Govaresh/ Vol. 24, No.3, Autumn 2019; 172-178.

Original Article

The Expression Levels of Matrix Metalloproteinases Inhibitors TIMP1, TIMP2 and Matrix Metalloproteinases MMP2, MMP9 in Iraqi Patients Diagnosed with Colon Cancer

Noah A. Mahmood [1], Zaynab S. Abdulghany [2,*], Israa M. Al-Sudani [3]

[1] PhD, Enzymology, Molecular Biology Department, Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University, Baghdad, Iraq
[2] PhD, Biotechnology, Vice of Molecular Biology Chairman, Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University, Baghdad, Iraq
[3] Assistant Professor, FICMS Pathology, Medical College, Ibn Sina University, Baghdad, Iraq

ABSTRACT

Background:
Colon cancer is the third most common cancer worldwide, responsible for more than half a million deaths every year. Metalloproteinases (MMP2 and MMP9) participate in tumor invasion and metastasis because of their ability to degrade the extracellular matrix (ECM).

Materials and Methods:
The expression levels of MMP2 and MMP9 as well as tissue inhibitor metalloproteinases TIMP1 and TIMP2 were determined by immunohistochemistry in 42 Iraqi patients diagnosed with colon cancer as well as in 18 benign tissues.

Results:
Immunohistochemistry revealed that the protein expression levels of MMP2 ,MMP9, TIMP1 and TIMP2 increased by 83%, 72%, 64%, and 57%, respectively, in patients with colon cancer tissues compared with 19%, 28%, 23%, and 16%, respectively, in benign tissues.

Conclusion:
MMP2, MMP9, TIMP1 and TIMP2 expression levels in Iraqi patients with colon cancer could be used as potential markers that can predict tumor behavior, progression, and prognosis.

Keywords: Iraqi patients, Colon cancer, MMP2/9, TIMP1/2, Immunohistochemistry.

please cite this paper as:

Mahmood N. A., Abdulghany Z. S., M. Al-Sudani I. The Expression Levels of Matrix Metalloproteinases Inhibitors TIMP1, TIMP2 and Matrix Metalloproteinases MMP2, MMP9 in Iraqi Patients Diagnosed with Colon Cancer. *Govaresh* 2019;24:172-178.

Corresponding author:
Zaynab S. Abdulghany, PhD
Behind Yarmook Teaching Hospital, Street 23, Sec. 603, Alqadissiya Q., Baghdad, Iraq.
Telefax: + 96 478 12218857
E-mail: Zaynab.saad@iccmgr.org

Received: 09 Jul.2019
Edited: 14 Sep 2019
Accepted: 15 Sep 2019

INTRODUCTION

In Iraq, the incidence of colon cancer ranks 3rd among the most commonly occurring cancers-after breast and lung cancers-according to the Iraqi cancer registry 2018 (1). The key role of metastasis is achieved by degradation of extracellular matrix (ECM) allowing tumor cells to invade local tissues, enter into the bloodstream, and then cause the development of a new tumor in other organs (2). Under normal conditions, matrix metalloproteinases (MMPs) play an important role in tissue remodeling, ovulation, wound healing, and angiogenesis (3). High MMP expression levels participate in disease development

such as cancer (4). MMPs are involved in the degradation of ECM, and play important roles in breast cancer invasion and metastasis (5). They are synthesized and released as zymogens, which can be activated by serine proteases and/or other metalloproteinases (4). The activities of MMPs are regulated by tissue inhibitor metalloproteinases (TIMPs) and stimulated by different microenvironment signaling molecules such as growth factors and cytokines (6).

Matrix metalloproteinases 2 and 9 (MMP2 and MMP9), also called gelatinases A and B (or type IV collagenases), were responsible for the degradation of basement membranes. Also they were implicated in cancer invasion and metastasis because of their ability to degrade collagen IV, which is the main component scaffold protein of basal membrane that plays an important role in separating epithelial cells from adjacent stroma (7, 8). When this protein is degraded, colon cancer cells are prone to infiltrate (9). The elevated MMP2 and MMP9 expression levels in cancer provide canals through which the cancer cells spread, thereby resulting in metastasis. Therefore, the expression of high levels of MMP2 and MMP9 is associated with cancer cell invasion, metastasis, and worse prognosis (10).

In this study, the expression levels of MMP2 and MMP9 and of TIMP1 and TIMP2 in Iraqi patients diagnosed with colon cancer as well as in benign tissues were examined by immunohistochemistry.

MATERIALS AND METHODS

1. Patients and tumor characteristics

This study involved 42 patients with colon cancer as well as 18 benign tissues surgically harvested from patients admitted to Alkarama Teaching Hospital in Baghdad city and also from a private laboratory, between November 2016 and April 2017. All patients with colon cancer recruited in this study had no chemotherapy or radiation therapy. The mean age of the patients was 53 years (range was 44 to 69 years). Fresh colon cancer tissues, as well as benign tissue samples, were collected and fixed in 10% formalin and embedded in paraffin to be used in immunohistochemical staining.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

2. Immunohistochemical staining

The protein expression levels of MMP2, MMP9, TIMP1, and TIMP2 were evaluated immunohistochemically. Four millimeters of the paraffin-embedded sections were cut and mounted on positively charged glass slides, deparaffinized in xylene, and rehydrated using an ethanol solution. The slides were incubated overnight at 4°C with the primary antibodies: anti-MMP2 antibody (M2420-52Q US-bio, USA), anti-MMP9 antibody (M2420-01R US-bio, USA), anti-TIMP1 (031291 US-bio, USA), and anti-TIMP2 (502589 US-bio, USA). Each slide was incubated for 30 minutes at room temperature with horseradish peroxidase conjugate detection reagent (DAKO biotechnology, USA) and with complex avidin-biotin. The slides were visualized using diaminobenzidine (DAB) and countered with hematoxylin, and dehydrated first with graduated ethanol and then with xylene. Finally, the slides were mounted with a water-free mounting medium (DPX) and then analyzed using a light microscope at 400x. Placenta tissue was used as a positive control. The negative control was treated with all of the above steps except the incubation with the primary antibody.

Evaluation of the immunohistochemical staining was carried out blindly to the patients' data and pathological features. The percentage and intensity of the staining were considered in this study. Normal cells that present in the whole tissue were scored as score 0 (no positive staining), score 1 (1-10%), score 2 (11-50%), and score 3 (51-100 %). The positive intensity was rated as 0 (none), weak positive, moderate, and strong positive. Both 0 and 1 scores were considered as low expression, whereas score 2 and score 3 were considered as high expression. An expression of each gene over 10% was considered positive.

3. Statistical Analysis

The comparison between colon carcinomas and benign tissues was performed by using Prism GraphPad version 6 (GraphPad Software Inc., San Diego, CA, USA). A Chi-square (χ^2) statistic was used to investigate whether expression values differed between colon cancer tissues and benign tissues. A value of $p < 0.05$ was considered as statistically significant.

Table 1: MMP2, MMP9, TIMP1, and TIMP2 expression levels in colon cancer tissues (n = 42) and benign tissues (n = 18).

Parameters	Tissue Types	High expression n (%)	Low expression n (%)	*p* value
MMP2	Cancer	32 (76.2%)	12 (23.8%)	0.001*
	Benign	6 (33.6%)	12 (66.4%)	
MMP9	Cancer	34 (81%)	8 (19%)	0.001*
	Benign	5 (27.7%)	13 (72.3%)	
TIMP1	Cancer	31 (73.8%)	11 (27.2%)	0.0001*
	Benign	4 (23.0%)	14 (77%)	
TIMP2	Cancer	29 (69%)	13 (31.0%)	0.0001*
	Benign	3 (17.0%)	15 (83%)	

*Mean significant expression of proteins in tissues with high probability rate.

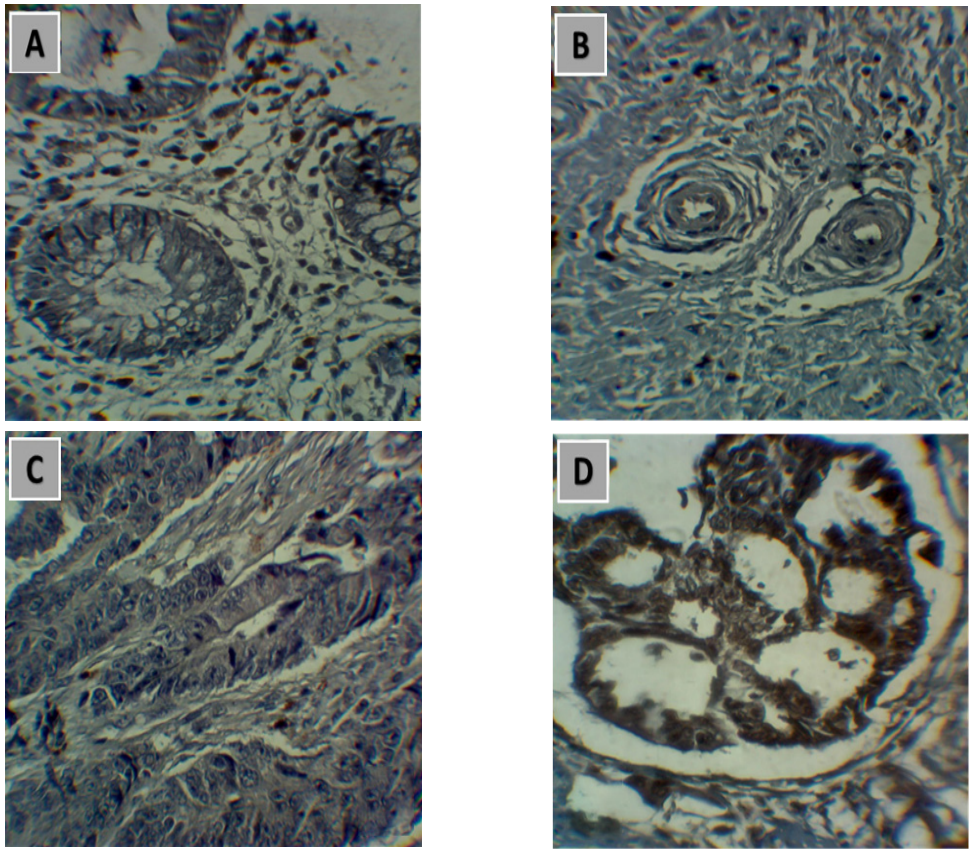

Fig. 1: Immunohistochemistry staining of MMP2 protein in colon cancer as well as in benign tissues (400X). (A) Benign tissues with slight MMP2 protein expression. (B) Colon cancer tissue stage I with positive MMP2 protein expression level. (C) Stage II colon cancer with moderate MMP2 protein expression level. (D) Stage III colon cancer with increasing MMP2 expression in the nuclei of cancer cells.

RESULT

Protein expression levels of MMP2, MMP9, TIMP1, and TIMP2 as determined by immunohistochemistry:

While MMP2 was mainly located in the epithelial cells of benign tissues, it was located in the cytoplasm and nucleus of colon cancer cells. The positive expression of MMP2 was observed in 71.9% of cancerous tissues, compared with 28.5% of benign tissues (p = 0.001, figure 1, table 1). Immunohistochemical analysis of colon cancer tissues showed high expression of MMP9 in the cytoplasm of colon cancer tissues compared with

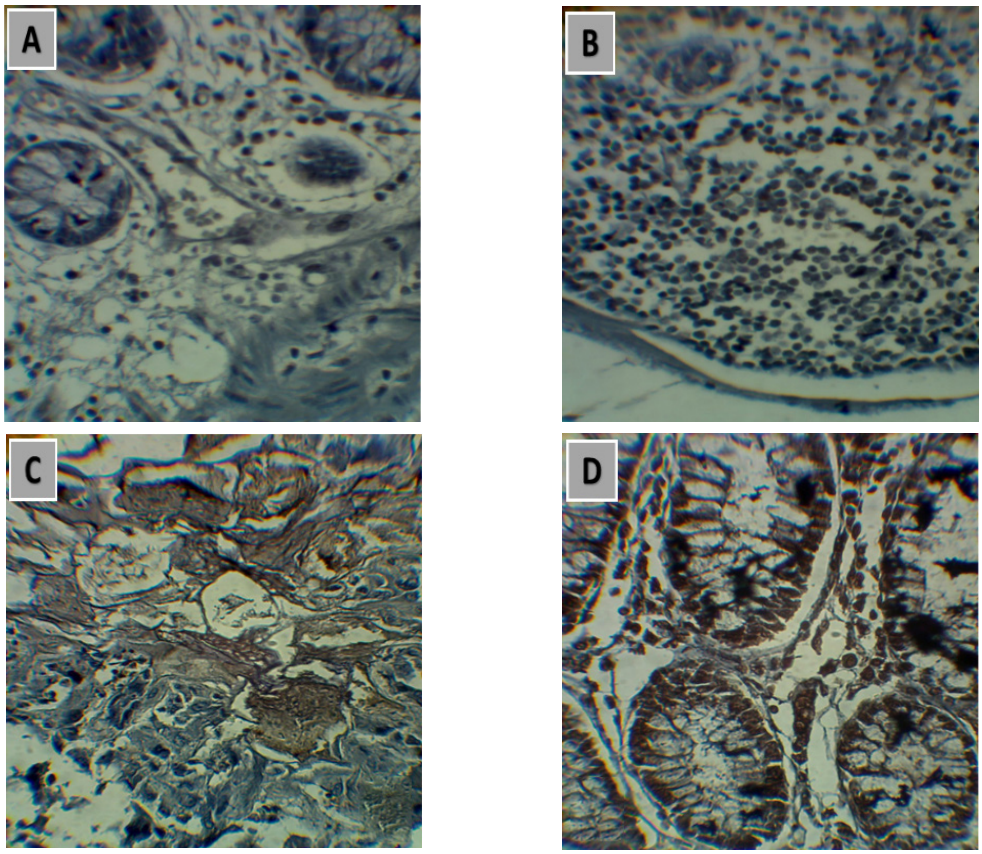

Fig. 2: Immunohistochemistry staining of MMP9 protein in colon cancer as well as in benign tissues (400X). (A) Benign tissues with slight MMP9 protein expression. (B) Stage I colon cancer tissue with positive MMP9 expression. (C) Stage II colon cancer tissue showing significant MMP9 protein expression. (D) Stage III colon cancer tissue with increasing MMP9 protein expression level.

that in benign tissues. MMP9 expression was found in 64.1% of the cases in cancer tissues and 23.8% of benign tissues ($p = 0.001$, figure 2, table 1). Regarding TIMP1, immunostaining of colon tissues revealed high expression in the cytoplasm and cell membrane of cancerous tissues. TIMP1 expression was found in 82.8% of colon cancer tissues compared with 19% of benign tissues ($p = 0.0001$, figure 3, table 1). On the other hand, immunostaining showed positive expression of TIMP2 in 69% of colon cancer tissues compared with 17% of benign tissues ($p = 0.0001$, figure 4, table 1).

DISCUSSION

For local and distance invasion, cancer metastasis occurs when the basement membrane was degraded. Degradation of the extracellular matrix (ECM) allows tumor cells to escape from their primary tumor, invade local tissues, enter into the bloodstream, and reach the secondary sites causing the formation of new tumors on other organs. Metalloproteinases play key roles in the metastatic process because of their ability to degrade matrix proteins (11). Cancer cell invasion and metastasis are controlled by genetic variation in tumor cells and by stromal cells that secret MMPs (12). Furthermore, the interaction between these factors may promote cancer cell invasion and metastasis. The basement membrane that surrounds tumor nests plays an important role in preventing cancer cells from invading (13).

In well differentiated tumors, the tumor cells have low invasion properties due to their relatively low expression of MMPs. However, in poorly differentiated tumors, there are high invasion properties and malignant activities resulting from overexpression of MMPs (14). The most important MMPs in colon cancer represent MMP2 and MMP9, which catalyze the breakdown of gelatin IV-the

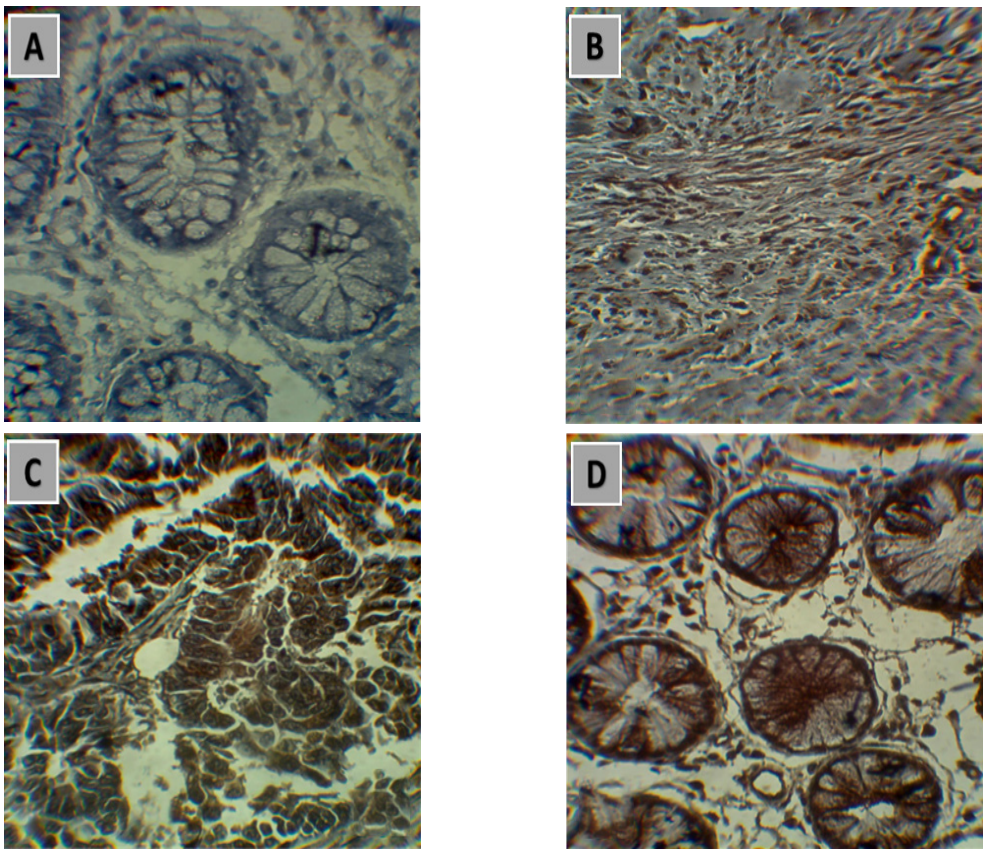

Fig. 3: Immunohistochemistry staining of TIMP1 in colon cancer as well as in benign tissues (400X). (A) Benign tissues with slightly positive TIMP1 protein expression. (B) Stage I colon cancer tissue with increasing TIMP1 expression. (C) Stage II colon cancer tissue showing significantly positive TIMP1. (D) Stage III colon cancer tissue with high TIMP1 protein expression level.

main component of the ECM. Therefore, MMP2 and MMP9 were implicated in colon cancer cell invasion and metastasis (12). Current results showed significantly elevated expression levels of MMP2 and MMP9 as well as TIMP1 and TIMP2 in the cancerous colon tissues compared with those in benign tissues. Increased levels of MMP2 and MMP9 were found to be stage-dependent in Iraqi women with colon cancer (data not shown). Moreover, MMP2 is localized in the nucleus of colon cancer cells while it is expressed only in the cytoplasm of normal colon epithelial cells (15). The current study presented that MMP2 was highly expressed in the nuclei of stage III colon cancer cells. This inter-localization of MMP2 may play a role in tumor cells' aggressiveness and metastasis in patients with colon cancer and requires further investigation.

In addition, TIMP1 and TIMP2 have gained great interest as a cancer biomarker in tissues and in corresponding plasma samples (16). Furthermore, Schrohl and his team have indicated that TIMP1 represent an important new target for anti-cancer therapy (17). However, currently little is known about the regulation of TIMP1 in cancer. The aim of this study was, therefore, to gain further insight then correlating the expression of TIMP1 with TIMP2 and MMP1 and MMP2 (18). According to the previous study that describes the role of TIMP1 in MMPs inhibition, TIMP1 has a major role in tumor progression, invasion, and metastasis (19). TIMP1 has multifunctional activities; one of these was based on a metalloproteinases-dependent, anti-proteolytic activity that could suppress colon cancer proliferation, migration, and metastasis, in addition to increase anti-apoptosis of colon cancer (19). The main findings of the present study that TIMP1 and TIMP2 proteins were significantly up-regulated in colon cancer. Furthermore, among the MMPs, MMP2

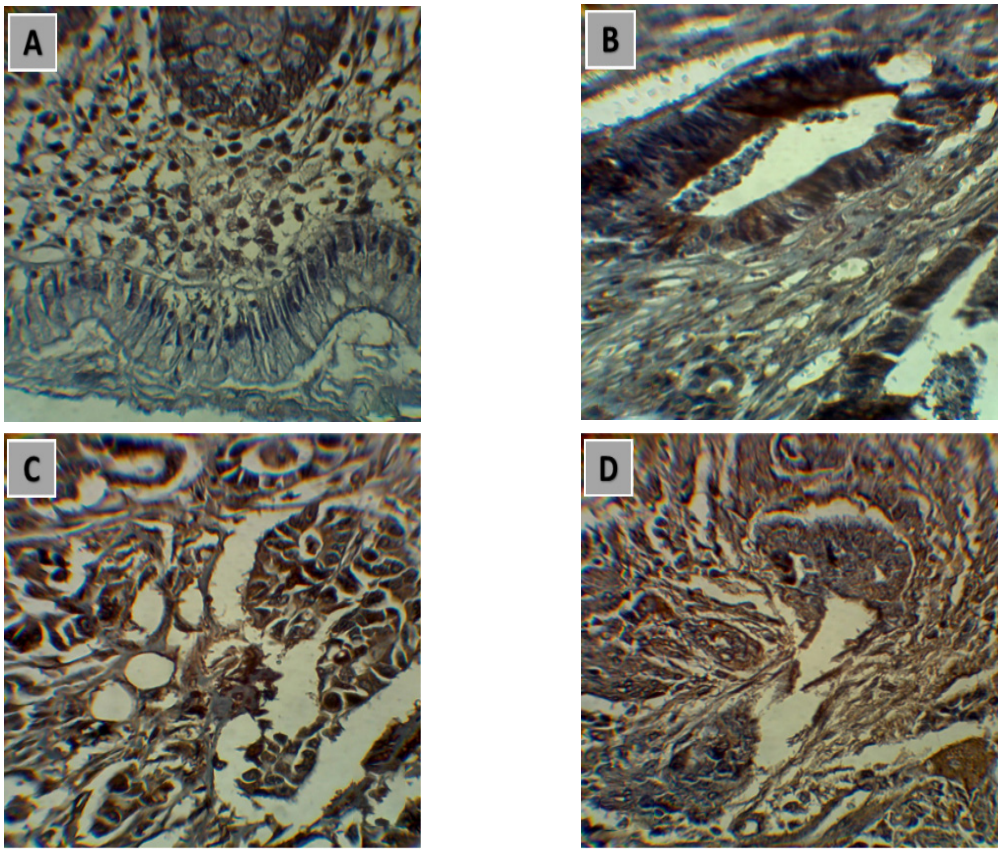

Fig. 4: Immunohistochemistry staining of TIMP2 in colon cancer as well as in benign tissues (400X). (A) Benign tissues with TIMP2 positive protein expression. (B) Stage I colon cancer showing increased TIMP2 expression. (C) Stage II colon cancer with high TIMP2 protein expression level. (D) Stage III colon cancer tissue with increasing expression TIMP2 protein level.

and MMP9 are up-regulated in colon cancer tissues compared with those in benign tissues.

On the other hand, when looking for interactions, a significant association was found between TIMP1, TIMP2, MMP2 and MMP9. Thus, it was clear that a broad range of MMPs involved directly in the breakdown of the ECM is up-regulated in the colon cancer tissue. In conclusion, the present results show that the expression levels of MMP2, MMP9, TIMP1 and TIMP2 increased in patients with colon cancer. MMP2 and MMP9, as well as TIMP1 and TIMP2, could be used as worthy markers for detection of colon cancer. The localization of MMP2 in the nucleus of colon cancer cells may play an important role in the progression and metastasis of colon cancer. The use of specific inhibitors targeting these MMPs in the treatment of colon cancer metastasis requires further investigation.

Ethics Committee Approval:

Ethics committee approval was received for this study from the Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University.

Informed Consent:

N/A.

Financial Disclosure:

The authors declared that this study received no financial support.

CONFLICT OF INTEREST

The authors declare no conflict of interests related to this work.

REFERENCES

1. Iraqi Cancer Registry, 2018. Ministry of health in Iraq.

2. Shah FD, Shukla SN, Shah PM, Shukla HK, Patel P. Clinical significance of matrix metalloproteinase -2 and -9 in breast cancer. *Indian J Cancer* 2009; 64:194-202.

3. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001; 17:463-516.

4. Pivetta E, Scapolan M, Pecolo M, Wassermann B, Abu-umeileh I, Balestreri L, et al. MMP-13 stimulates osteoclast differentiation and activation in tumor breastbone metastases. *Breast Cancer Res* 2011; 13: 105-20.

5. Ala-aho R, Kahari VM. Collagenases in cancer. *Biochem* 2005; 87:273-80.

6. Woessner JF. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB* 1991; 5:2145-54.

7. Iwasaki M, Nishikawa A, Fujimoto T, Akutagawa N, Manase K, Endo T. Anti-invasive effect of MMI-166 new selective matrix metalloproteinase inhibitor, in cervical carcinoma cell lines. *Gynecol Oncol* 2002; 85:103-7.

8. Kato Y, Yamashita T, Ishikawa M. Relationship between the expression of matrix metalloproteinase-2 and matrix metalloproteinase-9 and invasion ability of cervical cancer cells. *Oncol Rep* 2002; 9:565-9.

9. Duffy MJ, McGowan PM, Gallager WM. Cancer invasion and metastasis: Changing views. *J Pathol* 2008; 214:283-93.

10. Vizoso FJ, González LO, Corte MD, Rodriguez JC, Vasquez J, Lamelas ML. Study of matrix metalloproteinase and their inhibitors in breast cancer. *Br J Cancer* 2007; 96:903-11.

11. Rowe AG, Weiss SJ. Breaching the basement membrane: Who, when and how? *Trends Cell Biol* 2008; 18:283-93.

12. Jezierkska A, Motyl T. Matrix metalloproteinase-2 involvement in breast cancer progression: A mini-review. *Med Sci Monit* 2009; 15:32-40.

13. Hai-Xia F, Hai-Xia L, Dong C, Zhong-Xiuzi G, Jin-Hua Z. Changes in the expression of MMP2, MMP9 and COIIV in stromal cells in oral squamous tongue cell carcinoma. *Exp Clin Cancer Res* 2012; 31:90.

14. Eiseler T, Doppler H, Yan IK, Goodison S, Storz P. Protein kinase D1 regulates matrix metalloproteinase expression and inhibits breast cancer cell invasion. *Breast Cancer Res* 2009; 11:R13.

15. Eiró N, Fernandez-Garcia B, González LO, Vizoso FJ. Clinical Relevance of Matrix Metalloproteases and their Inhibitors in Breast Cancer. *J Carcin Mut* 2013:S13-004.

16. Holten-Andersen MN, Hansen U, Brunner N, Nielsen HJ, Illemann M, Nielsen BS. Localization of tissue inhibitor of metalloproteinases 1 (TIMP-1) in human colorectal adenoma and adenocarcinoma. *Inter J Cancer* 2005; 113: 198–206.

17. Schrohl AS, Meijer-van Gelder ME, Holten-Andersen MN, Christensen IJ, Look MP, Mouridsen HT, et al. Primary tumor levels of tissue inhibitor of metalloproteinases-1 are predictive of resistance to chemotherapy in patients with metastatic breast cancer. *Clin Cancer Res* 2006; 12:7054–8.

18. Sorensen, NM, Bystrom P, Christensen IJ, Berglund A, Nielsen HJ, Brunner N, et al. TIMP-1 is significantly associated with objective response and survival in metastatic colorectal cancer patients receiving a combination of irinotecan, 5-fluorouracil, and folinic acid. *Clin Cancer Res* 2007; 13: 4117–22.

19. Guohe S, Shifeng X, Hong Z, Yupeng W, Chao X, Tao Jiang. TIMP1 is a prognostic marker for the progression and metastasis of colon cancer through FAK-PI3K/AKT and MAPK pathway. *J Exper Clin Cancer Res* 2016; 35:148-60.