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USE OF SOME BONE-RELATED CYTOKINES AS PREDICTORS FOR RHEUMATOID ARTHRITIS SEVERITY BY NEURAL NETWORK ANALYSIS

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Abstract. *Background.* Rheumatoid arthritis (RA) is characterized by synovial membrane inflammation that results in joint damage. Many earlier studies have measured cytokines for a better diagnosis of RA. In the present study, three bone biomarkers [osteopontin, stromelysin-1 (MMP3), and vascular endothelial growth factor-A (VEGF)] are examined for their ability to estimate the severity of disease by using artificial neural network (NN) analysis and binary logistic regression analysis. *Methods.* The study enrolled 87 RA patients and 44 healthy control subjects. The biomarkers were measured by the enzyme-linked immunosorbent assay technique. Disease Activity Score (28 joints) and C-reactive protein (CRP) (DAS28-CRP) was calculated by using DAS28-CRP calculator. The patients with DAS28-CRP ≥ 5.1 αre considered as having high disease activity (HDA). While patients' group with DAS28-CRP < 5.1 are considered as moderate disease activity (MDA). The neural network (NN) analysis was used for the differentiation between groups. *Results.* Results showed that the most sensitive predictor for high disease activity (HDA) of RA is MMP3, followed by osteopontin and VEGF. These three biomarkers can differentiate significantly between HDA and MDA with a relatively high size effect (Partial $\eta^2 = 0.323$, $p < 0.001$). The HDA group has a significantly higher MMP3, CRP, RF, and anti-citrullinated protein antibodies (ACPA) than the MDA group. MMP3 is strongly associated with two inflammatory indicators; CRP and ESR. *Conclusion.* There was a significant elevation in the serum level of MMP3 in RA patients with HDA compared to the MDA and control groups. High DAS28, RF, CRP, and ACPA were found in HDA patients compared with the MDA group. The use of the NN analysis indicated that the measured biomarkers help predict the HDA state in RA patients. MMP3 and osteopontin are diagnostic biomarkers for the severity of RA and are related to many disease-related characteristics with a sensitivity of 88.9% and specificity of 68.4%.

Key words: *rheumatoid arthritis, inflammation, neural network analysis, stromelysin-1, ACPA, osteopontin.*

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ИСПОЛЬЗОВАНИЕ РЯДА ЦИТОКИНОВ, АССОЦИИРОВАННЫХ С КОСТНОЙ ТКАНЬЮ, В КАЧЕСТВЕ ПРЕДИКТОРОВ ТЯЖЕСТИ РЕВМАТОИДНОГО АРТРИТА ПРИ ПОМОЩИ НЕЙРОСЕТЕВОГО АНАЛИЗА

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Резюме. *История вопроса.* Ревматоидный артрит (РА) характеризуется воспалением синовиальной оболочки, приводящего к повреждению суставов. Многие более ранние исследования оценивали уровень цитокинов для улучшения диагностики РА. В настоящем исследовании для оценки тяжести заболевания с использованием нейронной сети и бинарного логистического регрессионного анализа были исследованы три костных биомаркера: остеопонтин, стромелизин-1 (MMP3) и фактор роста эндотелия сосудов А (VEGF). *Методы.* В исследовании приняли участие 87 больных РА и 44 здоровых человека контрольной группы. Уровень биомаркеров определяли методом иммуноферментного анализа. Показатель активности заболевания (28 суставов) и С-реактивный белок (CRP) (DAS28-CRP) рассчитывали с помощью DAS28-CRP-калькулятора. Пациенты с DAS28-CRP ≥ 5,1 считаются имеющими высокую активность заболевания (ВАЗ), в то время как при DAS28-CRP < 5,1 заболевание расценивается как умеренно активное (УАЗ). Нейросетевой анализ использовался для дифференциации между группами. *Результаты.* Результаты исследования показали, что наиболее чувствительным предиктором высокой активности заболевания (HDA) РА является MMP3, за которым следуют остеопонтин и VEGF. Эти три биомаркера могут существенно дифференцировать HDA и MDA с относительно высокой эффективностью (частичный $\eta^2 = 0{,}323$, $p < 0{,}001$). Группа с ВАЗ имеет значительно более высокий уровень MMP3, CRP, RF и антител к цитруллинированному белку (АСРА), чем группа с УАЗ. MMP3 тесно связан с двумя индикаторами воспаления: СРБ и СОЭ. *Выводы.* Отмечалось значительное повышение уровня MMP3 в сыворотке крови и уровней у пациентов с РА с ВАЗ по сравнению с группой с УАЗ и контрольной группой. Высокие уровни DAS28, RF, CRP и АСРА были обнаружены у пациентов с ВАЗ по сравнению с группой пациентов с УАЗ. Использование нейросетевого анализа показало, что измеренные биомаркеры помогают прогнозировать ВАЗ у пациентов с РА. MMP3 и остеопонтин являются диагностическими биомаркерами тяжести заболевания РА с чувствительностью 88,9% и специфичностью 68,4% и связаны со многими характеристиками заболевания.

Ключевые слова: *ревматоидный артрит, воспаление, нейросетевой анализ, стромелизин-1, АСРА, остеопонтин.*

Background

Rheumatoid arthritis (RA) usually begins between the ages of 30 and 50 years old. Women, smokers, and those with a family history of the disease are at higher risk. It causes discomfort and stiffness in various joints, most commonly the wrists, proximal interphalangeal joints, and metacarpophalangeal joints. RA rarely affects the distal interphalangeal or lumbar spine. Patients may also have weariness, weight loss, and anemia [55]. RA is an autoimmune-inflammatory illness that often affects females and older individuals. It is characterized by joint discomfort that develops with time as a result of an autoimmune-inflammatory response [34]. Despite the fact that the cause of RA is yet unknown, a set of environmental and genetic aspects is attributed [54]. A number of parameters have been evaluated as potential predictors of RA diagnosis, prognosis, or follow-up of RA disease. Because RA is characterized by an inflammation of the synovial membrane that leads to the reduction and destruction of joints [3, 36], adhesion molecules, soluble mediators, pro-inflammatory and anti-inflammatory cytokines [33, 53, 57], trace elements [1], adipokines [35] and various impacts of autoantibodies on joint inflammation and internal organ dysfunction, as well as structural abnormalities, are known [14]. Since the findings of the majority of criteria are not completely clear and definitive, the estimation of various inflammation-related cytokines continues to be an attractive subject of research. In the present work, the study focused on some of the less studied cytokines in RA including osteopontin (OPN), Stromelysin-1 (MMP3), and the vascular endothelial growth factor-A (VEGF). An OPN-rich extracellular matrix is present in mineralized tissues and extracellular fluids, especially at sites of inflammation [15, 61], where OPN combines osteoclasts and hydroxyapatite to enhance bone resorption [18]. In mice modeled with rheumatoid arthritis, OPN plays a critical role in the destruction of articular cartilage by promoting angiogenesis and inducing apoptosis in chondrocytes [60]. Earlier research showed a correlation between elevated levels of OPN and serum levels of inflammation markers, an increase in monocyte inflammatory molecules [63], and the number of T-helper 17 cells in the synovial fluid of RA patients [6, 37]. Furthermore,

in people with RA, plasma OPN was thought to be a biomarker of inflammatory bone damage [20]. Numerous studies have indicated that the OPN and its receptors, play key roles in RA pathogenesis [62].

The MMP3 enzyme is involved in the degradation of cartilage and bones and the destruction of extracellular matrix components in RA [41]. MMP3 level has been elevated in the synovium of RA patients where MMP3 concentration is around 250 times higher [7, 42]. A high MMP3 level correlates with the number of affected joints [59], and it could be used as a biomarker for both the diagnosis and the progress of the disease [7, 25]. MMP3 may be a biomarker for the disease, and it helps to break down extracellular matrix proteins in RA as the disease progresses. [39]. Serum levels of MMP3 were a sign of RA disease activity, bone and joint damage, medication susceptibility, and the outcome of the disease [25, 27]. After these findings, several researchers have recommended MMP3 testing as part of a routine examination to be used in conjunction with RA therapy choices [25].

There is an abundant expression of VEGF in synovial fluid and serum of RA patients [22] [58], which plays a key role in pannus formation and maintenance [58]. Patients with RA have high levels of VEGF in their blood and synovial fluid [58], which aids in the growth of blood vessels and their absorption by the synovial lining membrane in RA [31]. In a meta-analysis, significantly higher circulating VEGF levels in patients with RA were identified, and levels of VEGF are positively correlated with disease activity in RA [24]. The present study aims to use the abovementioned biomarkers (OPN, MMP3, and VEGF). in the current study to test the ability to differentiate between high disease activity (HDA) the moderate disease activity (MDA) of RA disease by using the artificial neural network analysis (NN) and binary and multivariate logistic analysis.

Methods

Participants. Eighty-seven RA patients (37 males and 50 females) and 44 age-matched healthy control subjects (18 males and 26 females) were recruited in the present case-control study. The samples were collected from the Ramadi Teaching Hospital in the Governorate of Anbar in Iraq, from June 2020 to January 2021. The European League Against Rheumatism and the American College of Rheumatology guidelines were used to diagnose RA in diseased subjects [2]. According to these diagnosis criteria, each patient should have a score > 6 based on the amount and location of painful joints, favorable serologic findings (anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF)), high C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)), and duration of RA symptoms. These criteria were found to be more accurate in predicting the likelihood of RA and have a higher specificity [9]. The clinical characteristics and sociodemographic data of all study subjects have been collected. Body mass index (BMI) was calculated by dividing subjects' body weight (kilograms) by their height squared (squared meter).

The Disease Activity Score was calculated by using (DAS28-CRP) calculator available online at https://www.mdcalc.com/disease-activity-score-28-rheumatoid-arthritis-crp-das28-crp. We divided patients into those with HDA when DAS28-CRP ≥ 5.1 and those with MDA when DAS28-CRP < 5.1 [52]. The institutional review board (IRB) of the University of Anbar supplied the ethical approval of the study (Document number 211C/2020), which complied with the "International Guideline for Human Research" guidelines established by the Declaration of Helsinki. A thorough medical profile was taken on all participants to rule out any existing systemic disorders that may affect the results of the studied parameters, especially diabetes mellitus, liver and kidney diseases, and cardiovascular incidents. The first step to ensure the work's quality is to recruit patients who have no kidney problems. All patients had normal urea and creatinine. These results are important to exclude any excretion of small-molecular weight proteins by diseased kidneys. Subjects that smoked were also excluded from the study.

Power analysis, using a 2-tailed test at $\alpha = 0.05$ and assuming an effect size of 0.5 with a power of 0.80, shows that the required sample size is 127 participants as calculated by the sample size calculator (https://www.ai-therapy.com/psychology-statistics/sample-size-calculator). Therefore, we used more than the calculated number (131 subjects). The sample size analysis was accomplished according to the equations and principles mentioned in Fahim et al. (2019) [11]. Patients were included by a convenience sampling technique.

Measurements. Five milliliters of venous blood were extracted without a tourniquet from all subjects, after overnight fasting, and centrifuged at 3000 rpm for 15 minutes after full clotting. Sera were separated and stored at −80°C until they were analyzed. Based on the latex agglutination concept, serum CRP and RF were assessed using semi-quantitative kits provided by the Spinreact® Co., Girona, Spain. Kits supplied by Hotgen Biotech Co., Ltd., Beijing, China, were used to conduct a semi-quantitative ACPA examination. Sandwich ELISA assay kits provided by Mybiosource®, Inc., CA, USA, were used to assess serum MMP3, OPN, and VEGF. All of the kits' intra-assay coefficients of variance were less than 10%. The sensitivities of the ELISA kits were OPN < 0.1 pg/ml, MMP3 < 0.068 ng/ml, and VEGF < 1 pg/ml.

Statistical analysis. Kolmogorov–Smirnov test revealed that all biomarker findings were normally distributed. As a result, all data are provided in terms of mean and standard deviation. To compare meas-

ured variables across categories, the analysis of variance (ANOVA) test was performed. While the Chi-square (2) test was employed to determine the statistical significance of the difference between nominal variables. Pearson's correlation analysis was utilized to evaluate correlations between biomarkers and clinical and demographic variables. We used Point-Biserial Correlation Analysis to analyze the correlation between dichotomous variables (CRP, RF, and ACPA) and continuous variables (biomarkers). The "multivariate general linear model" (GLM) was used to examine associations between RA severity and measured biomarkers while adjusting for confounding factors such as age and BMI. The influence of each biomarker on the severity of rheumatoid arthritis was then determined using a between-subject effects test. The partial eta-squared (η^2) effect size was used in the study. Based on the levels of the biomarkers, various z-unit weighted scores were determined. The essential biomarkers that predict indeed observed biomarkers were evaluated using multiple regression analysis. We have used NN processing to evaluate the predictability of the existence of RA in a subject using input variables and biomarkers. This analysis used an artificial feedforward model of two hidden layers of mini-batch training and gradient descent. The stopping criteria were one successive move with no further decrease in the error expression. The research sample was split into three categories: training, testing, and holdout. The reference for the correlation coefficient is the values that are not due to the chance at $p < 0.05$. The correlation coefficient was regarded as low when it ranged from 0.10 to 0.29, moderate when it ranged from 0.30 to 0.49, and high when it ranged from 0.50 to 1.0 [8]. Area under the curve (AUC) cut-off value should be more than 0.5. The tests are two-tailed, with a statistical significance level of 0.05. The IBM SPSS package for Windows 10, Version 25, 2017, IBM Corp., New York, USA was used for biostatistical analysis.

Results

Demographic and clinical biomarkers. The results in Table 1 showed the demographic details of RA patients in comparison with those of healthy controls. There were no statistically significant variations in BMI or age between the groups. Table 1 also indicates that serum MMP3, RF, CRP, and ACPA levels were substantially higher in RA patients compared

Table 1. The clinical and demographic data in high disease activity (HDA) rheumatoid arthritis patients and moderate disease activity (MDA) and healthy controls (HC)

Variables	HC^{A} (n = 44)	DAS < 5.1^{B} (n = 34)	DAS ≥ 5.1^{C} (n = 53)	F/χ^2	p
Age, years	48.41±5.26	48.09±5.25	49.72±6.01	1.084	0.341
Sex (Male/Female)	18/26	13/21	24/29	1.147	0.292
MI^{B}, kg/m^2	24.38±2.90	25.70±2.86	25.59±3.19	2.533	0.083
DAS28	N/A	3.81±0.79C	7.70±0.74^{B}	185.254	< 0.001
MMP-3, ng/ml	11.50±5.01^{B,C}	17.31±8.20^{A,C}	22.29±9.76^{A,B}	21.712	< 0.001
VEGF, pg/ml	202.97±56.35^{B,C}	128.92±42.82^{A}	132.29±45.35^{A}	31.941	< 0.001
OPN, ng/ml	3.03±1.20^{C}	3.82±1.66	4.25±1.87^{A}	6.897	0.001
Disease duration, years	0^{B,C}	12.65±4.71^{A}	11.06±5.13^{A}	123.133	< 0.001
CRP –/+	44/0^{B,C}	29/5^{A,C}	19/34^{A,B}	52.305	< 0.001
RF –/+	44/0^{B,C}	28/6^{A,C}	20/33^{A,B}	47.806	< 0.001
ACPA –/+	44/0^{B,C}	28/6^{A,C}	20/33^{A,B}	47.806	< 0.001

Notes. A, B, C: pair-wise comparisons (when a letter written on a number, it means that this number is significantly different from the values of the column that contain the letter over the column title), BMI: Body mass index, DAS28: Disease Activity Score-28, MMP3 (Stromelysin-1): Matrix metalloproteinase-3, VEGF: Vascular endothelial growth factor, RF: rheumatoid arthritis, CRP: C-reactive protein, ACPA: and anti-citrullinated protein antibodies, and OPN: osteopontin. The difference is considered significant when $p \leq 0.05$.

Table 2. The results of multivariate GLM analysis examining the differences in biomarkers between RA patients divided into those with high disease activity (HDA) and moderate disease activity (MDA)

Tests	Dependent variables	Explanatory variables	p	Partial η^2
Multivariate	All 3 biomarkers	Severity (HDA vs MDA)	< 0.001	0.323
		Sex	0.352	0.102
		BMI	0.002	0.163
		Age	0.152	0.063
Between-subject effects	MMP3	Severity (HDA vs MDA)	0.002	0.161
	VEGF	Severity (HDA vs MDA)	0.249	0.037
	Osteopontin	Severity (HDA vs MDA)	0.162	0.023

Note. Diagnosis: RA versus healthy controls, BMI: Body mass index, MMP3: Matrix metalloproteinase-3, and VEGF: Vascular endothelial growth factor.

Table 3. The binary logistic regression analysis results with high disease activity (HDA) and moderate disease activity (MDA) as dependent variables and bone biomarkers as explanatory variables

Dependent variables	Explanatory variables*	B (SE)	p	OR	95% CI
MDA versus HDA	MMP-3	0.107 (0.037)	0.004	1.113	1.036–1.197
	VEGF	0.011 (0.007)	0.138	1.011	0.997–1.025
	Osteopontin	0.450 (0.168)	0.007	1.568	1.128–2.180

Note. (*): Standardized values, OR: Odd ratio, SE: standard error, CI: confidence interval, MMP3: Matrix metalloproteinase-3, VEGF: Vascular endothelial growth factor.

to the control group with the highest level in DAS ≥ 5.1 patients' group. Serum VEGF showed a significant decrease in both patient groups in comparison with the control groups. While no significant difference between both patient groups. Patients with DAS ≥ 5.1 have significantly higher OPN than those with DAS < 5.1 and control groups. However, there was no substantial difference in the duration of disease between RA patient groups.

The results of multivariate GLM. Table 2 shows the effects of the multivariate GLM study, which showed that age (Partial $\eta^2 = 0.063$, $p = 0.152$) and sex (Partial $\eta^2 = 0.102$, $p = 0.352$) have no substantial impact on the three biomarkers. BMI had a slight effect on the levels of the three biomarkers (Partial $\eta^2 = 0.163$, $p = 0.002$). The severity of RA has a highly significant effect (Partial $\eta^2 = 0.323$, $p < 0.001$) on the biomarker levels. Between-subject tests revealed that the severity had the greatest impact on MMP3 (partial $\eta^2 = 0.161$, $p = 0.002$). OPN and VEGF demonstrated insignificant effects on the severity of the disease with a very small effect size.

Intercorrelation matrix. The most notable correlations were a significant moderate negative correlation between MMP3 and ACPA ($r = -0.379$, $p < 0.01$). The duration of illness is moderately related to VEGF ($r = 0.325$, $p < 0.01$) and OPN ($r = 0.383$, $p < 0.001$). CRP has a moderate negative correlation with VEGF ($r = -0.357$, $p < 0.01$) (results are not tabulated).

Results of binary logistic regression analysis. The binary logistic regression test recruited HDA state as a dependent variable (and MDA as the reference group) is seen in Table 3. The regression discriminated severity of RA patients and found that MMP3 and OPN substantially discriminated both study groups ($\chi^2 = 20.116$, $df = 3$, $p < 0.001$). The Nagelkerke's effect size was 0.350, and the classification precision was 74.2%, with a sensitivity of 66.7% and a specificity of 81.8%.

Effects of background variables. The univariate GLM analysis was used to assess the impact of medications on the serum levels of the studied parameters in RA patients. The study found no significant effects of naproxen, sulfasalazine, prednisolone, tofacitinib, or methotrexate on the blood levels of the three biomarkers tested. The other medications prescribed have little discernible impact. The cumulative effects of medication administration on the assessed parameters were just minor (partial $\eta^2 = 0.033$) (results are not tabulated).

Results of neural networks. As shown in Table 4, the neural network analysis was used to distinguish between individuals with HDA and MDA. One unit was employed in hidden layer 1, whereas hyperbolic tangent was used as an activation function, identity in the output layer, and sum of squares was used as an error term in the final neural network. Because the testing set had a significantly smaller sum of squares (5.331) and a lower relative error (23.6 percent versus 47.8 percent, respectively), the neural network model was able to generalize the trend. As a result, the holdout set had a 66.7 percent relative inaccuracy. As seen in Table 4, the sensitivity was 88.9%, and the specificity was 68.4%. The cut-off values of the serum biomarkers concentration at the above sensitivity and specificity were: MMP3 ≥ 17.75 ng/ml, VEGF ≥ 124.95 pg/ml, OPN ≥ 3.814 ng/ml. As shown in Figure, the input variables are ranked by relevance and relative importance, MMP3, OPN, and VEGF were the three most important predictors of the model's predictive power.

Table 4. The Results of neural networks with high disease activity (HDA) RA versus moderate disease activity (MDA) as reference group

	Models	HDA vs MDA
Input layer	Number of units	3
	Rescaling method	Normalized
Hidden layers	Number of hidden layers	1
	Number of units in hidden layer 1	3
	Activation Function	Hyperbolic tangent
Output layer	Dependent variables	HDA vs MDA
	Number of units	2
	Activation function	Identity
	Error function	Sum of squares
Training	Sum of squares error term	10.358
	% Incorrect or relative error	23.6%
	Prediction (sensitivity, specificity)	75.0%, 69.6%
Testing	Sum of Squares error	5.331
	% Incorrect or relative error	47.8%
	Prediction (sensitivity, specificity)	88.9%, 68.4%
	AUC ROC	0.81
Holdout	% Incorrect or relative error	66.7%
	Prediction (sensitivity, specificity)	60.0%, 45.5%

Note. AUC ROC: area under the curve of receiver operating curve.

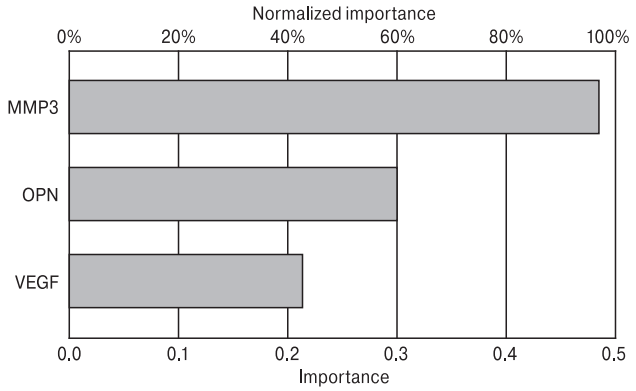

Figure. The importance of the biomarkers for differentiation between severity states of the rheumatoid arthritis patients by neural network analysis

Discussion

The current study's major finding is the elevation in serum MMP3 in RA patients when compared with the control group as seen in Table 1. The increase of MMP3 in RA is in accordance with other previous works [27, 29, 32, 48] that can be attributed to the secretion of MMP3, in higher amounts, from the synovial joints [42]. The contribution of pro-inflammatory cytokines in RA's pathogenesis is widely accepted [16]. Therefore, MMP3 has been proposed as a marker of inflammation in RA [41]. However, at high levels, MMP3 causes increases in the number of osteoclast precursors and osteoclast formation, resulting in inducing bone resorption [5]. Furthermore, MMP3 is correlated with the number of inflamed and painful joints in RA patients [59]. Serum MMP3 may be a possible diagnostic for histological synovitis and RA diagnosis [28], joint erosions in the early stages of the illness, and disease progression monitoring [26, 48]. MMP3 levels in the blood are an excellent predictor of bone damage, and MMP3 inhibition may be a significant treatment approach for individuals with early rheumatoid arthritis [47]. According to recent research, serum MMP3 levels predict clinical remission more accurately than CRP levels [17]. These results prompted several researchers to recommend MMP3 testing as part of a standardized evaluation to go along with RA treatment options [25].

In addition, a neural network method allowed for external validation of the clinical diagnosis of the HDA of RA against MDA with an AUC ROC curve of 0.814 and MMP3 and OPN as the most important discriminatory variable. Macrophage populations in synovial tissue are associated with articular damage, and a decrease in macrophage counts is a sensitive biomarker of therapy response in rheumatoid arthritis patients [56].

The multivariate GLM analysis was performed to estimate the effect of the cofounders on the serum levels of the measured parameters. As seen in Table 2, age and sex had no substantial impact on the levels of the assessed biomarkers. BMI has a slight effect on the variance of the serum level of the measured bone-related cytokines. The levels of biomarkers are substantially altered only by the magnitude of the severity in the RA patients, with a larger effect size (partial $\eta^2 = 0.323$). Tests for between-subject effects showed that 16.1 percent of the difference in the MMP3 concentration was attributed to RA. These analyses were performed to rule out the influence of cofounders on the values of the estimated parameters because there are some reports about the effect of BMI on MMP3 level [4], decreased expression of VEGF with increased patient age [12], and the effect of sex and age on OPN level [21]. However, these correlations were reported in diseases other than RA.

MMP3 is secreted by synovial fibroblasts or B cells, which are well-known MMP3 producers, that are responsible for the large rise in plasma levels [46]. The previously published study demonstrated a tight link between plasma OPN levels and MMP3 levels. Plasma OPN levels decreased considerably in responders after medication [20].

Table 3 showed that the severity of the RA is associated with the plasma level of MMP3. MMP3 is strongly associated with two inflammatory indicators; CRP and ESR [45]. These findings revealed a correlation between the levels of bone-related cytokines and biomarkers of inflammation. Other researchers, however, demonstrated that elevated serum biomarkers could not be a risk factor for reduced bone mineral density [44]. The same reasons apply: the inflammatory state associated with RA is the primary source of biomarker changes. ACPA levels are influenced by serum MMP3 levels. Previously, ACPA was found to have the best predictive importance for the production of RA [38, 51]. A strong association between ACPA positivity and arthritis development has also been established in several patients who later experienced RA [40]. Therefore, the increase in serum MMP3 may enforce the validity and sensitivity of ACPA in the diagnosis of RA in addition to estimating the severity of the RA disease [40].

There is a good predictive value of the serum MMP3 for the severity of RA subjects. MMP3 is a connective tissue biomarker. However, the diagnostic cut-off value is rather high. MMP3 expression is a good indicator of disease activity in people with rheumatoid arthritis [30]. MMP3 levels rose with advanced stage and RA class and gradually dropped following treatment effectiveness [49]. Serum MMP3 levels were shown to be strongly linked with serum CRP, RF levels, and joint damage [26]. Additionally, there was a statistically significant association between MMP3 and CRP, and ESR [17]. Elevated blood MMP3 levels in rheumatoid arthritis patients show inflammation [10, 32] and serve as an early predictor of increasing joint destruction as well as a powerful predictive indicator of rheumatoid

arthritis disease activity [10, 29]. In another study, serum concentrations of MMPs significantly correlated with markers of RA activity such as DAS28 and CRP levels [23].

Recently, it was shown that MMP3 serum levels correspond with the quantity of MMP3 generated by synovial mast cells [19] in inflamed joints, indicating the severity of rheumatoid synovitis [13]. With increasing RA severity, MMP3 and ACPA increase indicating the correlation of both biomarkers with the degree of disease activity [43, 50]. Therefore, the measured biomarkers, especially MMP3 can be used as a potential biomarker for RA activity with acceptable sensitivity and specificity. The main limitation of the current analysis is the limited size of the test sample. To ensure adequate generalization of the study findings, a greater sample size would be needed. The second drawback is the relatively high inter-assay coefficient of variance percent, which was less than 10% for all kits.

Conclusion

There was a significant elevation in the serum level of MMP3 in RA patients with HDA compared to the MDA and control groups. The NN analysis showed that the MMP3 is the most sensitive predictor for RA patients with HDA followed by OPN. In addition to the known high DAS28, RF, CRP, and ACPA in HDA patients, the binary logistic regression showed the potential use of OPN and MMP3 as differentiating factors for HDA from MDA.

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