecal Hemoglobin	54.36±1.35	56.71±3.06	63.51±3.65	66.61±3.05	77.75±3.59	$p<0.001^{***}$
MIF protein	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	224.44±19.47	262.26±23.11	318.92±18.06	371.32±17.81	452.51±29.11	$p<0.001^{***}$

Table no. 3 Summarizes levels of Biochemical parameters in CRC with recurrences and controls with their significant values

Parameters	Case of CRC with Recurrences Mean ± SD	Control Mean ±SD	p value
1.CEA	52.82± 7.01	2.2 ± 0.72	p<0.001***
2.CA 19-9	54.64±4.80	22.68± 8.37	p<0.01**
3.Fecal Hemoglobin	62.78±13.31	31.13±13.85	p<0.01**
4.MIF protein	261.72±37.61	8.14±4.11	p<0.001***
5.TIMP-1 Protein	302.87±39.64	132.31±19.37	p<0.001***

Table 4. Correlation of MIF and TIMP -1 protein with other biochemical parameters

	r value
1] MIF/ CEA	0.84
MIF/ CA199	0.73
MIF/FECAL Hb	0.74
MIF/ TIMP-1	0.82
2] TIPM-1/CEA	0.85
TIPM-1/CA199	0.78
TIMP-1/FECAL Hb	0.84

It is observed that Fecal hemoglobin can also serve as a screening test for diagnosis. TIMP-1 and MIF protein inhibits the ability of cancer cell to metastasize. In our study we found that there is significant rise in TIMP-1 and MIF samples between stages 0, I, II, III, and IV as compared to control. The highest level of expression was observed at stage IV. Further significant rise was observed when CRC patients were compared with recurrence patient. TIMP-1 is a glycoprotein involved in cell survival and tumorigenesis. It's 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 counter parts may promote accumulation of cancer associated fibroblast within the colon cancer tissues. TIMP-1 may be connected with degree of malignancy and survival rate of CRC patients. V.Surlin *et al* and Mitsuya Murashige *et al* . (2011) (Mitsuya Murashige1, 1996) also described higher TIMP-1 level as being associated with adverse prognosis. Mitsuya Murashige *et al* . said that TIMP-1 is independent predictor of worse survival. In additional to this we found there is statistically significant correlation between TIMP-1 with other biochemical parameters. Similarly MIF is known to be involved in the fundamental process that control cell proliferation, differentiation angiogenesis, tumor progression, and metastasis. MIF is a multifunctional cytokine whose dysregulation plays a pivotal role in a wide variety of inflammatory and autoimmune diseases.

MIF protein also implicated in the early stages of colorectal carcinogensis and it has been highly expressed in the gastrointestinal tracts and sporadic human colorectal adenomas (Anderson, 2011). MIF also facilitate the tumorgensis in the adenomatous polyposis and genetic deletion of MIF resulted in reduce tumor microvessel density. Xing- Xiang He and Ken Chen et.al. also observed significant MIF level increased in both serum and tumor specimens of patients with colorectal cancer compared to healthy controls. The author demonstrate that increased MIF expression correlates with an increase in both tumor differentiation and the extent of metastasis (lymph node) suggesting that MIF may play a crucial role in colorectal carcinogenesis and metastasis. Our findings strongly suggest that increased MIF expression is associated with enhanced proliferation of colon cancer cells in response to growth factors and loss of cell differentiation and lymph node metastases (Gong *et al* ., 2013; Lin Yang, 2018; Shu-Lin Chen, 2011) Hence the assessment of TIPM-1 and MIF protein in the serum may be of great prognostic value.

Conclusion

The present study elucidates that exceptionally high serum levels of TIMP-1 and MIF proteins may have strong association with poor prognosis of CRC. Hence the early detection of CRC by evaluation of diagnostic, and prognostic, genetic markers may serve as screening tools for CRC in

clinical practice. In conclusion our results may provide new approaches in the diagnosis, prognosis, and monitoring the risk factors of colorectal cancer, and may play a vital role in management of CRC.

Ethics approval and consent to participate: This study was approved by the Institutional Ethical Committee, and each patient provided written informed consent form to donate blood sample after diagnostic procedures.

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