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The Role of MMP-3 Serum Level in both Colorectal and Gastric Cancer in Iraqi Patients

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Abstract.

Background:

The gastrointestinal tract cancer (GIT cancers) is a worldwide problem, In Iraq, Colorectal Cancer (CRC) is among the Top Ten Cancers (4th) but Gastric Cancer (GC) is not included. Matrix metalloproteinase-3 belongs to the MMP family and is capable of degrading a wide spectrum of substrates. Increased serum concentrations of certain MMP-3 in different types of cancer have been identified, indicating their utility as biomarkers. MMP-3 known with the role of apoptosis creation, angiogenesis modulation, invasion and metastasis in tumor.

Materials and Methods:

Thirty eight patients with CRC and GC (CRC=21,GC=17) and 20 healthy individuals as a control group were collected, 5-10ml were collected from them.MMP-3 ELISA test was performed to the serum of both, the patients and the control group.

Results:

Serum MMP-3 levels of the both GITs cancers (CRC+GC) and separately were significantly higher than the control group ($P > 0.05$). Also, non correlation or association presented between the serum level of MMP-3 and Dukes as well as TNM staging in both GIT cancers.

Conclusion:

MMP-3 serum level can be a predictive marker for GITs cancers in both CRC and GC collectively or separately.

Keywords. MMP-3 Serum Level, Gastrointestinal Tract Cancers (GIT Cancers) Colorectal Cancer (CRC), Gastric Cancer (GC)

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INTRODUCTION

The gastrointestinal tract cancer (GIT cancer) is a worldwide problem, about 4500 to 6000 new cases are registered in UAS each year [1,2]. Data of the estimated numbers of the top 20 Leading Cause of Death in the Eastern Mediterranean Region between 2015 to 2030 showed that Colorectal Cancer (CRC) will jump from the 19th in 2015 up to 15th in 2030 while Gastric Cancer (GC) will be the 14th in 2030 after it was the 17th in 2015. In Iraq, CRC is among the Top Ten Cancers (4th) but GC is not included, in males top ten cancers the CRC is the 5th while GC is the 9th, in the females top ten cancers the CRC is the 3rd but GC is not included in the list [3]. Identifying molecular markers involved in the propagation and progression of GIT cancers like CRC can promote early diagnosis, successful personal care and reliable prognostic evaluation [4].

One approach can revolve around the relationship between cancer cells and the normal tissue around them, involving a enormouse group of proteolytic enzymes-proteases. Proteases work as a key role in different biological processes in many diseases like cancer [5]. Among the broad spectrum of proteases, the matrix metalloproteinases (MMPs) played a significant role in the development of cancer effectors because of their potential to degrade the protein components of the extracellular matrix (ECM) and the basement membranes, It gives access to the vascular and lymphatic networks of tumor cells and promotes metastasis generation [6].

MMPs exisited in the latent phase and at decreased concentration under normal physiological conditions. They normally have a prodominance, a catalytic domain, a hinge region and a hemopexin domain [7]. To date, there have been 23 MMPs detected and described in humans. Based on the specificity of the substrates, sequence similarity and structure of the domain; MMPs can be classified into six groups: collagenases, gelatinases, stromelysins, matrilysins, MMPs (MT-MMPs) form membranes, and other MMPs [8].

Increased serum concentrations of certain MMPs in different types of cancer have been identified, indicating their usefulness as biomarkers. Improved expression of several MMPs (MMP-1, −2, −7, −9 and −13) [9].

Matrix metalloproteinase-3 (MMP-3, stromelysin-1) is a member of the MMP family, capable of degrading a wide variety of substrates comprising collagen type II, IV, IX, X and XI, fibronectin, gelatin, elastin, proteoglycanase, E-cadherin and osteopontinin [10, 11]. MMP-3 manifactured by various cell kinds including synovial cells, monocytes, macrophages, fibroblasts and chondrocytes. Matrix metalloproteinase-3 (MMP-3) and metalloproteinase-3 (TIMP-3) tissue inhibitor are the two keys *in vivo* enzymes that are deep related with tumor cell invasion and metastasis during extracellular matrix synthesis and destroying metabolism balance control [12]. The expression MMP-3 has been used as an eventual diagnostic and/or prognostic marker for certain cancers [13, 14]. MMP-3 has been identified with the role of apoptosis induction, angiogenesis control, invasion and metastasis in cancer [15, 16, 17].

The aim of our research is to learn the Effect and Association of MMP-3 Serum Level on both Gastric and Colorectal Cancer in Iraqi Patients separately and together comparing to the control samples as well as the dukes and TNM staging .

MATERIALS AND METHODS

Sample collection

Blood sample (5-10) ml were drawn from veins at cubital fossa thirty eight patients with GC (H.pylori+ve as in histopathological reports) and CRC patients (GC=17, CRC=21) and 20 healthy individuals were collected (after definitive diagnosis and before taking the chemotherapy) at the Oncology clinic/ Baghdad Teaching Hospital and the Teaching Hospital for GIT and liver disease /Medical city starting from the 1st of January till the mid of March 2017.

A questionnaire was made to obtain the demographic data (name, address, gender, ABO, RH, tobacco smoking, Alcohol consumption, food type and family history), while the histopathological data (cancer type, staging and grading) were taken from the patients' files. Blood samples were centrifuged at 2000 g for (10-15) minutes to obtain serum used to detect MMP-3 levels by ELISA technique.

Detection of MMP-3 levels by ELISA

ELISA kit (Ray Bio) was applied by using the manual of instructions. In short, the microtiter plate was pre-coated with an antibody specific to MMP-3 then standards and samples were added to the appropriate microtiter plates wells.

A biotin conjugated antibody preparation specific for MMP-3 and avidin conjugated to Horseradish peroxidase (HRP) were added to each well. After incubation, 3, 3', 5, 5' tetramethyl-benzidine (TMB) substrate solution was added to all wells. Only those wells that contain MMP-3 biotin-conjugated antibody avidin will exhibit a change in color. The enzyme substrate reaction was terminated by adding of (according to the manual), 3 M sulphuric acid solution then the color change was measured spectrophotometrically (ASYS, Australia) at a wavelength of 450 nm ± 2 nm. Finally, MMP-3 concentration was determined by comparing the optical density (O.D.) of each sample to the standard curve.

Data Analysis

We applied SPSS and used descriptive statistics in addition to differences tests using the t test, and the relationships were studied through correlation coefficient and multiple linear regression.

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RESULTS

Demographic and Histopathological Data

The demographic data that in Figure (1) are in the mean age of the patients, which was 55.6 years, their sexes were 16 males (42%) and 22 females (58%), their ABO system was categorized as the following: A 12 (31%), B 10 (27%), AB 6 (15%) and O 10 (27%), while the Rh factor was positive in 8 (22%) and negative in 30 (78%).

Figure (2) showing Patients who were non tobacco smokers were 22 (57%), mild tobacco smoker were 2 (5%) and heavy tobacco smokers were 14 (38%), Alcohol consumers were 4 (10%) and non-alcohol consumers were 34 (90%), Patients when their food mainly vegetarian were 4 (10%), meat 1 (3%) and mixed diet were 33 (87%). The family history was positive in 17 (45%) patients who 7 (41%) have relatives suffer from GIT cancers while the rest 10 (59%) have relatives that suffer from other organ cancers.

The histopathological data in Figure 3 showed that the cancer types were adenocarcinoma 36 (95%) and others 2 (5%) were diagnosed as a signet ring and mucinous carcinomas.. The tumor cell differentiation was classified as follows: well differentiated 1 (2%), moderately differentiated 20 (52%) and poorly differentiated 17 (46%).

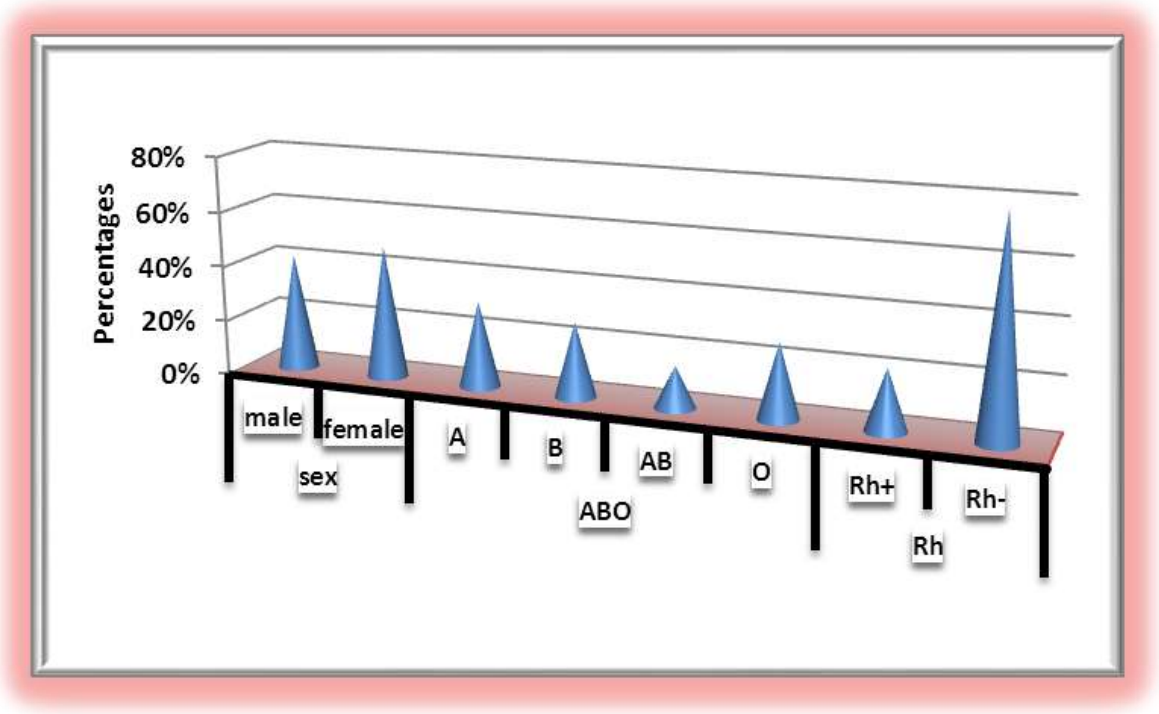

Figure 1. Sex, ABO system and Rh data

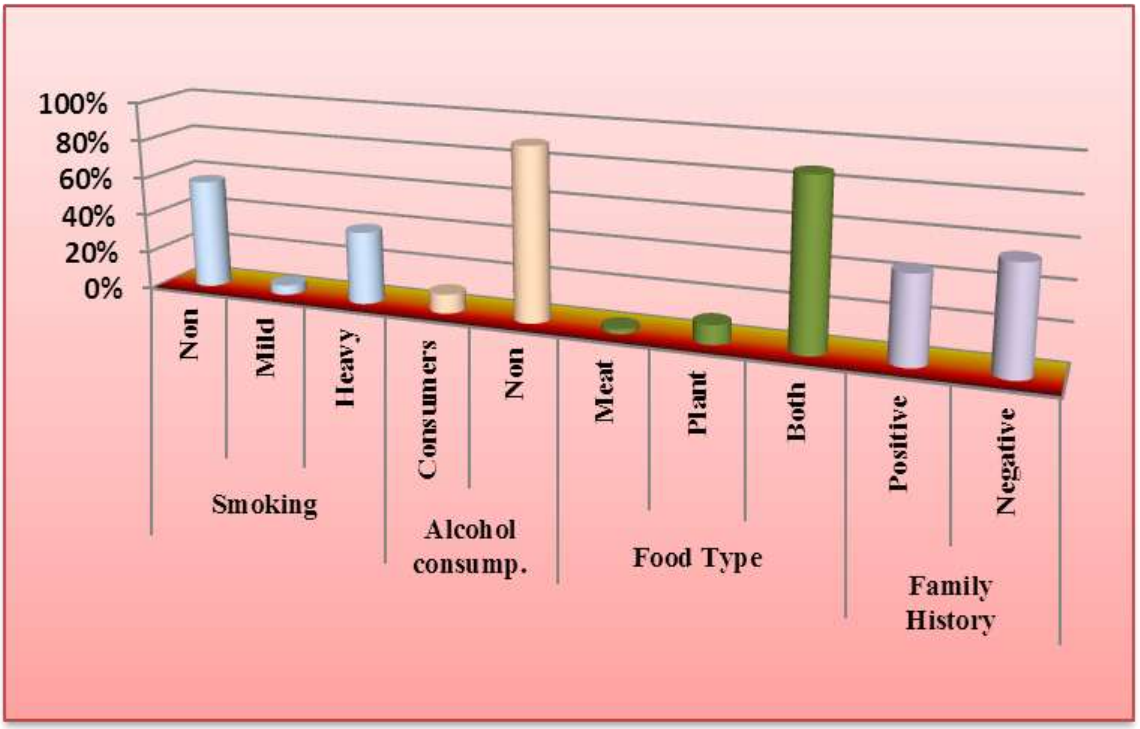

Figure 2. Data of tobacco smoking, alcohol consumption, food intake and family history of the GIT cancer patients

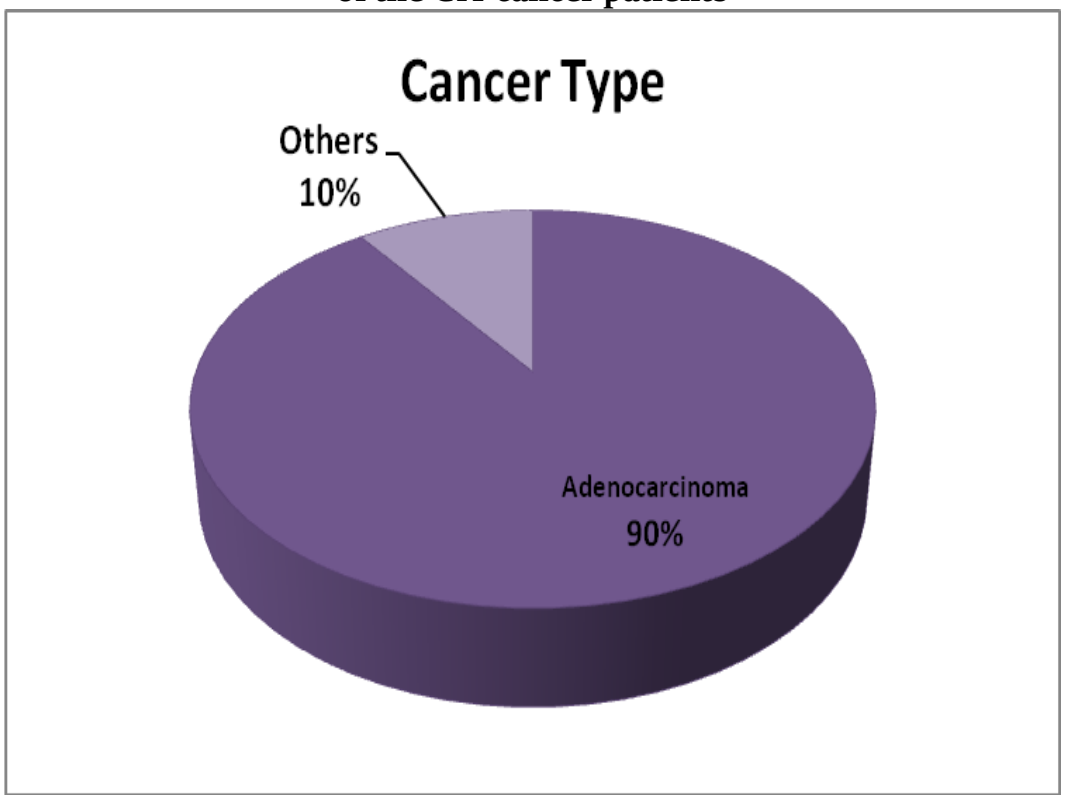

Figure 3. Histological cancer types in GIT cancers patient

MMP-3 serum level for both GIT cancers (CRC & GC)

In Table 1 the mean of the GIT cancer samples for MMP-3 serum level was (3.11±1.07) while the mean of the control samples for the MMP-3 serum level was (1.99±0.56). Obviously, the dispersion data of the GIT cancer samples were higher than the control sample.

Table 1. Descriptive statistics of MMP-3 serum level of the samples

Variable		N	Mean±SD	95% (C.I.) for Mean	
				Lower Bound	Upper Bound
MMP-3	GIT cancers	38	3.11±1.07	2.7578	3.4633
	Control	19	1.99±0.56	1.7201	2.2629

A *t* test was used in the case of two independent samples to determine whether there was a difference between the GIT cancer samples and the control samples. Table 2 presents the results of the test where the value of *t* for MMP-3 serum level is 4.24 with significant level in both cases (P > 0.05). A clear indication of significant differences between the two samples.

Table 2. t test study between GIT cancers samples and control samples depending on the MMP-3 serum level for the sample

Variable	Mean± SE	*t*	DF	Sig. (2-tailed)
MMP-3 serum level	1.11±0.263	4.24	55	0.000

Kolmogorov-Smirnov test was conducted to find out if the depend variable represented by MMP-3 serum level for the sample is follow the normal distribution, so the test statistics appeared 0.068, and since the significance level (P< 0.05), this indicates that the variable follows the normal distribution.

A measurement of the correlation between the variables (Differences, Dukes, T, N and M) and MMP-3 serum level for the sample, as it is shown in (Table 3) that there is no significant relationship between the variables (Differences, Dukes, T, N and M) and MMP-3 serum level for the sample, where the level of significance (P> 0.05).

Table 3. Pearson Correlation between the variables (Differences, Dukes, T, N and M) and MMP-3 serum level of the samples

MMP-3 serum level	Differences	Dukes	T	N	M
Pearson Correlation	-0.159	0.036	-0.053	-0.141	-0.068
Sig. (2-tailed)	0.340	0.832	0.753	0.398	0.685

In order to study the effect of independent variables (Differences, Dukes, T, N, M) on MMP-3 serum level, multiple linear regression analysis was used. The results observed that the value of the coefficient of determination is 0.043, which indicates the weak relationship between the variables. The analysis of variance in Table 4 shows that there was no effect of independent variables (Differences, Dukes, T, N, M) on an MMP-3 serum level where the level of significance (P> 0.05).

Table 4. ANOVA[a] to study the impact of independent variables on MMP-3 serum level for the GIT samples

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.830	5	0.366	0.287	0.917[b]
	Residual	40.791	32	1.275		
	Total	42.621	37			
a. Dependent Variable: patient concentration MMP-3						
b. Predictors: (Constant), Differences, Dukes, T, N, M						

MMP-3 serum level and Colorectal Cancer (CRC)

The descriptive indicators of the variable MMP-3 serum level of CRC samples (in case of CRC samples and control samples) are depicted in the following Table 5.

Table 5. Descriptive statistics of MMP-3 serum level of CRC samples

Variable		N	Mean±SD	95% (C.I.) for Mean	
				Lower Bound	Upper Bound
MMP-3 serum level	CRC	22	3.16±1.07	2.68	3.63
	Control	19	1.99±0.56	1.72	2.26

The study sample consisted of 22 patients with CRC cancer, as well as the control sample consisted of 19 persons. Table 5 shows that the mean of the CRC samples for the MMP-3 serum level was (3.16±1.07) while the mean of the control sample for MMP-3 was (1.99±0.56). Obviously, the dispersion data of the CRC samples were higher than the control samples.

A *t* test was used in the case of two independent samples to determine whether there was a difference between the CRC samples and the control samples. Table 6 presents the results of the test where the value of *t* is 4.27 with significant level (P< 0.05), A clear indication of significant differences between the two samples.

Table 6. t test study between CRC samples and control samples depending on the MMP-3 serum level for CRC samples

Variable	Mean±SE	*t*	DF	Sig. (2-tailed)
MMP-3 serum level	1.17±0.27	4.27	39	0.000

Kolmogorov-Smirnov test was conducted to find out if the depend variable represented by MMP-3 serum level for CRC samples is follow the normal distribution, so the test statistics appeared 0.102, and since the significance level (P> 0.05), this indicates that the variable follows the normal distribution.

Table 7 shows a measurement of the correlation between the variables (Differentiation, Dukes, T, N and M) and MMP-3 serum level of CRC samples, as it shows that there was no significant relationship between the variables (Differentiation, Dukes, T, N and M) and MMP-3 serum level of CRC samples, where the level of significance (P< 0.05).

Table 7. Pearson Correlation between the variables (Differetiation, Dukes, T, N and M) and MMP-3 serum level of CRC samples

MMP-3 serum level	Differentiation	Dukes	T	N	M
Pearson Correlation	-0.167	0.040	-0.055	-0.181	-0.099
Sig. (2-tailed)	0.457	0.861	0.808	0.420	0.660

In order to study the effect of independent variables (Diffrentiation, Dukes, T, N, M) on the MMP-3 serum level of CRC samples, multiple linear regression analysis was used. The results observed that the value of the coefficient of determination is 0.127, which indicates the weak relationship between the variables. The analysis of variance shown in Table 8 shows that there is no effect of independent variables (Differentiation, Dukes, T, N, M) on MMP-3 serum level for CRC samples where the level of significance (P> 0.05).

Table 8. ANOVAa to study the impact of independent variables on the MMP-3 serum level of CRC samples

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3.080	5	0.616	0.467	0.795 [b]
	Residual	21.081	16	1.318		
	Total	24.161	21			

a. Dependent Variable: patient concentration MMP-3

b. Predictors: (Constant), Differences, Dukes, T, N, M

MMP-3 serum level and Gastric Cancer(GC)

The descriptive indicators of the variable MMP-3 serum level for GC samples (in case of GC samples and control samples) are depicted in the following table 9.

Table 9. Descriptive statistics of MMP-3 serum level for GC samples

Variable		N	Mean±SD	95% (C.I.) for Mean	
				Lower Bound	Upper Bound
MMP-3 serum level	GC	16	3.04±1.10	2.45	3.62
	Control	19	1.9±0.56	1.72	2.26

The study sample consisted of 16 patients with GC, as well the control samples consisted of 16 persons. Table 9 shows that the mean of the GC samples for the MMP-3 serum level was (3.04±1.10) while the mean of the control samples for MMP-3 was (1.9±0.56). Obviously, the dispersion data of the GC samples were higher than the control samples.

A *t* test was used in the case of two independent samples to determine whether there was a difference between the GC samples and the control samples. Table 10 presents the results of the test where the value of *t* is 3.62 with significant level (P> 0.05), A clear indication of significant differences between the two samples.

Table 10. t test study between GC samples and control samples depending on the MMP-3 serum level for GC samples

Variable	Mean±SD	t	DF	Sig. (2-tailed)
MMP-3 serum level	1.04±0.28	3.62	33	0.001

Kolmogorov-Smirnov test was conducted to find out if the depend variable represented by MMP-3 serum level for GC samples is follow the normal distribution, so the test statistics appeared 0.107, and since the significance level (P> 0.05), this indicates that the variable follows the normal distribution.

A measurement of the correlation between the variables (Differentiation, Dukes, T, N and M) and MMP-3 serum level for GC samples, as it is shown in (Table 11) that there is no significant relationship between the

variables (Differentiation, Dukes, T, N and M) and MMP-3 serum level for GC samples, where the level of significance (P< 0.05).

Table 11. Pearson Correlation between the variables (Differentiation, Dukes, T, N and M) and MMP-3 serum level for GC samples

MMP-3 serum level	Differentiation	Dukes	T	N	M
Pearson Correlation	-0.124	0.031	-0.065	-0.080	-0.106
Sig. (2-tailed)	0.649	0.909	0.812	0.769	0.696

In order to study the effect of independent variables (Differentiation, Dukes, T, N, M) on MMP-3 serum level for GC samples, multiple linear regression analysis was used. The results observed that the value of the coefficient of determination is 0.030, which indicates the weak relationship between the variables. The analysis of variance shown in Table 12 shows that there is no effect of independent variables (Differentiation, Dukes, T, N, M) on MMP-3 serum level for GC samples where the level of significance (P< 0.05).

Table 12. ANOVAa to study the effect of independent variables on the MMP-3 serum level of GC samples

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.555	5	0.111	0.062	0.997[b]
	Residual	17.769	10	1.777		
	Total	18.323	15			
a. Dependent Variable: patient concentration MMP-3						
b. Predictors: (Constant), Differences, Dukes staging system, T, N, M						

DISCUSSION

MMPs are zinc-dependent endopeptidases which destruct protein-collagen, laminin, and fibronectin extracelliualr during the invasion and metastasis of cancer.MMPs are produced primarily by reactive stromal and inflammatory cells surrounding tumors rather than by tumor cells [18]. MMP-3 is produced by interstitial connective cells, fibroblasts, capillary endothelial cells, macrophages and tumor cells with extensive substrates and can degrade the basement membrane, proteoglycans, laminin, fibronectin, and collagen II, III, IV, V and VI [19, 20]. MMP-3 has the special feature of triggering certain MMP types, such as MMP-2 and MMP-9 [21]. Plasmin activates proMMP-3 to active MMP-3 which in effect encourages proMMP-9 to active MMP-9, leading to a development of colorectal cancer [22].

In our study, the total cases of GIT cancers showed significant increase in MMP3 serum level compairing with the control samples which is the same results which presented by other researchers who showed that MMP-3 gene over expression as well as increased serum level have been seen in different GIT cancers [23,24,25] which mentioned by Verma et al. and others who claimed that considerable evidence has shown progression of the disease in chronic GIT diseases like inflammatory bowel disease,Crohns disease and Ul creative colitis as well as experimental animal models of cancer invasion and metastasis related with boosted secretion of MMP3 by tumor cells and/or stromal cells which eventually will cause increased serum level of MMP3[24,25,26,27,28]. However, our findings showed a significant rise in MMP-3 serum levels in CRC samples relative to similar controls in other

research that showed that higher levels of MMP-1, -2, -3, -7, -9, -13 and MT1-MMP expression in human colorectal cancer have been reported. [22,23] which can be explained that MMP-3 is over- expressed in colorectal tumors [29].

In GC cases, there is a significant rise in the serum level of MMP-3 comparing to the control which already presented in multiple scientific observations, in each of the 24 cancer cases, the activities of MMP-2, -3, -9 and -10 enzymes were observed. MMP over expression was documented in tumors compared to normal tissue; the activated form of MMP-3 and -10 levels were elevated in cancers.

Also, the levels of MMP-3 and -10 mRNA in tumors were significantly more than those of normal tissues in both the stromal and epithelial components of tissues [23,29]. One of the serious concerns in colon cancer patient management is the determination of prognostic factors in the early stages of the disease in order to determine which patients are at risk of recurrence or poor prognosis and then would benefit from a chemotherapy regimen[30].

According to our study results there were no correlation or association presented between the serum level of MMP-3 and the Differentiation, Dukes staging as well as TNM staging in both GIT cancers, which coincide with other authors [31,32,33,34,35] who showed the same information in Colorectal and Gastric cancers.

CONCLUSIONS

We concluded that MMP-3 serum level can be a biomarker for GITs cancers in both CRC and GC collectively or separately.

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