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Validity of Serum Matrix Metalloproteinase 9 in Diagnosis of Rheumatoid Arthritis: A Case-Control Study from Iraq

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Abstract
Objective: To assess validity of serum matrix metaloprotinase 9 (s.MMP 9) in diagnosis of rheumatoid arthritis (RA)**Patients and methods:** This case control study was conducted on 50 RA patients (10 males and 40 females) diagnosed by a rheumatologist according to American College of Rheumatology (ACR) 1987 revised criteria or ACR- European League Against Rheumatism (EULAR) 2010 Criteria and compared with 25 healthy controls (5 male and 20 female) matched in age and sex. Demographic and clinical characteristics of patients were recorded. S.MMP9 was measured using Enzyme Linked Immunosorbent Assay (ELISA) KIT in both groups.**Results:** No significant difference between patients and controls in age, sex, and body mass index (BMI) ($p>0.05$). Serum MMP9 was significantly higher in patients with RA than controls (188.4 ± 24.5 ng/ml vs 21.98 ± 7.87ng/ml, $p<0.05$).The optimum cut-off value of serum MMP9 that can differentiate between patients with RA from controls was 78.522ng/ml using Receiver Operating characteristic curve (ROC) test, with P value <.0001 and AUC was 1.00) with nearly 100% sensitivity and specificity. At cut-off value ≥34.038 we got the highest sensitivity 100% with specificity 96% and Accuracy 98.7%. And At cut-off value ≥125.479 we had got the highest specificity 100% with sensitivity 98% and Accuracy 98.7%.**Conclusions:** serum MMP9 was significantly higher in patients with RA than healthy controls. Serum MMP9 was a valid measure to differentiate between RA patients and healthy controls. This may be beneficial for early diagnosis of RA and subsequent a new promising treatment.
Keywords: Matrix metalloproteinase 9. Rheumatoid arthritis, inflammatory arthritis, Matrix metalloproteinase

1. Introduction

Rheumatoid arthritis (RA) is common disabling autoimmune disease characterized by progressive joint disorder, significant pain, and functional disability [1]. Genetic factors, including the human leukocyte antigen (HLA) shared epitope, hormonal factors, and environmental exposures as tobacco smoke or infectious agents may predispose to the development of RA [2-5]. Rheumatoid arthritis primarily starts as a state of persistent cellular activation leading to autoimmunity and immune complexes in both joints and other organs where it manifests [6]. Various phases of progression of RA include: Initiation phase due to non-specific inflammation, amplification phase due to T cell activation, and chronic inflammatory phase with tissue injury due to cytokines like interleukin 1 (IL1), Tumor Necrosis Factor-alpha(TNF-alpha) and interleukin 6 (IL–6) [7].

Matrix metallopeptidase 9 (MMP-9) is a matrixin, a class of enzymes that belong to the zinc-metalloproteinases family involved in the degradation of the extracellular matrix. In humans the MMP9 gene encodes for a signal peptide, a propeptide, a catalytic domain with inserted three repeats of fibronectin type II domain followed by a C-terminal hemopexin-like domain [8]. They are strongly involved in normal and pathological processes [9]. Several studies have reported significant association of MMP9 with RA [10-12]. This study was designed to evaluate the validity of serum MMP-9 in the diagnosis of RA.

2. Patient and Method

2.1 Study design

This case control study was conducted at the Rheumatology Consultation Clinic of Baghdad Teaching Hospital / Medical City from February 2015 to the end of April 2015.Ethical permission to conduct the research was obtained from Department of Medicine and a signed consent was taken from all patients and controls to admit the study.

2.2 Sample selection

A total of 50 Iraqi patients with RA diagnosed by a rheumatologist according to ACR 1987 revised criteria or ACR-EULAR 2010 Criteria [13, 14] compared with 25 healthy control subjects matched in age and sex were enrolled in the study. Patients were excluded from the study if they had overlapping connective tissue disease, or other inflammatory arthritis or those who have other comorbid diseases.

2.3 Clinical and laboratory evaluation

Full history and clinical examination were done for all participants in the study. Complete blood count (CBC)

and erythrocyte sedimentation rate (ESR), rheumatoid factor and anticitrolinated peptide antibody (ACPA), C reactive protein were recorded. Serum MMP9 was measured using enzyme linked Immunosorbent assay (ELISA) KIT (MYBIOSOURSE UNITED STATE).

2.4 Statistical Analysis

Statistical analysis was done using statistical package for social sciences version 18 (SPSS V.18, Chicago, IL, USA). Anderson darling test was used to assess the normality of distribution of continuous variables. Data that follow normal distribution presented by mean and standard deviation. The normally distributed variables were compared by independent t-test mean. Receiver operator curve (ROC) was used to find a cut off value for MMP-9 to see the validity of MMP9 in separating patient from normal control and area under the curve i.e. AUC and its p value prescribe this validity (if AUC ≥ 0.9 mean excellent test, 0.8 – 0.89 means good test, 0.7 – 0.79 fair test otherwise unacceptable) in addition to other validity parameters: Sensitivity, Specificity, Positive predictive value (PPV), Negative predictive value (NPV). P-values less than 0.05 were considered statistically significant.

3. Results

The mean of age of patients was 45.5 ± 14.6 year and the mean of age of controls was 46.1 ± 11 year (P = 0.84). The patients were 40 females (80%) and 10 males (20%), and in controls 20 females (80%) and 5 males (20%) (P = 1.00). Mean body mass index (BMI) for patients was 24.94 ± 2.28 kg/m^2 and for controls was 24.5 ± 1.06 kg/m^2 (P = 0.27) .

Mean serum MMP9 was statistically and significantly higher in patients with RA (188.4 ± 24.5ng/ml) than controls (21.98 ± 7.87ng/ml), p<0.0001 as figure 1.

In table 1 the cut-off value of serum MMP9 that can differentiate between patients with rheumatoid arthritis from controls was 78.522ng/ml using ROC (Receiver Operator curve) test, with P value <.0001 and AUC was 1.00.

Table 2 shows that at a cut-off value ≥34.038ng/ml we got the highest sensitivity 100% with specificity 96% and Accuracy 98.7%, and at cut-off value ≥125.479 ng/ml we got the highest specificity 100% with sensitivity 98% and Accuracy 98.7%.

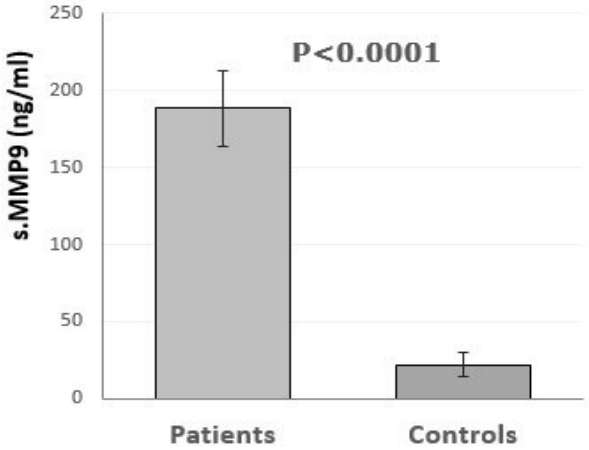

Figure1: Comparison of S. MMP9 level between patients and controls**.** sMMP9 serum matrix metallo proteinase enzyme 9.

Table 1: Cut –off value of S. MMP9 level that can differentiate between patients and controls using ROC test**.**

Variable	AUC (95%CI)	P value	Optimum Cut off value
Serum MMP9	1.00	<.0001	78.522ng/ml

ROC (Receiver Operating Curve), AUC (Area Under Curve), MMP9(Matrix Metallo Proteinase 9).

Table2: Validity parameters for S. MMP9 when used as a test to differentiate between patients and controls**.**

Positive if ≥Cut off value	Sensitivity	Specificity	Accuracy	PPV at pretest probability =		NPV at pretest probability
				50%	90%	10%
≥34.038ng/ml (highest sensitivity)	100%	96%	98.7%	96.2%	99.6%	100.0%
≥125.479ng/ml (highest specificity)	98%	100%	98.7%	100%	100%	99.8%

PPV, Positive Predictive Value; NPV, Negative Predictive Value.

4. Discussion

This case control study evaluated validity of s.MMP9 for diagnosis of RA. It showed that mean serum level of

MMP-9 was significantly higher among patients with RA than healthy people. At the optimum cut-off value of ≥78.522ng/ml, serum MMP9 can significantly differentiate between patients with RA from controls. In addition, at a cutoff value ≥34.038ng/ml, s.MMp9 has highest sensitivity and at cut off ≥125.479ng/ml we got the highest specificity. This is clinically significant because it can help us in early diagnosis and subsequently early treatment.

Several studies have reported significant association between s.MMP9 and RA however the validity in diagnosis of RA has not been mentioned. Kim et al [15] assessed which MMPs, among the gelatinases MMP-2 and MMP-9 and the collagenases MMP-1 and MMP-13, play a more proactive role in the angiogenic process in arthritic joint. They suggested that in RA, MMP-9 and MMP13 may play more important role in angiogeneic process of arthritic joint than MMP2 and MMP1.

Xue et al [16] investigated the effect of endogenous MMP-9 on the invasive characteristics of RA synovial fibroblasts and concluded that MMP-9 contributed to RA synovial fibroblast survival, proliferation, migration and invasion, with potent effects. Additionally, MMP-9 stimulated RA synovial fibroblast-mediated inflammation and degradation of cartilage. This indicate that MMP-9 derived from RA synovial fibroblasts may directly contribute to joint destruction in RA.

de Rooy et al [17] identified the genetic risk factors for the severity of joint damage in RA by studying genetic susceptibility loci of several autoimmune diseases and demonstrated that two loci that confer risk to other autoimmune diseases also affect the severity of joint destruction in RA. Rs11908352 may influence joint destruction via MMP-9 production.

Recently, Shevchenko et al [18] studied the promoter regions of the matrix metalloproteinase (MMP9 genes to assessed their associations with the risk of RA and with the types of its clinical course in women and found that presence of the allelic variants of the MMP genes may be one of the genetic factors that predispose to RA in women.

The current study has some limitations: First, the small sample size of the participants and second the short duration of the study. However, this can be solved by a larger sample with a longer follow up prospective study. The strength of the study is strict inclusion criteria for the individuals to enroll in the study in addition to being the first study that assessed validity of MMP9 in diagnosis of RA.

In conclusion, serum level of MMP9 was a valid biomarker to differentiate between RA patients and controls. These results may help us in early diagnosis of RA and subsequently promising new medications for a better treatment.

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Conflict of Interest: None

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