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# MATRIX METALLOPROTEINASES-3 (MMP-3) SERUM LEVEL AND GENETIC POLYMORPHISMS ASSOCIATED WITH RHEUMATOID ARTHRITIS

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**Abstract.** *Introduction*. MMP-3 plays a crucial role in the process of bone erosion in the pathomechanism of rheumatoid arthritis (RA). It acts by removing the outer osteoid layer, which allows the osteoclasts to tightly connect and carry out the subsequent damage to the underlying bone. MMP-3 can trigger the production of other MMPs like MMP-1, MMP-7, and MMP-9, it plays a pivotal role in the remodeling of connective tissues. Aim of the study: to assess the influence of MMP-3 serum levels and single-nucleotide polymorphisms of rs679620 in the rheumatoid arthritis patients' group in comparison to the control group. Subjects: eighty eight samples, 45 rheumatoid arthritis patients after being referred by their treating physician for regular RA test. The remaining 43 samples all represent apparently healthy people. The present study investigated the serum concentration of MMP-3 and rs679620 SNPs in the group of patients with RA, in comparison to the control group. *Results*. The results indicated a significant elevation in MMP-3 levels in RA patients in comparison to healthy individuals (12.75±0.38 vs 9.69±0.37) and the findings of rs679620 SNPs appeared that the patient group has a non-significant increase in both allele frequency A and genotype frequency AA when compared to the control group (66.2 vs 52.2%; p = 0.172; OR = 1.79 and 35.3 vs 17.4%; p = 0.229; OR = 2.59), but a non-significant decrease in both allele frequency C and genotype frequency CC when compared to the control group (2.94 vs 4.4%; p = 1.0; OR = 0.67 and 2.9 vs 4.3%; p = 1.0; OR = 0.67), as well as a non-significant decrease in allele frequency G and both genotypes frequency GG and AG when compared to the control group (30.9 vs 43.5%; p = 0.233; OR = 0.58, 0.0 vs 8.7%; p = 0.159; OR = 0.12 and 61.8 vs 69.6%; p = 0.585; OR = 0.71). Patients carrying the AA and AG genotype, had significantly higher serum levels of MMP-3 compared to control (P = 0.005 and 0.004) respectively. *Conclusion*. Rs679620 may influence joint destruction via increase MMP-3 production.

***Key words:*** *Rs679620, MMP-3, RA patients, autoimmune diseases, matrix metalloproteinase, gene polymorphism.*

## УРОВЕНЬ МАТРИКСНОЙ МЕТАЛЛОПРОТЕИНАЗЫ-3 (MMP-3) В СЫВОРОТКЕ И ГЕНЕТИЧЕСКИЙ ПОЛИМОРФИЗМ, СВЯЗАННЫЙ С РЕВМАТОИДНЫМ АРТРИТОМ

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**Резюме.** *Введение.* MMP-3 играет решающую роль в процессе эрозии кости в патогенезе ревматоидного артрита (РА), которая истощает внешний остеоидный слой, что позволяет остеокластам плотно соединяться и далее повреждать подлежащую костную ткань. MMP-3 может запускать выработку других MMP, таких как MMP-1, MMP-7 и MMP-9, и играет ключевую роль в перестройке соединительных тканей. Цель исследования: оценить влияние уровня MMP-3 в сыворотке крови и однонуклеотидного полиморфизма rs679620 в гене

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MMP-3 в группе больных ревматоидным артритом по сравнению с контрольной группой. Материалы: изучены восемьдесят восемь образцов от 45 пациентов с ревматоидным артритом, направленных лечащим врачом на регулярное обследование на РА. Остальные 43 образца получены от практически здоровых людей. В настоящем исследовании изучалась сывороточная концентрация MMP-3 и SNP rs679620 в группе пациентов с РА по сравнению с контрольной группой. *Результаты.* Показано значительное повышение уровней MMP-3 у пациентов с РА по сравнению со здоровыми людьми (12,75±0,38 против 9,69±0,37), а уровень представленности rs679620 в группе пациентов был незначительно повышен для аллеля A и генотипа AA по сравнению с контрольной группой (66,2 против 52,2%; p = 0,172; ОШ = 1,79 и 35,3 против 17,4%; p = 0,229; ОШ = 2,59), но незначительное снижена как для частоты аллеля C, так и частоты генотипа CC по сравнению с контрольной группой (2,94 против 4,4%; p = 1,0; ОШ = 0,67 и 2,9 против 4,3%; p = 1,0; ОШ = 0,67), а также и недостоверно снижена частоты аллеля G и частота обоих генотипов GG и AG по сравнению с контрольной группой (30,9 против 43,5%; p = 0,233; ОШ = 0,58, 0,0 против 8,7%; p = 0,159; ОШ = 0,12 и 61,8 против 69,6%; p = 0,585; ОШ = 0,71). Пациенты с генотипами AA и AG имели значительно более высокие уровни MMP-3 в сыворотке крови по сравнению с контролем (P = 0,005 и 0,004 соответственно). *Вывод.* Rs679620 может влиять на разрушение суставов за счет увеличения продукции MMP-3.

***Ключевые слова:*** *Rs679620, MMP-3, больные РА, аутоиммунные заболевания, матриксная металлопротеиназа, полиморфизм генов.*

## Introduction

Extracellular matrix (ECM) components can be degraded by a group of zinc-dependent endopeptidases known as matrix metalloproteinases (MMPs) [24]. Since ECM breakdown is linked to embryonic development and angiogenesis, it is of critical importance. It also plays a role in cellular healing and tissue remodeling. Abnormal degradation of the ECM can result from changes in MMP expression. This is the root cause of diabetes-related vascular problems and other chronic degenerative illnesses [6]. Matrix metalloproteinases (MMPs) have been linked to chemokine activation and leukocyte infiltration during inflammatory responses [12]. MMP family has been implicated in tumor cell invasion and metastasis [23]. Rheumatoid arthritis (RA), is a persistent inflammatory disease that predominantly impacts the synovial membrane lining of the joints that ultimately lead to joint destruction [22]. In rheumatoid arthritis, your immune system attacks the tissue lining the joints on both sides of your body, other parts of the body may also be affected [16]. The risk of developing rheumatoid arthritis has been associated with HLA-DRB1 which contain 5 amino acids known as "shared epitope" [8]. Researchers are especially interested in viral causes [11][13] and immunity had been suggested to be involved in the pathophysiology of of autoimmune diseases [2][20]. RA patients have elevated levels of matrix metalloproteinase-3 (MMP-3), also called (stromelysin-1). Based on screenings conducted over the past three decades, it appears that blood levels of MMP-3 predict disease outcome and medication response and positively reflect RA disease activity, bone and joint injury, and radiographic erosion [17]. The gene of MMP-3, which is part of a cluster of MMP genes, is located on human chromosome 11q22.3. During tissue remodeling, MMPs have a role in both normal physiological processes like reproduction and embryonic development and pathological processes like arthritis and tumor spread. The majority of MMPs are released in their inactive pre-protein form and become active only after being cleaved by extracellular proteinases. In the joints, there are synovial fibroblasts and chondrocytes, produce the proteinase MMP-3. It plays a crucial role in the degeneration of joints in RA patients. Proteoglycans, fibronectin, laminin, and elastin, as well as collagens type II, III, IV, IX, and X, are all targets of the MMP-3 enzyme. Because MMP-3 can trigger the production of other MMPs like MMP-1, MMP-7, and MMP-9, it plays a pivotal role in the remodeling of connective tissues [5].

## Materials and methods

*Sample collection*. In total, 88 samples were taken, 45 rheumatoid arthritis patients (18 in high disease activity and 27 in moderate disease activity, from the Baghdad-Teaching Hospital/Baghdad Province were enrolled in this study after being referred by their treating physician for regular RA tests [14]. The remaining 43 samples all represent apparently healthy people (with age and sex matched with the patients). 5 ml of venous blood was obtained from the subject. After letting 3 ml of blood clot for 30 minutes at room temperature (25°C) [25], then centrifugation for 15 minutes at 3000 RPM/min, the serum was collected and frozen at –20°C for further use in ELISA procedure, according to [21]. Serological examination of Human matrix metalloproteinases (MMP-3) concentrations was applied using (MMP-3) sandwich ELISA. The remaining 2 ml was placed in an EDTA tube for genomic tests involving the MMP-3 gene polymorphisms Rs679620. In terms of research ethics, the current study was greenlit in accordance with the Helsinki Declaration on the Human Protection in Experimental Research (World Medical Associa-

**Table 1. The condition and primer information of Rs679620 MMP-3 gene polymorphisms**

| Primer | | Sequence | Target gene | Size of product |
|---|---|---|---|---|
| rs679620 | F | 5'-CTTGCTTTGGAACAGCTTCAG-3' | MMP-3 (Chen et al., 2012) | 634 bp |
| | R | 5'-CTCTTCCCAGACTTTCAGAGC-3' | | |

tion2013). To identify the existence of chronic and inflammatory ailments among all participants, a questionnaire was employed, and those individual with such conditions were subsequently not included in the study's results [15].

*DNA extraction*. DNA extraction was performed according to the manufacture instructions of (HiGenoMB, HIMEDIA, India), the protocol of DNA extraction from whole blood: The level of DNA samples that showed an adequate of level integrity was estimated by using a Nanodrop spectrophotometer (Thermo Fisher Scientific) with basic computerized software control and data recording, which was preceded by the use of TE buffer as a blank solution. 2 microliters of DNA were loaded to the Nanodrop to determine the concentration in ng/µl. The concentration was in the range of 40–120 ng/µl. In the DNA purity, the absorbance of the sample was measured at (260 and 280 nm) wavelengths using the nanodrop spectrophotometer. A260/A280 ratios between 1.7 and 1.9 indicate the presence of pure DNA.

*Primer preparation*. The NCBI-primer blast website was utilized for the primer design process. The information and condition of the primers were presented in Table 1.

*Polymerase chain reaction*. All PCR reactions were carried out in a 25 µl final volume and according to the manufacturer's instructions. A quantity of 25 µl of the reaction mixture (12 µl of green master-mix, 2 µl of DNA, 2 µl of primers 9 µl of nuclease free water) was added to each PCR tube. Following this, the tubes were sealed and subjected to a brief centrifugation to remove any air pockets and spin the contents down. The tubes were then transferred onto a miniopticon PCR thermocycler. Primer annealing temperature optimization: by applying gradient temperature (54, 55, 56, 57, 58, 59, 60, 61, 62, 63 and 64)°C, until reach to optimum annealing temperature. The annealing temperature of 62°C for MMP-3 was optimum for producing clear and sharp bands in agarose gel (Fig.). The PCR cycler conditions were set according to the optimum primer annealing temperature and PCR Go Taq Green Master Mix kit instructions

*Statistical analysis*. Prior to computing the mean, Student's T-test and standard deviation, the normality, homogeneity, and distribution of the parametric data assessed using the software version 26.0 of the IBM SPSS application. A level of significance of 0.05 was used to assess the statistical significance of the calculated probability. Pearson's coefficient of chi_ square was employed to compute the probability associated with the not parametric data. In addition, the odd ratio, 95% confidence interval and Fisher's exact probability were calculated by WinPepi version 11.65 [1] for the genotyping and alleles frequencies. Such for the genotyping and alleles frequencies calculations, an online Hardy-Weinberg calculator was used [4].

## Results and discussion

*Serum level of MMP-3*. The result showed that concentrations of MMP-9 were significantly elevated in RA patients as appeared in Table 2.

Table 2 shows that MMP-3 concentrations were significantly elevated in patients ($P = 0.000003$), this is indicate that MMP-3 plays a pivotal role in bone destruction and cartilage components degradation in RA [10]. Matrix metalloproteinase 3 (stromelysin-1) is a protein-degrading enzyme that has been linked to RA-related joint destruction through its ability to degrade collagen (XI, IX, V, IV and III types), matrix proteins, and proteoglycans, as well as to activate other pro-MMPs (7,8 and 9) [19]. Serum matrix metalloproteinase-3 (MMP-3) is an objective, practical, and disease-specific marker of ongoing illness and joint damage in rheumatoid arthritis (RA) patients, as shown here; these findings are consistent with those of [9], who found that MMP-3

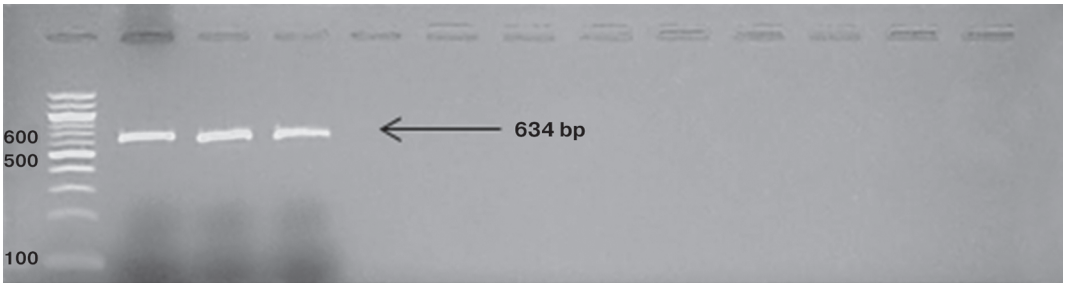


**Figure. Gel electrophoresis for MMP-3 PCR product (Agarose 2%, at 100 volts for 45 min) visualized under U.V. light after staining with Red Safe Stain**

**Table 2. MMP-3 level in Patients and control groups**

| Group | MMP-3 level means±SE (pg/ml) | | Probability |
|---|---|---|---|
| | Patients group | Control group | |
| Total | 12.75±0.38 | 9.69±0.37 | 0.000003 |

**Note.** * is significant at P ≤ 0.05.

**Table 3. Serum levels of MMP-3 in relation to CDAI score in RA patients**

| Disease activity | MMP-3 level means±SE (pg/ml) | | Probability |
|---|---|---|---|
| | Patients males | Patients females | |
| High | 12.73±1.21 | 13.49±0.87 | 0.649 |
| Moderate | 13.23±0.45 | 12.21±0.50 | 0.432 |
| Probability | 0.793 | 0.156 | |

**Note.** * is significant at P ≤ 0.05.

**Table 4. The Hardy-Weinberg equilibrium (HWE) and frequency distribution of the MMP-3 gene genotypes, rs679620, in blood samples from the patient and control groups**

| Genotyping of MMP-3 rs679620 | Patients group No. (%) (n = 34) | | Control group No. (%) (n = 23) | |
|---|---|---|---|---|
| | Observed | Expected | Observed | Expected |
| AA | 12 (35.3) | 14.89 (43.8) | 4 (17.4) | 6.3 (27.2) |
| AC | 0 (0.0) | 1.32 (3.9) | 0 (0.0) | 1.0 (4.5) |
| CC | 1 (2.9) | 0.03 (0.09) | 1 (4.3) | 0.04 (0.2) |
| AG | 21 (61.8) | 13.9 (40.9) | 16 (69.6) | 10.4 (45.4) |
| CG | 0 (0.0) | 0.62 (1.8) | 0 (0.0) | 0.9 (3.8) |
| GG | 0 (0.0) | 3.24 (9.5) | 2 (8.7) | 4.4 (18.9) |
| Total | 34 (100.0) | 34 (100.0) | 23 (100.0) | 23 (100.0) |
| P-HWE | Uncountable | | Uncountable | |

**Notes.** P-HWE: Probability of Hardy-Weinberg equilibrium. I degree of freedom (d.f.) for Chi-squared distribution.

**Table 5. Genotype and allele frequencies of MMP-3 gene rs679620 of patients group and control group in blood samples**

| Genotyping of MMP-3 rs679620 | Patients group No. (%) (n = 34) | Control group No. (%) (n = 23) | OR (95% CI) | Fisher's exact probability |
|---|---|---|---|---|
| A | 45 (66.2) | 24 (52.2) | 1.79 (0.84–3.83) | 0.172 |
| C | 2 (2.94) | 2 (4.4) | 0.67 (0.09–4.82) | 1.0 |
| G | 21 (30.9) | 20 (43.5) | 0.58 (0.27–1.25) | 0.233 |
| AA | 12 (35.3) | 4 (17.4) | 2.59 (0.73–9.15) | 0.229 |
| AC | 0 (0.0) | 0 (0.0) | – | Uncountable |
| CC | 1 (2.9) | 1 (4.3) | 0.67 (0.04–10.66) | 1.0 |
| AG | 21 (61.8) | 16 (69.6) | 0.71 (0.23–2.13) | 0.585 |
| CG | 0 (0.0) | 0 (0.0) | – | Uncountable |
| GG | 0 (0.0) | 2 (8.7) | 0.12 (0.01–2.59) | 0.159 |
| OR: odd ratio, 95% CI: 95% confidence intervals | | | | |

**Table 6. Level distribution of MMP-3 in patients group and control group according to the genotypes (A/C/G rs679620)**

| Genotyping of MMP-3 rs679620 | MMP-9 level mean±SE (pg/ml) | | Probability |
|---|---|---|---|
| | Patients group | Control group | |
| AA | 10.89±0.72 B | 7.04±0.60 B | 0.005 |
| AC | – | – | – |
| CC | 12.72 AB | 12.71 AB | Uncountable |
| AG | 13.33±0.59 A | 10.39±0.42 A | 0.004 |
| CG | – | – | – |
| GG | – | 6.23 | – |
| ANOVA table: the similar letters referred to a non-significant difference (P > 0.05) among the genotyping of the same group. | | | |

was an important indicator for evaluating RA disease, stratifying disease activity, and predicting prognosis [18]. MMP-3 could be useful as a biological markers for rheumatoid arthritis disease activity assessment [3].

According to disease activity the MMP-3 mean values were insignificantly correlated with CDAI score in RA patients (Table 3), since both stages showed an increment with enzyme compared to healthy individuals. While Ahmed and Salloom. Indicated that patients with a high disease activity score had greater mean values of MMP-3 than those with a moderate or low disease activity score [2].

*MMP-3 gene (rs679620).* The SNP of MMP-3 gene (rs679620); located on Chromosome11 (chr11:102842889) was expected with six genotypes (AA, AC, CC, AG, CG and GG) and three alleles (A, C and G) in patients and control group. It was observed that genotypes frequencies in both groups of subjects disagree with Hardy–Weinberg (H-W) equilibrium because more than one genotypes were absent in both studied groups, so P value was uncountable. The genotypes (AC, CG and GG) weren't observed in patients group, while the two genotypes (AC and CG) were absent in control group (Table 4).

*P-HWE: Probability of Hardy-Weinberg equilibrium. I degree of freedom (d.f.) for Chi-squared distribution*. The patient group has a non-significant increase in both allele frequency A and genotype frequency AA when compared to the control group (66.2 vs 52.2%; p = 0.172; OR = 1.79 and 35.3 vs 17.4%; p = 0.229; OR = 2.59), but a non-significant decrease in both allele frequency C and genotype frequency CC when compared to the control group (2.94 vs 4.4%; p = 1.0; OR = 0.67 and 2.9 vs 4.3%; p = 1.0; OR = 0.67), as well as a non-significant decrease in allele frequency G and both genotypes frequency GG and AG when compared to the control group (30.9 vs 43.5%; p = 0.233; OR = 0.58, 0.0 vs 8.7%; p = 0.159; OR = 0.12 and 61.8 vs 69.6%; p = 0.585; OR = 0.71). Table 5 also showed that the AA and A allele exhibited a higher value of odd ratio (2.59 and 1.79) respectively, so it might be consider as a potential risk factor, while the genotypes CC, AG, GG and alleles C and G showed a lower value (0.67, 0.71, 0.12, 0.67 and 0.58) respectively, and this reflecting a protective property of these factors

The present findings appeared that there was a significant difference between the AA genotype and AG genotype in both studied groups. The two genotypes AA and AG showed a significant increase level of MMP-3 in the patients group compared to the control (10.89±0.72 vs 7.04±0.60 and 13.33±0.59 vs 10.39±0.42 pg/ml). In both patient and control groups the AG genotype was observed with the highest mean (13.33±0.59 and 10.39±0.42 pg/ml) respectively, compared to other genotypes (Table 6).

Patients and control carrying the AG genotype, had significantly higher serum levels of MMP-9 compared to AA genotype with in both studied groups. Patients carrying the AA and AG genotype, had significantly higher serum levels of MMP-3 compared to control (P = 0.005 and 0.004) respectively, so Rs679620 may influence joint destruction via increase MMP-3 production which was also associated with more severe joint damage. This agree with Chen et al. who indicated that Rs679620 have an important role in determining the circulating levels of MMP-3 in RA, and that MMP-3 polymorphism is associated with the level of disease activity over time [7].

## Conclusion

Rs679620 may enhance MMP-3 production and joint destruction. Patients carrying the AA and AG genotype, had significantly higher serum levels of MMP-3 compared to control. AA and A allele exhibited a higher value of odd ratio (2.59 and 1.79) respectively, so they might be consider as potential risk factors, while the genotypes CC,AG,GG and alleles C and G showed a lower value of odd ratio (0.67, 0.71, 0.12, 0.67 and 0.58) respectively, and this reflecting their protective properties.

## Additional information

**Ethical approval.** This study was approved by Baghdad University/College of Science Ethics committee (Ref: CSEC/0922/0079). Everyone signed a study-related informed consent. In compliance with the Helsinki Declaration, all human rights have been observed.

**The Declaration of Competing Interest.** The authors have declared no conflict of interest.

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