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Association Risk of Metalomatrix Proteinase Enzymes Levels (MMP-1,MMP-9 And MMP-13) with Development of Rheumatoid Arthritis

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Abstract

Matrix metalloproteinase are family of metalloendopeptidases enzymes play a critical role in degrade of extracellular matrix components in rheumatoid arthritis patients . This study aims to assess the association of MMP1,9,13 with the development of rheumatoid arthritis disease . The study included 69 RA patients positive for RF factor .MMPs concentration is measured by sandwich ELISA technique .The results showed that the MMP-1, 9 ,13 levels are significant increase in rheumatoid arthritis patients compared with control group . Only MMP1 showed a significant differences regarding the period of rheumatoid arthritis disease. However ,no association between the levels of MMP-1, 9 ,13 with gender, age group of patients , activity of disease and associated diseases with RA . Highly positive correlation was showed between MMP-1 and MMP-9 levels in RA patients. Our study concluded there was an association between the increase of MMP-1, 9 ,13 levels with development of rheumatoid arthritis in Iraqi patients , while MMP1 is associated with the early detection of rheumatism.

Key words: Rheumatoid arthritis , MMP-1,MMP-9 and MMP-13 ,diabetes , hypertension , autoimmune disease

Introduction

Rheumatoid arthritis(RA) is one of the life-threatening autoimmune diseases characterized by continuous destruction to the joints with loss of bone and cartilage and synovial fluids (SFs) [1-3] . RASFs secrete various proteases, including MMPs that degrade extracellular matrix (ECM) components, mainly proteoglycans and collagens, of articular cartilage in the affected joints [4]. MMPs play a critical role in the pathogenesis of RA [5] .Since MMP-1,3, 9, ,13 expression is upregulated in RASFs, MMPs are considered to play a critical role in the degeneration of cartilage in RA joints [6] . MMP-1 (collagenase 1) and MMP-13 (collagenase 3) cleave collagens, whereas MMP-3 (stromelysin 1) and MMP-9 (gelatinase B) target proteoglycans that are comprised of aggrecan . The degeneration of proteoglycans at the surface and the subsequent degradation of collagen fibrils in the deep zone together result in the destruction of articular cartilage. MMPs may thus play a distinct role in joint destruction in RA. Rheumatoid arthritis treatment requires an understanding of the mechanisms that regulate the gene expression of MMPs ,so the advanced knowledge of the genetic mechanisms and molecular events that control MMPs gene activation in RASFs is perhaps help in the understanding of rheumatic pathology and assist in the development of new treatment strategies for this disease. This study aimed to assessment the risk of metalomatrix proteinase enzymes levels (MMP-1, 9 ,13) with development of rheumatoid arthritis.

Materials and Methods

Sixty nine patients (6 (8.69%) males and 63(91.30%) females) with positive rheumatoid factor and twenty healthy individuals as control group are included in this study. The patients are attended to the Rehabilitation Center for the Disabled and Artificial Limbs and Al Zahra'a and Al Karama Teaching Hospitals in Wasit Province from 1 December 2019 to 1 December 2020 . The ages of the patients ranged from twenty two to ninety-three years. The selected patients were stratified according to the severity of RA into three stages. In addition the laboratory test Erythrocyte Sedimentation Rate (E.S.R) was checked for each patient . The patients were divided to subgroups according to age groups ,gender ,disease severity, disease duration, line of treatment and associated diseases . The healthy individuals had no pathological state at time of this study, and without any history of systemic diseases , immunological disorders ,and cancer diseases . Blood samples and their sera were taken and separated in jell tubes from RA patients with different etiology and from control group .

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MMP-1,MMP-9 and MMP-13 analysis

The levels of MMP-1, 9 ,13 were measured according to the instructions of manufacture company (Elabscience), using Sandwich- ELISA kit . All reagents were preheated at room temperature (18~25℃) before used 15 min. The micro ELISA plate is covered by antigen specific to human MMP-1, 9 ,13 . The results were read by ELIZA reader at 450 nm wavelength.

Statistical analysis

SPSS V.20 program was used to test the effect of difference factors in study parameters .Quantitative data were calculated as mean, SE and SD .The difference between means assessed by analysis of variance (one and two way - ANOVA). P-value for all tests was considered significant if <0.05.

Results and Discussion

The Levels of MMP-1, 9 13 in Rheumatoid Arthritis Patients and Control Group

The levels of MMP-1, 9 ,13 in rheumatoid patients and control group were summarized in table(1) , the mean levels of MMP-1, 9 ,13 were recorded, in patients as 172.7 ±(3.7) ±(4.4) IU/ml, 169.5±(3.3) ±(4.0) IU/ml and 167.9±(4.1) ±(4.9) IU/ml respectively and in control group 83.5 ±(1.2) ± (2.8) IU/ml, 88.8±(7.3) ± (1.6) IU/ml and 94 ±(8.8) ±(1.9) IU/ml respectively with very highly significant increase in RA patients compared to control group (p value < 0.0001, 0.001 and 0.001 respectively) . These results were coming in agreement with many researches [7] who study a total of 30 patients with RA and 20 as control group and they conclude that patients with early RA are characterized by high serum levels of degrading metalloproteinase such as MMP-1, MMP-9, and MMP-13with significantly increase in patients (p < 0.001 ,0.001 and 0.01) respectively than control group. Also, [8] , [9] showed high levels of MMP-1 and MMP-3 in RA patients than in control group (P<0.05). However, down regulation of this gene was found in patients in comparison to control group showed in other study [10]. Furthermore, MMP inhibitor that preferentially inhibits MMP-13 has been shown to block the degradation of explanted human osteoarthritic cartilage [11]. MMP-13 inhibitors such as the ones described here will help further define the role of this protease in arthritis and other diseases and may soon lead to drugs that safely halt cartilage damage in patients .Also, [12] reported that the enzyme level in the serum of control group was lower when compared with the RA patients, and the activity of the enzyme was significantly different between the SF and RA patients (P< 0001). This is probably due to the fact that the enzyme in normal human cartilage was bound to an inhibitor named tissue inhibitor of metalloproteinase (TIMP). Generally, the results of this study confirm that increase of MMP-1, 9 ,13 in patients serum are associated with RA, and this suggests an association between the increase of MMP-1, 9 ,13 levels with development of rheumatoid arthritis in Iraqi patients .

Table 1. Levels of MMP-1,MMP-9 and MMP-13 in RA patients and control group

Cases	**No.**	**MMP1 mean concentration Mean SD±SE±(IU/ml)**	**MMP9 mean concentration Mean SD±SE±(IU/ml)**	**MMP-13 mean concentration MeanSD±SE±(U/ml)**
Patients	69	172.7 ±(3.7) ±(4.4)	169.5±(3.3) ±(4.0)	167.9±(4.1) ±4.9)
Control group	20	83.5 ±(1.2) ± (2.8)	88.8±(7.3) ± (1.6)	94 ±(8.8) ±(1.9)
P value		0.0001	0.001	0.001

Estimation of MMP-1,MMP-9 and MMP-13 Levels in Rheumatoid Arthritis Patients Based on Their Gender and Age Group

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Assessment the levels of MMP-1, 9 ,13 in RA patients according to their gender were summarized in tables (2,3). Our study included 69 patients ; 6 men (8.69%) and 63 women(91.30%) .The mean concentration of MMP1, 9 ,13 in male patients were 186.2±(1.7) ±(7.3)**,** 180±(1.9) ±(7.8)and 170±(4.3) ±(1.7)respectively , while female gender showed 171.4±(3.8) ±(4.8), 168±(3.4) ±(1.7)and 167.6±(4.1) ±(5.2) respectively with no significant differences between both sexes (p value >0.5) (table 2) . These results were coming in agreement with many researches [13].Also,[14] were found no significant difference between male and female patients regarding the level of MMP. Moreover, [12] when study (158) RA patients (132 female, 26 male) showed no significant association between the gender and the activity of enzyme MMP-1 in patients ,as well as, [9] showed the same result .From the results of this study, it appears that sex hormones do not effect on the level of MMP1, 9 and 13 production in RA patients.

Table 2. Distribution of MMP-1,MMP-9 and MMP-13 Levels in rheumatoid patients according to their gender

Cases	**No %**	**MMP-1 mean concentration Mean SD± SE±(IU/ml)**	**MMP-9 mean concentration Mean SD± SE±(IU/ml)**	**MMP-13 mean concentration Mean SD± SE±(IU/ml)**
Male patients	6(8.69%)	186.2±(1.7) ±(7.3)	180±(1.9) ±(7.8)	170±(4.3) ±(1.7)
Female patients	63(91.30%)	171.4±(3.8) ±(4.8)	168±(3.4) ±(1.7)	167.6±(4.1) ±(5.2)
P value		0.3	0.4	0.8

Table (3) showed the distribution of MMP-1, 9 ,13 Levels in rheumatoid patients according to age group . The results showed that the first group of patients (22-31 Y) recorded 159.6 ±(2.7)±(1)IU/ml , 157.1±(1.9)±(7.3) IU/ml and 144.4±(2.6)±(1) IU/ml respectively , while the second group (32-41 Y) recorded 180.5±(3.3)±(7.3)IU/ml, 173.5±(2.2)±(4.9) IU/ml and 170.8±(3.6)±(7.9) IU/ml respectively ,the third group (42-51 Y) ,recorded 150.6±(2.7)±(7.9)IU/ml , 160.6±(3.4)±(9.8) IU/ml and 167.5±(4.7)±(1.3) IU/ml respectively , and the fourth group (52-61 Y) ,recorded 180.0±(3.7)±(1)IU/ml , 172.5±(4.6)±(1.2) and 172.3±(5.1)±(1.3) , respectively while the last group (61+ Y) recorded 178.6±(4.6)±(1.2)IU/ml , 178.6±(4.6)±(1.2) IU/ml and 170.7±(3.9)±(1) IU/ml with no significant differences (p> 0.05) between the five age groups regarding MMP-1, 9 ,13 levels .Our results indicated to no effects of age on MMPs concentration in RA patients . [12], showed that the number of rheumatoid arthritis females were more than males by (5) times and it is most common after the age between 40th and 50th years, with no significant effect of age on the enzyme activity.

Table 3. Distribution of MMP-1,MMP-9 and MMP-13 Levels in rheumatoid patients according to age group

AGE GROUP	**Patients No %**	**MMP1 mean concentration**	**MMP9 mean concentration**	**MMP-13 mean concentration**

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		Mean SD± SE±(IU/ml)	**Mean SD± SE±(IU/ml)**	**Mean SD± SE±(IU/ml)**
22-31 Y	7 (10.14%)	159.6 ±(2.7)±(1)	157.1±(1.9)±(7.3)	144.4±(2.6)±(1)
32-41 Y	21 (30.43%)	180.5±(3.3)±(7.3)	173.5±(2.2)±(4.9)	170.8±(3.6)±(7.9)
42-51 Y	12 (17.39%)	150.6±(2.7)±(7.9)	160.6±(3.4)±(9.8)	167.5±(4.7)±(1.3)
52-61 y	14 (0.28%)	180.0±(3.7)±(1)	172.5±(4.6)±(1.2)	172.3±(5.1)±(1.3)
62+ Y	15 (21.74%)	178.6±(4.6)±(1.2)	173.8±(3.7)±(9.7)	170.7±(3.9)±(1)
P value		0.13	0.66	0.64
Total	69	172.7±(3.7)±(4.4)	169.5±(3.3)±(4)	167.9±(4.1)±(4.9)

Estimation of MMP-1,MMP-9 and MMP-13 Levels in Rheumatoid Arthritis Patients According to Activity of Disease

Distribution of MMP-1, 9 ,13 levels according to the RA disease activity in patients was showed in table**(** 4). The results showed the distribution of the MMP-1, 9 ,13 levels in three categories for disease activity . The mean level of low activity was recorded as 170.6 ±(3.9) ±(1.1)IU/ml , 161.9 ±(2.6) ±(7.9) IU/ml and 147.4±(3.1) ±(9.4) IU/ml respectively, moderate activity 175.9±(3) ±(5.7)IU/ml , 172.3±(3.4) ±(6.5) IU/ml and167.8±(4.2) ±(8.1) IU/ml respectively ,and sever activity were 170.4±(4.2) ±(7.8) IU/ml, 169.6±(3.3) ±(4) IU/ml and 175.4±(4.1) ±(4.9) IU/ml respectively . The results showed no significant difference between three categories of disease activity regarding the concentration mean of MMP-1, 9 ,13 in patients. Another study done by [15], noted that mild is not significantly different from sever progression regarding pro MMP-8 and MMP-9. Also , [16] showed that untranslated regions in the MMP-8,9 gene that contain AUUUA sequences contribute to joint destruction in RA disease. The role of MMP1,9,13 in cartilage destruction was reported by [6]. Also,[17] reported there was association between activity of RA disease and MMP-1 and MMP-3.[18] also reported the increased expression of miR-203 leads to a considerable increase in MMP-1 levels in rheumatoid arthritis synovial. Other study indicated there was direct association between MMP-9 levels and the activity RA disease [9] . The enzyme MMP9 showed an increase in RA patients and its concentration showed an increase in the joints of patients with severe rheumatism compared to the mild to moderate [15,19,20]. Our results confirm the increased activity of MMP-9 correlated with rheumatism. In the same direction, MMP-9 gene is among the most studied MMPs gene, is directly correlated to RA disease activity [21]. Although RA is a non-curable disease, but if diagnosed and treated well in time, it improves quality of life. To achieve this aim, it is recommended that MMP-9 gene expression be included in the workup of all RA patients or at least those RA patients who are not responding to medication to modify treatment regimes in order to prevent permanent deformities. The results of another study suggested that increased levels of MMPs in the serum of rheumatism patients is a useful marker for disease activity in the early stage [7] .

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Table 4. Distribution of MMP-1,MMP-9 and MMP-13 Levels in rheumatoid patients according to activity of disease

Activity of disease	**No. of patients**	**MMP1 mean concentration Mean SD± SE±(IU/ml)**	**MMP9 mean concentration Mean SD± SE±(IU/ml)**	**MMP-13 mean concentration Mean SD± SE±(IU/ml)**
low	11	170.6 ±(3.9) ±(1.1)	161.9 ±(2.6) ±(7.9)	147.4±(3.1) ±(9.4)
moderate	28	175.9±(3) ±(5.7)	172.3±(3.4) ±(6.5)	167.8±(4.2) ±(8.1)
sever	30	170.4±(4.2) ±(7.8)	169.6±(3.3) ±(4)	175.4±(4.1) ±(4.9
P value		0.8	0.6	0.1

Estimation of MMP-1,MMP-9 and MMP-13 Levels in Rheumatoid Arthritis Patients According to Type of Treatment

Relationship between of MMP-1, 9 ,13 titer and treatment of disease in rheumatic patients were summarized in table(5) .Out of 69 rheumatoid patients , seven patients were treated with vitamins and showed MMP-1, 9 ,13 levels as 169.7(4.1)± (1.5) IU/ml, 160.9(2.6)± (1) IU/ml and 141.7(2.7)± (1) IU/ml respectively, 9 patients treated with Painkiller 176.1(2.6)± (8.8)IU/ml 175.7(4.3)± (1.4) IU/ml and171.3(4.1)± (1.3) IU/ml, respectively ,40 patients treated with chemotherapy, 171.1(3.9)± (6.3)IU/ml , 171(3.4)± (5.5) IU/ml and172.6(4.4)± (6.9) respectively ,and 13 patients of them were treated with steroid ,showed 176.8(3.5)± (9.8)IU/ml , 165.1(2.6)± (7.2) and 165(3.7)± (1) respectively . The results showed no significant differences between all groups. These results indicate that there was no significant relationship between the treatments used for rheumatism patients and the levels of MMP1,9,13 in the patients' serum, and these drugs did not modulate or alter MMP-1, 9 13 production in rheumatic patients. The same results were showed by [7]. As well as [21] showed DMARDs are effective in the treatment of RA patients in the Pakistani population as MMP-9 gene expression was down regulated. Response to DMARDs was equally seen in both genders. Therefore, no treatment modification was indicated in the study group. Moreover, [22], reported the estradiol and progesterone decrease the amount of active MMP-13 by inhibiting the conversion of proMMP-13 into active form and accelerating the active MMP-13 fragmentation.

Table 5. Distribution of MMP-1,MMP-9 and MMP-13 levels in rheumatoid patients according to line of treatment

Type of treatment	**No.**	**MMP1 mean concentration Mean SD±SE±(IU/ml)**	**MMP9 mean concentration Mean SD±SE±**	**MMP-13 mean concentration Mean SD±SE±(**

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			(IU/ml)	**IU/ml)**
Vitamins	7	169.7(4.1)± (1.5)	160.9(2.6)± (1)	141.7(2.7)± (1)
Painkiller	9	176.1(2.6)± (8.8)	175.7(4.3)± (1.4)	171.3(4.1)± 1.3)
Chemotherapy	40	171.1(3.9)± (6.3)	171(3.4)± (5.5)	172.6(4.4)±(6.9)
Steroid	13	176.8(3.5)± (9.8)	165.1(2.6)± (7.2)	165(3.7)± (1)
P value		0.9	0.7	0.3
Total	69	172.7 (3.7)±(4.4)	169.5±(3.3)±(4)	167.9(4.1)±(4.9)

Estimation of MMP-1,MMP-9 and MMP-13 Levels in Rheumatoid Arthritis Patients According to Period of Disease

Distribution of MMP-1, 9 ,13 levels in RA patients according to period of disease were summarized in table (6). The results showed that the first period of disease (1M - 8 y) was recorded 169.8±(3.3)±(4.7)IU/ml , 169.3±(3.1)±(4.3)IU/ml and 169.7±(4)±(5.6) IU/ml for MMP1, 9 ,13 respectively while the second group (9 - 16 Y) 169.8±(3.3)±(1)IU/ml, 161.0±(2.7)±(8.3)IU/ml and 159.4±(4.3)±(1.2)IU/ml respectively, the last group (17 +) , 212.1±(3.7)±(4.4)IU/ml , 186.0± (5.8)± (2.3) IU/ml and 167.2±(5.1)±(2.1)IU/ml respectively , with significant differences (p> 0.05) for MMP1only between the three period of disease groups ,while MMP-9 and MMP-13 levels showed no significant regarding period of disease .Our results indicates to no effects of disease duration on MMP-9 ,13 concentration in patients serum [23] indicated no significant differences in patients with morphological types of RA and those with OA regarding sex ratio age or disease duration. Finally, [24] showed no association between SF MMP-3 or MMP-1 and RA disease duration.

Table 6. Distribution of MMP-1,MMP-9 and MMP-13 levels in rheumatoid patients according to period of disease

Period of disease	**No.**	**MMP1 mean concentration MeanSD±SE±(IU/ml)**	**MMP9 mean concentration MeanSD±SE±(IU/ml)**	**MMP-13 mean concentration MeanSD±SE±(IU/ml)**
1M - 8 y	52	169.8±(3.3)±(4.7)	169.3±(3.1)±(4.3)	169.7±(4)±(5.6)
9 - 16 Y	11	169.8±(3.3)±(1)	161.0±(2.7)±(8.3)	159.4±(4.3)±(1.2)
17 +	6	212.1±(3.7)±(4.4)	186.0±(5.8)±(2.3)	167.2±(5.1)±(2.1)

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P value		0.02	0.3	0.7
Total	69	172.7±(3.7)±(4.4)	169.5±(3.3)±(4)	167.9±(4.1)±(4.9)

Estimation of MMP-1,MMP-9 and MMP-13 Levels in Rheumatoid Arthritis Patients According to Associated Diseases

Distribution of MMP-1, 9 ,13 levels in RA patients according to associated diseases was shown in table (7). The mean levels of MMP-1, 9 ,13 in rheumatoid arthritis patients with diabetic disease were (174.9 ±(3.2)±(9.7), 171.3±(3.4)±(1) and163.2±(2.8)±(8.6)) IU/ml respectively, with hypertension (164.4±(3.4)±(9.7), 173.2±(4.5)±(1.7) and 146.6±(3)±(1.1))IU/ml respectively, with other disease were (192.4±(7.6)±(3.4), 171.6±(3.8)±(1.7) and 175.5±(5.1)±(2.2))IU/ml respectively and rheumatoid arthritis patients only were (171.2±(3.3)±(4.9), 168.2±(3.1)±(4.6) and 171.4±(4.4)±(6.5) IU/ml respectively. The results showed no significant difference between all groups regarding the concentration levels of MMP-1, 9 ,13 in RA patients. [9] reported that the excess syntheses of MMPs lead to the accelerated matrix degradation associated with many diseases [25,26]. The overproduction MMP-9 may induce micro vascular damage may facilitate the movement of inflammatory cells across the basement membrane. Also, [10] indicated the excess MMP-13 activity causes cartilage degradation in osteoarthritis, making this protease an attractive therapeutic target. However, clinically tested MMP inhibitors have been associated with painful, joint-stiffening musculoskeletal side effect that may be due to their lack of selectivity. Insofar as aberrant MMP-13 activity contributes to other pathologies such as cancer, heart failure, rheumatoid arthritis, and liver fibrosis [27]. MMP-13 selective inhibitors will be useful in further characterizing the role of this protease in other diseases. Moreover, [13], in their study showed an association of MMP1 levels with a wide variety of disorders, including polycystic kidney disease, rheumatoid arthritis, idiopathic pulmonary fibrosis, congestive heart failure, and acute myocardial infarction

Table 7. Distribution of MMP-1,MMP-9 and MMP-13 Levels in rheumatoid patients according to associated diseases

Type of associated diseases	**No.**	**MMP1 mean concentration Mean SD± SE±(IU/ml)**	**MMP9 mean concentration Mean SD± SE±(IU/ml)**	**MMP-13 mean concentration Mean SD± SE±(IU/ml)**
Rheumatoid arthritis patients with diabetes	11	174.9 ±(3.2)±(9.7)	171.3±(3.4)±(1)	163.2±(2.8)±(8.6)
Rheumatoid arthritis patients with hypertension	7	164.4±(3.4)±(9.7)	173.2±(4.5)±(1.7)	146.6±(3)±(1.1)
Rheumatoid arthritis patients only	46	171.2±(3.3)±(4.9)	168.2±(3.1)±(4.6)	171.4±(4.4)±(6.5)
Rheumatoid arthritis patients with Other disease	5	192.4±(7.6)±(3.4)	171.6±(3.8)±(1.7)	175.5±(5.1)±(2.2)
P value		0.6	0.9	0.4
Total	69	172±(3.7)±(4.4)	169.5±(3.3)±(4)	1.967±(4.1)±(4.9)

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Correlation between MMP1,MMP9 , MMP-13 levels in rheumatoid patients

The correlation between MMP1, MMP9 and MMP-13 levels in rheumatoid patients, was showed in table (8) . The results showed highly positive correlation between MMP-1 and MMP-9 levels (P=0.001) . MMP1 belongs to the subfamily of MMP called collagenases while MMP9 belongs to the subfamily of gelatinases . Both of them work on common substrate such as collagen VII and additional substrates such as aggrecan and gelatin [6].

Table 8. Correlation between MMP1,MMP9 and MMP-13 levels in rheumatoid patients

correlation	**MMP1**	**MMP9**	**MMP-13**
MMP1 **R/P value**	 ----------	P=0.001 R= 0.5	P=0.9 R= -0.004
MMP9 **R/P value**	P= R=	 ----------	P=0.1 R= 0.1
Total	**69**		

Conclusion

The study concludes that high levels of MMP-1, 9 ,13 may be correlated with the development of rheumatoid arthritis disease, while MMP1 is associated with the early detection of rheumatism.

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