SciMedCentral

Journal of Cancer Biology & Research

Research Article

Clinical Correlation of Clinical Stage of Carcinoma Colon with Tissue Expression of MMP 2, MMP 9 AND TIMP 2

Murali Mohan Soma*

Kamineni Hospital, India

***Corresponding author**

Murali Mohan Soma, Kamineni Hospital, Hyderabad, Telangana, India

Submitted: 23 September 2022

Accepted: 30 November 2022

Published: 30 November 2022

ISSN: 2373-9436

OPEN ACCESS

Keywords

- Matrix Metalloproteinase; Gelatinase; Carcinoma Colon; Immunohistochemistry; Tumour Prognosis

Abstract

Objectives: Metastasis of cancer is an intricate and well-coordinated interaction of numerous cellular and molecular factors. Matrix metalloproteinases are zinc dependent proteolytic enzymes that mediate destruction of extracellular matrix. MMP2 & 9 plays a major role in normal homeostasis at cellular level. These enzymes are often overexpressed in malignant tissues, resulting in degradation of collagen in basement membrane, leading to enhanced tumour growth, invasion and metastasis. This study is to find correlation between tissue expression of MMP 2, MMP 9 and TIMP 2 in cancinoma colon with stage of cancer, metastasis and survival.

Methods: Prospective observational study of 33 patients with carcinoma colon who underwent surgical treatment. Full thickness biopsy of tumour tissue was examined for tissue expression MMP 2, 9 and TIMP 2 and its correlation with cancer stage, metastasis and survival.

Results: Tissue expression of MMP 2, MMP 9 and TIMP 2 were significantly more than adjacent normal tissue. MMP 9 had significant correlation with tumour stage, but MMP 2 & TIMP 2 had no significant correlation. MMP 2, MMP 9 and TIMP 2 had no significant correlation with metastasis or survival.

Conclusions: Matrix metalloproteinases are enzymes at cellular level which play a key role in tissue homeostasis. Even though tissue expression of tumour tissue is more than adjacent normal tissue, they did not predict the clinical outcome (metastasis, survival) of the patients.

ABBREVIATIONS

MMP: Matrix Metalloproteinases; TIMP: Tissue Inhibitor of Matrix Metalloproteinase

INTRODUCTION

Carcinoma is one of the leading causes of death. It is largely preventable, with appropriate screening and awareness [1]. Pre-existing adenomas or carcinoma in situ progress gradually to frank malignancy, becomes invasive, and disseminates through lymphatic and/or vascular channels.

Metastasis is an intricate process requiring coordinated interaction of many tumour and host factors at both cellular and molecular levels. A critical early step in tumour dissemination is cleavage of basement membrane (Collagen IV) and destruction of extracellular matrix, liberating tumour cells into circulation.

Matrix metalloproteinases (zinc dependent proteolytic enzymes) mediate controlled destruction of extracellular matrix (Collagen IV, Laminin 5, etc) during homeostasis and participate in normal development, wound healing, inflammatory process, etc [2]. Numerous MMPs are identified in humans till date. Gelatinase A (MMP-2) and B (MMP- 9) play a main role in degradation of collagen IV of basement membrane, leading to invasion of basement membrane by tumours. MMP activity is regulated by tissue inhibitors of metalloproteinases (TIMPs) [3].

In malignant tissues, these enzymes are either over expressed or aberrantly regulated, resulting in widespread destruction of extracellular matrix and enhanced tumour growth, angiogenesis, tumour invasion and metastasis along with cell adhesion molecules, cytoskeletal proteins, growth factors [4]. A direct association has been proposed between the increased expression of these MMPs and negative clinical outcome.

Colorectal cancer is the second commonest cancer in India. Surgical resection is the best treatment option. 40% of them undergo curative resection. They develop local, regional, or distant tumour recurrence or relapse [5].

S. Papadopoulou et al. [5], Zeljko Sundov et al. [6], have demonstrated that expressions of these MMPs are correlated with stage of the tumour and metastasis of colorectal cancers.

MATERIALS

Prospective, observational study of 33 consecutive patients with proved colorectal cancer underwent surgery in the

department of General surgery, Kamineni hospital, Hyderabad from October 2009 to October 2011.

Inclusion criteria

All stages (modified Duke's and TNM staging) of histologically proved colorectal cancers who underwent surgical treatment were considered.

Exclusion criteria

Synchronous malignancies, Colon disease with benign pathology, Cancers treated with palliative support

METHODOLOGY

Biopsy tissue

Full thickness biopsies from edge of the tumour, preserved in formalin, routine histopathological examination and Immunohistochemical staining for tissue expression of MMP2, MMP 9 and TIMP2 by standard Avidin – Biotin peroxidise Complex technique.

Reagents

Novocastra™ Lyophilised Mouse monoclonal antibody, Matrix Metalloproteinase – 2 {Product code: NCL-MMP2-507, Clone: 17B11, IgG2a, κ), Matrix Metalloproteinase – 9, Product code: NCL-MMP9-439, Clone: 15W2. Antigen used for immunisations: Prokaryotic recombinant protein corresponding to 132 amino acids of C-terminus of the matrix metalloproteinase 2 molecule.

Hybridoma partner

Mouse myeloma (p3-NS1-Ag4-1).

Preparation

Lyophilised tissue culture supernant containing 15mM sodium azide, reconstituted with sterile distilled water.

Novolink polymer detection system, Product no.: RE7150-K.

Principle of use

Based on polymerisation technology to prepare polymeric HRP – linker antibody coagulates.

Positive & Negative tissue control protocol was strictly maintained.

Immunohistochemistry scoring system

Slides were examined under bright field light microscopy, 400 x magnifications by 2 different pathologists. Positivity for the expression is defined by membrane staining above the background level as negative – 0, weak intensity – +, mild - ++, moderate - +++, strong- ++++ [2, 5]. When discrepancy in opinion arises, slides were re-evaluated and scored.

Statistical analysis

Tissue expression of MMP-2, MMP-9 TIMP-2 in tumour tissue and normal tissue were compared with Wilcoxon rank sum test. Comparison with tumour staging was done using Correlation coefficient, univariate analysis and multiple regression tests. And outcome parameters like metastasis and survival with correlation coefficient using "Medcalc" 12.1.0.0.

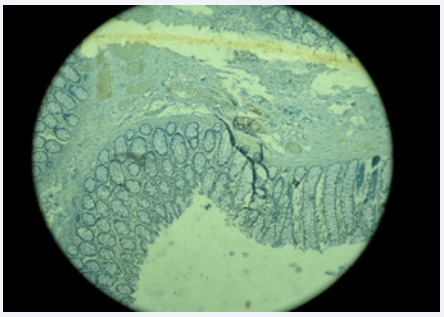

Figure 1 0 – Negative Staining.

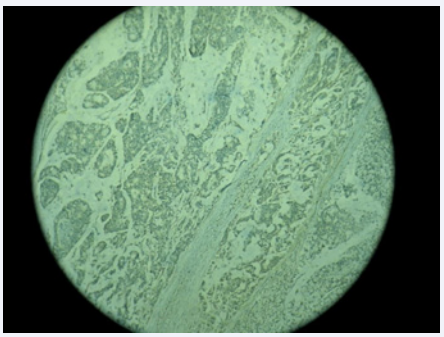

Figure 2 1+ Weak Staining.

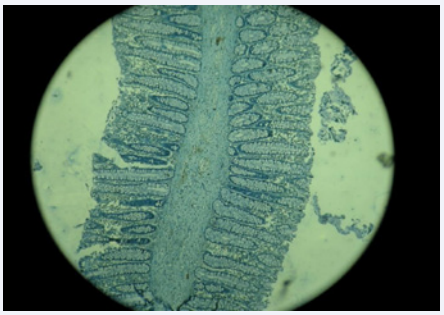

Figure 3 2+ Mild Staining.

RESULTS

18 male (54.55%) and 15 female (45.45%) patients with age distribution from 24 to 79 (mean - 55 ± 13.17) years.

Tissue Expression of MMP-2, MMP-9 & TIMP-2: Tissue expression of MMP 2 levels were analysed with Wilcoxon Ranksum test (paired samples): Number of positive differences -5, negative differences -19, Z score -2.785714, P= 0.0053. Level of expression is significant.

Tissue expression of MMP 9 levels were analysed with Wilcoxon test (paired samples): Number of positive differences -2, negative differences -17, Z score -11.00, P= 0.0002. Level of expression is significant.

Tissue expression of TIMP 2 levels were analysed with Wilcoxon test (paired samples): Number of positive differences

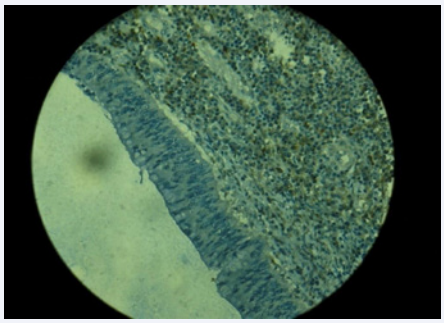

Figure 4 3+ Moderate Staining.

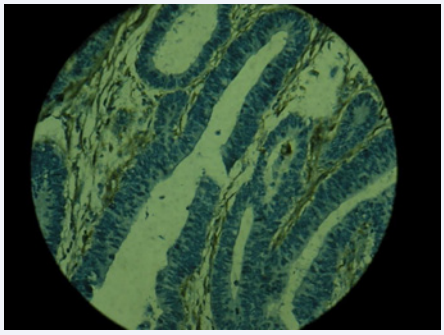

Figure 5 4+ Strong Staining.

-4, negative differences -16, Z score -28.00, P= 0.0027. Level of expression is significant.

CORRELATION COEFFICIENT

MMP-2, 9 & TIMP-2 vs. Stage of Cancer

Correlation coefficient was measured to assess strength of the linear relationship between staging (y-axis) and tissue expression of various MMPs and TIMP (x-axis) (Table 1).

By taking tissue expression of MMP-9 on x-axis and various Dukes staging on y—axis, the correlation coefficient was measured and found to be statistically significant (p = 0.0354), whereas tissue expression of MMP-2 & TIMP-2 were not statistically significant (p = 0.2269 & 01444),

MMP-2, 9 & TIMP-2 vs. Metastasis: Correlation coefficient of tissue expression of MMP 2, 9 & TIMP 2 vs metastasis were not statistically significant (p= 0.9879, 0.7507, 0.5807) (Table 2).

MMP-2, 9 & TIMP-2 vs. Survival: Correlation coefficient of tissue expression of MMP 2, 9 & TIMP 2 vs survival were not statistically significant (p= 0.9527, 0.5522, 0.3193) (Table 3).

Multiple regression analysis: However, when all factors (confounding) were taken into account, none of the individual parameter is correlating with the stage of the disease (Table 4,5).

Only tissue expression of MMP-9 is correlating with Stage of the tumour. Tissue expression of MMP-9 is not correlating with metastasis or outcome.

Whereas the tissue expression of various MMP-2 levels and TIMP-2 levels were not correlating with the outcome i.e., metastasis, survival, when analysed by using Univariate analysis, multivariate analysis and correlation coefficient.

DISCUSSION

Dynamic equilibrium exists between the various enzymes involved in the synthesis and degradation of extracellular matrix components to maintain tissue homeostasis [2]. Disruption of this balance results in tumour growth and metastasis. Activated forms of MMP 2 and 9 in higher concentrations results in colorectal cancer invasion and metastasis [7,8] S. Papadopoulou et al., observed higher concentrations of Gelatinase-A (MMP-2) in cancer tissue than corresponding normal colonic mucosa. Positive correlation between MMP-2 antigenic concentration and clinicopathological parameters – Grade, Duke's stage, but not Lymph node involvement. Staining intensity increased from

Table 1: Linear relationship between staging (y-axis) and tissue expression of various MMPs and TIMP (x-axis).

With Staging	Correlation Coefficient (r)	P	95% confidence interval
MMP 2	0.2162	0.2269	-0.1373 to 0.5208
MMP 9	0.3675	0.0354	0.02772 to 0.6312
TIMP 2	0.2597	0.1444	-0.09179 to 0.5537

Table 2: Correlation coefficient of tissue metastasis expression.

With Metastasis	Correlation Coefficient (r)	P	95% confidence interval
MMP 2	0.002791	0.9879	-0.3462 to 0.3511
MMP 9	-0.05844	0.7507	-0.3990 to 0.2963
TIMP 2	0.1014	0.5807	-0.2563 to 0.4348

Table 3: Correlation coefficient of tissue survival expression.

With Survival	Correlation Coefficient (r)	P	95% confidence interval
MMP 2	0.01073	0.9527	-0.3338 to 0.3527
MMP 9	0.1153	0.55229	-0.2374 to 0.4411
TIMP 2	0.1788	0.3193	-0.1752 to0.4920

Table 4: Correlation coefficient of tissue survival expression.

Coefficient of determination R^2	0.2231
R^2-adjusted	0.04386
Multiple correlation coefficient	0.4724
Residual standard deviation	1.0916

Table 5: Stage of the Disease.

Regression Equation Independent variables	Coefficient	Std. Error	P
(Constant)	1.4388		
Tissue expression of MMP-2 tumour	0.1752	0.2861	0.5456
Tissue expression of MMP-2 normal	-0.2570	0.3209	0.4305
Tissue expression of MMP-9 tumour	0.3521	0.2294	0.1369
Tissue expression of MMP-9 normal	0.3208	0.3605	0.3817
Tissue expression of TIMP tumour	0.1470	0.2623	0.5800
Tissue expression of TIMP normal	-0.04011	0.3172	0.9003

adenoma to adenocarcinoma. Similar findings were observed in our study i.e., significant expression of MMP-2 is noticed in tumour tissue when compared with normal tissue (p= 0.0053), but not correlating with stage or lymph node involvement p=0.22 [5].

Yoshito Matsuyama, Sonshin Takao et al., studied relationship between MMP–2 and MMP-9 enzymatic activity by Gelatin zymography and liver metastasis using xenograft tumours. Type IV collagenase activity and TIMP-2 levels were higher in colorectal and pancreatic cancers with liver metastasis than in cancers without liver metastasis. They concluded that MMP-2, MMP-9 and TIMP-2 expression in tumours with liver metastasis may be relevant to tumour invasion and metastasis. But in our study, MMP-2, MMP-9 or TIMP did not correlate with metastasis or survival which is against the above study [3].

Stephanie Curran, Sinclair R Dundas et al, MMP/TIMP phenotype identified involvement in tumour invasion and poor prognosis in colorectal cancers. These enzymes and their physiologic inhibitors, act in a coordinated manner to form an integrated system. Studied MMP-1, 2, 3, 7, 9, 13, MT1-MMP and MT2-MMP, TIMP-1, 2, 3 in Duke's stage C colorectal cancers using immunohistochemical score based on intensity of immunoreactivity and proportion of immunoreactive cells. There was no significant difference between the 2 groups in distribution of tumour site, degree of differentiation, nodal status, patient age, gender. MMP-2, MMP-3, MMP-9, TIMP-2 and TIMP-3 with significantly altered expression in group 2 compared with group 1. In all cases, expression of these MMPs and TIMPs was reduced in tumours of group 2 compared with group 1 tumours. Survival analyses showed that TIMP-2, MMP-2, and MMP-3 demonstrated significant differences in outcome between group 1 and group 2. Whereas in our study, statistically significant difference in expression of MMPs and TIMP in tumour tissue and normal tissue, but only tissue expression of MMP-9 correlating with tumour staging. Others did not correlate with stage, metastasis or survival of colorectal cancer [4].

Zeljko Sundov et al., Immunohistochemically detected high expression of MMP-2 as predictor of poor prognosis in Duke's B colon cancer. They studied expression of MMP-2 semi quantitatively. Univariate analysis showed High histological grade, tumours, vascular invasion, male sex, age> 60 years associated with shorter survival in Duke's B colon cancer. Multivariate analysis showed MMP-2 over expression and vascular invasion are associated with shorter survival. MMP-2 is independent indicator of shorter survival. Whereas the present study did not reveal statistically significant correlation with survival of colorectal cancer [6].

P Ring, K Johansson et al., investigated immunohistological staining patterns of MMP-2, MMP-9 and TIMP-2 in full thickness biopsy, to predict tumour stage and survival in colorectal cancer patients using MMP-2, MMP-9 and TIMP-2. Positive expression of MMP-2 restricted to tumour epithelium, no staining of interstitial stroma or basement membrane. Intensity and distribution of MMP-2 staining did not correlate with Dukes stage. Tumour epithelium, basement membrane, interstitial tissue, all were negative for MMP-9. Positive expression of TIMP-2 was found in basement membrane ($p<0.01$), stroma ($p<0.05$) and sub glandular region ($p<0.05$) in localised tumour than in rumour with regional or distant metastasis. Correlation with survival time reached near significance ($p<0.07$). Neither pattern correlated with tumour differentiation, death. MMP-2 expression was independent of tumour stage. In our present study, the tumour marker expression did not correlate with metastasis which is against the above study. The tumour marker expression did not correlate with survival which in correlating with the above study [9].

Mitsuya Murashige, Masaki Miyahara et al., studied MMP-2, MMP-9, TIMP-1, TIMP-2 expression in colorectal cancer with liver metastasis. They found that expression of TIMP 1 and TIMP 2 are closely correlated with progression of human colorectal cancer. The levels of expression of MMP-2, MMP-9, TIMP-1 were significantly higher in primary colorectal cancer than in adjacent normal tissue. They studied Enhanced expression of TIMP in human colorectal tumours. MMP 2 and 9 play important roles in tumour invasion and metastasis. Higher levels of TIMP-1 mRNA positively correlated with lymph node metastasis and 5 year survival, higher levels of TIMP-1, TIMP-2 mRNA were positively correlated with Dukes classification. Expression of TIMP- 1, 2 closely correlated with progression of human colorectal cancer, which is well correlated with our present study [10]. ET Waas et al., observed significant correlation between Pro MMP 9 levels and tumour location. Rectal tumours showed lowest pro MMP 9, left colon tumours showed highest. Neither age, tumour size, histopathological differentiation, tumour cell mucin, T stage, lymph node involvement correlated with gelatinolytic MMP activity. In normal mucosa, male patients displayed higher levels of Pro MMP 2 than females. In present study, though statistically significant difference of expression of various MMPs and TIMP were observed, but it appears that MMP-2 expression is slightly more in males, MMP-9 expression is slightly more in females and TIMP is equal in both [11].

Lukas JAC Hawinkels, Kim Zuidwijk et al. Studied VEGF release by MMP-9 mediated heparin sulphate cleavage induced colorectal cancer angiogenesis undergoing resection for colorectal cancer and found significant correlatio. VEGF and MMP-9 levels significantly enhanced in tumours ($p<0.0001$) and were mutually correlated ($R=0.405$, $p<0.0005$). MMP-2 levels were not enhanced compared to normal mucosa, did not correlate with VEGF. MMP-2 and MMP-9 were weakly correlated with each other ($R=0.250$, $p=0.019$). MMP-9 correlated with significantly with circulating VEGF concentration ($R=0.379$, $p=0.009$). They concluded that Neutrophil derived MMP-9 release biologically active VEGF from extracellular matrix of colon cancer cells by cleavage of Heparan sulphate. VEGF plays a major role in angiogenesis, which is crucial for progression of carcinoma colon. Significant correlation between MMP-9 and VEGF, but not with MMP-2 [12].

Satoshi Takeha, Yoshide Fujiyama, et al., studied Stromal expression of MMP-9 and Urokinase receptor is inversely associated with liver metastasis and infiltrating growth in human colorectal cancer. Their observations were significant correlation between μ-PAR and MMP-9 ($R=0.296$, $p<0.001$), MMP-9 and CD68 ($R=0.287$, $p<0.001$). MMP-9 (gelatinase B) and urokinase-type plasminogen activator receptor (u-PAR)

are predominantly expressed by immune/inflammatory cells in human colorectal cancers, are inversely related to cancer cell invasion and metastasis. Whereas in our 5 metastatic cases, there is no significant inverse relation between the tumour invasion and MMP expression [13].

Takanobu yamada et al., studied clinicopathological significance of relative expression of MMP 7, MMP 9, MMP 13, and TIMP 1, β-actin genes in colorectal cancers. Expression levels are higher in cancer tissue than adjacent normal tissue, correlated with liver metastasis. MMP 13 is a useful predictor of liver metastasis in colorectal cancer [14].

Ida Pucci Minafra, Salvatore Minafra et al., studied zymographic analysis of circulating and tissue forms of colon carcinoma gelatinase A (MMP 2) and B (MMP 9) separated by mono- and two- dimensional electrophoresis. Extracellular regulation is achieved mainly through the balance between proenzyme activation and inhibition, which altered in cancer patients. MMP 2, 9 are overproduced in carcinomas. Several oligomeric circulating and tissue forms of MMP 9 are preferentially found in oncologic sera. Activated forms of MMP 2, 9 are uniquely present in primary tumour extracts confirming the involvement of tissue microenvironment in gelatinase activation and function [15].

Zhao-Shi Zeng et al., explored the relationship between MMP-2 and 9 activities and pattern of type IV collagen during human colorectal tumorigenesis. Type IV collagen expression was continuous in normal mucosa, adenoma, carcinoma in situ, Limited/absent type IV collagenin100% and 23% of colorectal cancers with and without metastases. By Double immunostaining, MMP 9 protein expression was localised within areas of limited type IV collagen staining. Type IV collagen staining was greatest in areas devoid of MMP9 expression. They concluded that loss of basement membrane type IV collagen in colorectal tumorigenesis and are associated with elevation of active forms of MMP 2, 9 expressions. Type IV collagen degradation correlates with local elevation in MMP-9 expression, supporting the notion that control of type IV collagen degradation by preventing the type IV collagenase activation may be beneficial in preventing human colorectal tumour progression. But in our study, MMP-9 expression correlating with stage of the tumour. Our study is against this study, as MMP expression does not predict metastasis or survival [16].

CONCLUSION

The present study is a group of 33 patients of all stages of colorectal cancer. This study is to use molecular markers to predict the stage of the colorectal cancer and outcome.

1. The statistically significant tissue expression of MMP-2, MMP-9 and TIMP- 2 were observed in tumour tissue when compared with normal tissue with p values 0.0053, 0.0002, 0.0027 respectively.
2. The tissue expression of MMP-9 is well correlating with the advancing stage of the disease. p= 0.0354.
3. The tissue expression of MMP-2 and TIMP-2 were not correlating with the stage of the disease with p values 0.22, 0.144 respectively.
4. None of the studied variables (MMP-2, MMP-9 and TIMP-2) were predictive of outcome (recurrence, metastasis and mortality). P> 0.05 in all.

ACKNOWLEDGEMENTS

I acknowledge my professors, Dr. Satyanarayana G, Dr. Raghavendra Prasad and my mentor Dr. Vimalakar Reddy for guiding me, helping me throughout my study.

REFERENCES

1. Nick Mulcahy. Cancer to become leading cause of death worldwide by 2010. World cancer report - International Agency for Research on Cancer (Medscape medical news). 2008.
2. Angela G. Mysliwiec, Deborah L. Ornstein: Matrix metalloproteinases in colorectal cancer. Clin Colorectal Cancer. 2002; 4: 208-19.
3. Yoshito Matsuyama, Sonshin Takao, Takashi aikou. Comparison of matrix metalloproteinase expression between primary tumours with or without liver metastasis in pancreatic and colorectal carcinomas. J Surg Oncol. 2002; 80: 105-110.
4. Stephanie Curran, Sinclair R Dundas, Jenny Buxton, Matthew F Leeman, Robin Ramsay, Grame I Murray. Matrix metalloproteinase/ Tissue inhibitor of matrix metalloproteinase phenotype identifies poor prognosis colorectal cancers. Clin Cancer Res. 2004; 10: 8229-34.
5. S Papadopoulou, A Scorilas, N Arnogianaki, B Papapanayiotou, A Tzimogiani, N Agnantis, et al. Expression of Gelatinase-A (MMP-2) in human colon cancer and normal colon mucosa. Tumour Biol. 2001; 22: 383-89.
6. Zeljko Sundov, Snjezana Tomic, Katarina Vilovic, Nena Kunac, Marija Kalebic, Losko Bezic. Immunohistochemically detected high expression of matrix metalloproteinase-2 as predictor of poor prognosis in Duke's B colon cancer. Croat Med J. 2008; 49:636-42.
7. Lance A Liotta, Elise C Kohn. Invasion and metastasis. Holland-Frei cancer medicine 5th edition, 2000; chapter 8.
8. Mary Jo Murnane, Jinguo Cai, Sania Shuja, David Mc Aneny, Veronica Klepeis, John B Willet. Active MMP-2 effectively identifies the presence of colorectal cancer. Int J Cancer. 2009; 125: 2893-902.
9. P Ring, K Johanson, M Hoyhtya, K Rubin, G Lindmark. Expression of tissue inhibitor of metalloproteinases TIMP-2 in human colorectal cancer - a predictor of tumour stage. British Journal of Cancer. 1997; 76: 805-11.
10. M Murashige, M Miyahara, N Shiraishi, T Saito, K Kohno, M Kobayashi. Enhanced expression of tissue inhibitors of metalloproteinases in human colorectal tumours. Jpn J Clin Oncol. 1996; 26: 303-09.
11. ET Waas, RMLM Lomme, J DeGroot, Th Wobbes, T Hendriks. Tissue levels of active matrix metalloproteinase-2 and -9 in colorectal cancer. Br J Cancer. 2002; 86: 1876-83.
12. Lukas JAC Hawinkels, Kim Zuidwijk, Hein W Verspaget, Eveline S M de Jonge-Muller, Wim van Duijn, Valérie Ferreira, et al: VEGF release by MMP-9 mediated heparin sulphate cleavage induces colorectal cancer angiogenesis. Eur J Cancer. 2008; 44: 1904-13
13. S Takeha, Y Fujiyama, T Bamba, T Sorsa, H Nagura, H Ohtani. Stromal expression of MMP- 9 and urokinase receptor is inversely associated with liver metastasis and with infiltrating growth in Human colorectal cancer: A novel approach from immune/inflammatory aspect. Jpn J Cancer Res. 1997; 88: 72-81.
14. Takanobu Yamada, Takashi Oshima, Kazue Yoshihara, Shuzo Tamura, Amane Kanazawa, Daisuke Inagaki, et al. Over expression of MMP-13

gene in colorectal cancer with liver metastasis. Anticancer Res. 2010; 30: 2693-99.

15. Ida Pucci-Minafra, Salvatore Minafra, Giampiero La Rocca, Marilisa barranca, Simona Fontana, Giuseppina Alaimo, et al. Zymographic analysis of circulating and tissue forma of colon carcinoma gelatinase A (MMP-2) and B (MMP-9) separated by mono- and two-dimentional electrophoresis. Matrix Biol. 2001; 20: 419-27.

16. Zhao-Shi Zeng, Alfred M Cohen, Jose G Guillem. Loss of basement membrane type IV collagen is associated with increased expression of metalloproteinases 2 and 9 (MMP-2 and MMP-9) during human colorectal tumourigenesis. Carcinogenesis. 1999; 20: 749-55.

Cite this article

Soma MM (2022) Clinical Correlation of Clinical Stage of Carcinoma Colon with Tissue Expression of MMP 2, MMP 9 AND TIMP 2. J Cancer Biol Res 9(1): 1134.