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Study the Effect of Methotrexate and Etanercept Therapies on the Serum Levels of Galectin-3 and Matrix metalloproteinase-3 in Iraqi Patients with Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder characterized by inflammatory joint damage, leading to stiffness, swelling, and pain. The biomarkers galectin-3 (Gal-3) and matrix metalloproteinase-3 (MMP-3) have been implicated in RA pathogenesis. This study aimed to observe the effect of Methotrexate (MTX) and Etanercept (ETA) medications on serum Gal-3 and MMP-3 levels of Iraqi RA patients. Gal-3 and MMP-3 were measured in sera of 92 RA patients and 38 control using ELISA assay. Patients were categorized into groups according to received treatment: MTX, ETA, combined MTX plus ETA, and untreated patients (Un-T). The clinical disease activity index (CDAI) was calculated for patients. The Gal-3 levels for MTX reduced significantly to control (P=0.031) and ETA (P=0.002). MMP-3 level elevated in ETA than control (P=0.005), and decreased in MTX than ETA (P=0.008). MTX monotherapy decreases the Gal-3 and MMP-3 levels without attaining the desired mild activity. ETA monotherapy reduces Gal-3 levels to within the normal range and achieves mild disease activity, but it does not significantly affect MMP-3 levels. Both biomarkers are useful for monitoring the effectiveness of MTX treatment more accurately than simply observing disease activity. Gal-3 and MMP-3 association was slightly affected in treatment groups but still statistically significant.

Keywords: Etanercept, Galectin-3, Matrix metalloproteinase-3, Methotrexate, Rheumatoid arthritis.

دراسة تأثير علاجات الميثوتريكسيت والايتانرسبت على مستوى مصل الكالكتين -3 و الماتركس ميتالوبروتينيز -3 لمرضى عراقيين بالتهاب المفاصل الرثوي

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الخلاصة:

يعد التهاب المفاصل الروماتويدي RA هو اضطراب التهابي مناعي ذاتي مزمن يتميز بتلف المفاصل ويؤدي الى التيبس , التورم والالم. وقد ثبت تورط العلامات الحيوية الجالكتين-٣ (Gal-3) و الماتريكس ميتالوبروتينيز-٣ (MMP-3) في التسبب في مرض الـ RA. هدفت الدراسة الى ملاحظة تأثير علاجي الميثوتريكسيت (MTX) والايتانرسبت (ETA) على مستوى مصل الـ Gal-3 والـ MMP-3 لمرضى

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عراقيين بالتهاب المفاصل الروماتويدي حيث تم قياسهما في مصل 92 مرضى و 38 من الاصحاء بتقنية الاليزا. تم تقسم المرضى الى مجاميع تبعا للعلاج المستهلك الى: MTX, ETA, , خليط من (MTX مع ETA) مع مجموعة مرضى بدون علاج. تم قياس نشاط المرض السريري لجميع المرضى. اظهرت النتائج ان مستوى الـ Gal−3 منخفضاً في الـ MTX مقارنة بالمجموعة الضابطة (P=0.031) ومجموعة العلاج ETA (P=0.002) . ارتفع مستوى MMP−3 في ETA بالمقارنة مع الكونترول (P=0.005) وانخفض في MTX بالمقارنة بـETA (P=0.008) . تستخلص الدراسة بأن العلاج الاحادي MTX يقلل من الـ Gal−3 والـ MMP−3 دون تحقيق نشاط خفيف للمرض المرغوب به. يخفض العلاج الاحادي ETA من مستوى الـ Gal−3 الى القيم طبيعية ويعطى نشاط خفيفا للمرض ولكنه لا يوثر بشكل كبير على مستوى الـ MMP−3 . كلا العلامتين الحيوية هي مفيدة لمراقبة نشاط دواء الـMTX بشكل أكثر دقة من مجرد مراقبة نشاط المرض.تأثر الارتباط مابين الـ Gal−3 والـMMP−3 بشكل طفيف في مجاميع العلاج ولكنها لاتزال ذات اهمية احصائية.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease of unknown etiology, that causes joint stiffness, swelling and pain leading to disability and poor quality of patient's life [1]. The disease primarily affects articular joints, particularly the wrists, hands, and feet, with less frequent non-articular organs [2]. Epidemiological data indicate a global prevalence of 0.5-1% [3], with similar rates (approximately 1%) observed in the Iraqi population [4]. Both genetic and ecological factors are involved in the pathogenesis of RA and implicated in the activation the immune cells [3]. Many biomolecules like galectin-3 (Gal-3) [5] and matrix metalloproteinase-3 (MMP-3) [6] are implicated in RA pathogenicity. Gal-3 belongs to a group of lectins family proteins called galectins that recognize the β-galactoside structures of carbohydrates at the cell surface and extracellular environment [7]. It is known as a chimera-type, structurally different from other members of galectins, which contains only one preserved carbon recognition domain (CRD) and one non-lectin N-terminal domain that facilitates its oligomerization [8]. Gal-3 can meditate many processes during inflammations and activate the immune response through cell-to-cell or cell-to-matrix communications [5]. It elevates in membranes of human synovial and synovial fibroblasts in arthritic joints and in serum RA patients. Further, it is associated with the severity of the disease [8]. The MMP-3 belongs to matrix metalloproteinase enzymes that are stimulated in response to some cytokines releasing. It is responsible for the irreversible devastation of joint tissues including cartilage, bone and tendons [2]. It serves as a strong predictive marker for the disease activity of RA and the gradual joint damage [9].

The growing comprehension of RA pathophysiology has enabled the creation of various treatment approaches. Those treatments are "nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs)" [10], [11]. The DMARDs treatment comprised conventional synthetic (cs-DMARD), targeted synthetic (t-DMARD) and biological (b-DMARD) [10]. The first line of cs-DMARD treatment is methotrexate (MTX). It is widely used to treat both rheumatic and non-rheumatic diseases including malignant [12]. It is an antagonist to folic acid and inhibits the dihydrofolate reductase enzyme [13]. The European League Against Rheumatism (EULAR) guidelines on pharmacological therapy recommend taking MTX either alone or in combination with another cs-DMARD or with a low dosage of glucocorticoids unless there are no interferences [14]. The patients who did not achieve a low or remission level of disease activity are advised to be treated by b-DMARD such as Etanercept (ETA) even if they are receiving the cs-DMARDs treatment such as MTX [15]. ETA is a fusion protein of the recombinant human tumor necrosis factor (TNF-α) receptor which is a safe and effective treatment for patients with active RA. It blocks the cell-surface receptor of TNF-α and acts as a competitive inhibitor for

the TNF-α thus suppressing its activity in RA patients' joints [16]. In addition, the combination MTX with ETA could greatly reduce the disease activity and the radiographic changes, thus improving the physical activity of patients [17].

The patients with RA who test negative for RF and/or ACPA may still experience disease progression and bone erosions without receiving adequate attention. The absence of these traditional serological tests in this subset of patients poses a challenge in initiating early and appropriate treatment. Therefore, research into alternative biomarkers is necessary and required for both the disease diagnosis and also for monitoring the efficacy of the received treatments. The current study stated a hypothesis that supposes some anti-rheumatic drugs may have some effect on the serum level of Gal-3 and MMP-3 and aimed to observe the effect of monotherapies of MTX and ETA, and also the combined treatment of (MTX+ ETA) on the serum levels of Gal-3 and MMP-3 biomarkers in patients with RA.

2. Materials and Methods

2.1 Patients and Control

This study involved a convenience sample of 92 RA patients, divided into four groups based on their treatment types. The cohort included 18 males (19.5 %) and 74 females (80.5%) who met the RA classification criteria of the 2010 American College of Rheumatology /European League Against Rheumatism (ACR/EULAR) [18]. Participants were recruited at the outpatient clinic of Baghdad Teaching Hospital between November 2023 to April 2024. The control group were 38 healthy persons termed as HG composed of 10 males (26%) and 28 females (73%) their age matched to RA patients. Patients' samples were collected on three kinds of DMARDs, MTX was 20 (22%), ETA 40 (43%), combined ETA and MTX medication 25 (27%) and the remaining patients: 7 (8%) were never addressed termed as (Un-T) group.

2.2 Exclusion criteria

The study excluded patients with comorbid conditions such as acute and chronic infections, malignancies, serious pulmonary disease, hepatic disease, thyroiditis and other overlapping autoimmune diseases like Psoriasis/psoriatic arthritis, Multiple sclerosis MS, Inflammatory bowel disease IBD, Systemic lupus erythematosus SLE, Type 1 diabetes T1DM.

2.3 Materials

The kits utilized in this study included the human Galectin-3 ELISA kit (BT LAB Bioassay Technology Co., Cat. No. E1951Hu), human matrix metalloproteinase-3 ELISA kit (BT LAB Bioassay Technology Co., Cat. No. E0907 Hu), human anti-Cyclic Citrullinated Peptide Antibody ACPA ELISA kit (Elk Biotechnology Co., Ltd Cat. No. ELK9202). C-reactive protein CRP (NycoCard™ CRP Ref. 1116807 Abbott Diagnostics Technologies AS). ESR was according to haematology siemens system (Siemens Healthineers, Germany) analyzer, Rheumatoid Factor RF kit: SPINREACT, S.A./S.A. U Ctra. Santa Coloma (Ref. no.1200202).

2.4 Methods

Blood samples were collected from participants and allowed to clot at room temperature for 15 min, followed by centrifugation for 5 min in 3000 rpm/mint. The serum was then separated, aliquoted into multiple 0.5 mL Eppendorf tubes, and stored at -4°C until the completion of the RA diagnostic tests and at -80°C for subsequent enzyme-linked immunosorbent assay ELISA analysis. Clinical laboratory tests were measured during the collection of sample period for all participants including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and rheumatoid factor RF according to their manufacturer's references. The clinical disease activity index (CDAI) was evaluated [19], and demographic information was obtained. Gal-3, MMP-3, and ACPA antibody levels were assessed using the ELISA method according to their manufacturer's references.

2.5 Statistical Analysis

SPSS version 24 software was used for statistical analysis of the obtained data which were introduced as (mean ± SD), median and interquartile range (IQR) or frequency and percentage n (%). Significant differences between the two groups were analyzed using a T-test, either assuming equal variances or unequal variances based on their variances assessed by Levene's test for equality of variances. For comparisons involving more than two groups, either One way ANOVA test for significant differences of equal variances of groups or the Welch test for unequal variances was appropriate to use. The significant difference was considered as $p < 0.05$.

3. Results

3.1 Demographic and clinical characteristics of study participants

As shown in Table 1, demographic variables such as age, gender, BMI, and serum Gal-3 levels did not differ significantly between patients and healthy controls. However, high statistical differences were observed between the studied groups in terms of the traditional laboratory tests for RA disease ESR, CRP, RF, ACPA and serum level of MMP-3.

Table 1: The demographic and clinical characteristics of Rheumatoid Arthritis patients and healthy subjects

Demographic Variables	**RA patients (n= 92)**	**Healthy group (n=38)**	**P value**
Age (years) Mean ±SD	44.13 ±11.517	45.16 ±9.42	0.627
Gender: n (%) Male Female	18 (19.5%) 74: (80.5 %)	10 (26%) 28 (73%)	0.394
Disease duration (year) Median (25^{th}-75^{th} percentiles)	7 (4-11.75)	-	-
CDAI score Median (25^{th}-75^{th} percentiles)	16 (10.25-22)	-	-
BMI (kg/m^2) Mean ±SD	30.16 ± 6.04	28.64 ± 4.75	0.169
RF: n (%) Negative Positive	37 (40%) 55 (60%)	38 (100%) 0	**< 0.0001**
ESR (mm/ hour) Median (25^{th}-75^{th} percentiles) Male: Female:	16.5 (8.75-51.5) 25 (15- 42.75)	9 (6 - 12) 16 (13.25 - 20)	**0.00**7 **< 0.0001**
CRP (mg/L) Median (25^{th}-75^{th} percentiles)	5.5 (2.4 - 8.6)	2 (0.87- 3.2)	**< 0.0001**
ACPA (ng/ml) Median (25^{th}-75^{th} percentiles)	27.25 (18.3-34.82)	19.9 (14.7-23.7)	**< 0.0001**
Gal-3 (pg /ml) Median (25^{th}-75^{th} percentiles)	460.7 (347.4 – 629.7)	511.4 (405.3 – 654.3)	0.776
MMP-3 (ng/ml) Median (25^{th}-75^{th} percentiles)	2.7 (2.1 – 3.4)	2.3 (1.9-2.9)	**0.006**
Treatments			
DMARDs Treatment type n (%)	**Dose** Median (25^{th}-75^{th} percentiles)	**Treatment duration** (Mean ±SD) year	
MTX: 20 (22%)	20 (20-25) mg/week	3.1 ± 2.67	
ETA: 40 (43%)	# 50 mg/ 2 weeks	2.31 ± 2.16	
ETA + MTX: 25 (27%)	MTX: 20 (15-25) mg/week Eta: # 50 mg/ 2 weeks	2.62 ± 1.64	

Bold p-values are Significance level at p<0.01, level, # Constant dose of drug for all patients.

3.2 Treatment Groups Results

Both Gal-3 and MMP-3 levels, as presented in Table 2, showed a significant difference within the five studied groups, (control, untreated (Un-T), ETA, MTX, and combination MTX plus ETA).

Table 2: The mean ± SD of Gal-3 and MMP-3 in treatment groups.

Variables	HG (n=38)	Un-T (n=7)	ETA (n=40)	MTX (n=20)	(MTX + ETA) (n=25)	P -value
Gal-3 pg /ml	521.37 ± 185	570.37 ± 304.8	579 ± 232.5	390.7 ± 140	485.2 ± 255.4	**0.03***
MMP-3 ng/ml	2.36 ± 0.79	2.88 ± 1.557	3.33 ± 1.49	2.11 ± 0.995	2.86 ± 1.54	**0.009***
* *Significance level at p<0.05, collected data introduced as a mean± SD.*						

Figure 1a demonstrates that Gal-3 expression was markedly lower in the MTX group versus the control and ETA groups ($p<0.05$), though the control and ETA groups showed comparable Gal-3 levels. Furthermore, the MMP-3 level, as seen in Figure (1b), was elevated significantly in the ETA treatment group when compared to the control and reduced in the MTX group when compared with the ETA group. The other two compared groups showed a non-significant difference.

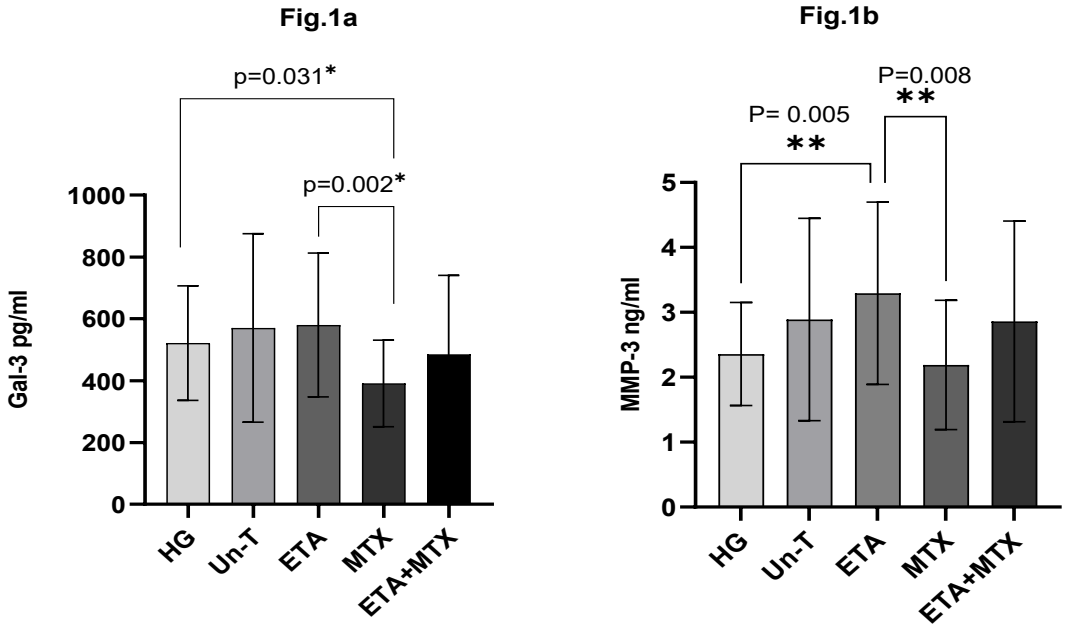

Figure 1: The Bar chart of mean ±SD for Gal-3 and MMP-3 in the studied groups

3.3 Correlation Results between Gal-3 and MMP-3 in Treatment Groups

The association between Gal-3 and MMP-3 revealed a significant strong positive correlation in the control and Un-T groups. However, this relation showed a moderate level of association in three treatment groups (MTX, ETA, and combined treatment) and also the overall RA patient group as seen in Table 3.

Table 3: P and r- values of Pearson coefficient correlation between Gal-3 and MMP-3 in studied groups

Correlation between Gal-3 and MMP-3	Groups					
	HG	Un-T	MTX	ETA	MTX + ETA	All RA patients
r- value	0.81	0.94	0.564	0.633	0.62	0.67
p- value	<0.0001	0.0016	0.01	0.00	0.009	<0.0001

3.4 Disease Activity Levels in Treatment Groups

Patients with RA were stratified into three subgroups based on their disease activity levels. The frequency (%) of each level was 23 (25 %) mild activity, 47(51%) moderate activity, and 22 (24%) severe activity. The mean ± SD of CDAI score in disease levels was (8.13 ± 1.45, 15.89 ± 3.52, and 32.31± 5.89) respectively and showed significant differences (P< 0.001). We found no variations in the mean ±SD of Gal-3 between the mild, moderate and severe levels of disease activity (P=0.064). The mean ± SD was (438.7± 145.2, 566 ±259, and 459.6± 266 pg/ml) respectively. In addition, MMP-3 revealed a nonsignificant difference in disease activity levels (p= 0.269, with mean ± SD was (2.67± 0.9, 3.14 ± 1.56, and 2.64 ± 1.51 ng/ml) respectively.

On the other hand, Table 4 illustrates, the n (%) of each level in the three treatment groups. The chi-square test showed a significant correlation between the type of treatment and the level of disease activity (p=0.02), 50% of patients receiving MTX monotherapy have severe activity of disease while ETA and combined therapies groups showed a percentage of 15 % and 12% respectively. Furthermore, a higher ratio of mild activity (30 %) was noticed in the ETA treatment group and a lower ratio was observed in the MTX group.

Table 4: The n (%) of the RA patients with mild, moderate, and severe disease activity levels in Treatment groups.

Medications Groups	Disease Activity Levels Frequency (%)		
	Mild	Moderate	Severe
ETA (n=40)	12 (30 %)	22 (55 %)	6 (15 %)
MTX (n=20)	3 (15 %)	7 (35 %)	10 (50 %)
ETA + MTX (n=25)	7 (28 %)	15 (60 %)	3 (12 %)
The data introduced as n (%).			

4. Discussion

Either monomer or generated oligomers of Gal-3 in the extracellular, cytoplasmic, or nuclear microenvironment can cause tissue inflammation [5]. It stimulates different cytokines and chemokines including IL-6 and MMP-3 [20]. In general, we found non-significant differences in Gal-3 levels between RA patients and healthy individuals, and this result contradicts that obtained in several previous studies that found elevated levels of Gal-3 in RA patients more than in healthy people [21-23]. Notably, Mendez-Huergo et al. demonstrated reduced Gal-3 concentrations in patient groups compared to control subjects. The researchers reported that this decrease could be possibly due to DMARD and /or corticosteroid therapies [24]. Our results discovered that Gal-3 serum level was decreased in patients who received the MTX treatment at the recommended dosage (median 20 mg/week) in comparison to both control and ETA treatment groups. This lower level could be attributed to the antirheumatic effect of the MTX drug that inhibits the expression of Gal-3 in RA patients. For more evidence, Oshima et al. study reported that the intracellular level of Gal-3 was increased upon

treatment by TNF-α in vitro, and it is consistent with the observed higher concentration in serum and synovial of RA patients of his study [25]. The study also reported that Gal-3 level had returned to levels within the normal range after receiving biologic anti-TNF-α therapy (infliximab) and this situation was sustained for several months. It was concluded that infliximab had a salutary influence on the decreasing level of Gal-3. This result agreed with our finding despite the different types of medications used and supported our hypothesis that suggests some anti-rheumatic drugs may have somewhat effect on the level of Gal-3 as we found it decreased. The MTX treatment is the first-line strategy for the treatment of many autoimmune diseases including RA and systemic sclerosis (SSC) [26, 27]. Our results are consistent with prior clinical research involving different rheumatologic diseases. The study of Sundblad et al. found that Gal-3 serum level was significantly reduced in patients with SSC who were receiving MTX treatment compared to those not treated with such treatments including biologic DMARDs [28]. The mechanism of MTX in decreasing the expression of Gal-3 is still poorly understood but may be the same for both diseases. It was known that Gal-3 has multifunction in different cell tissues such as increasing the induction of IL-2 production in T cells, activation of many types of lymphoid and myeloid cells, stimulation of superoxide oxidative species secretion from neutrophils and monocytes, enhancement of the IL-1 synthesis by monocytes. Further, it stimulates 5-hydroxytryptamine (5-HT) released from mast cells and basophils. Also, it can bridge the cells and extracellular matrix (ECM) to potentate the chemotaxis and reservation of macrophages and neutrophils [29].
Remarkably, the level of Gal-3 was (485.2 ± 255.4) for the combined treatment group (ETA plus MTX) and was statistically decreased insignificantly when looking at to level of ETA monotherapy that is (579 ± 232.5). Also, it was insignificantly elevated when compared to MTX monotherapy that is (390.7 ± 140) as seen in Figure (1.a). At this point, we believe that this oscillation of Gal-3 concentration within the three treatment groups confirms the effect of the MTX drug in the downregulating of Gal-3 expression. On the other hand, the effect of MTX therapy was clear and sufficient to overcome the proinflammatory Gal-3 effect in the combined treatment group, in which the Gal-3 level was at the normal level and showed a non-significant difference with control subjects.

In our study, MMP-3 levels were elevated in RA patients compared to the healthy group. However, MMP-3 levels decreased in patients treated with MTX compared to those receiving biologic ETA therapy, approaching nearly normal levels similar to the control group. Remarkably, the MTX is thought to have an anti-rheumatic effect through the increase in the release of extracellular adenosine [30]. Adenosine is suggested to negatively regulate MMP-3 generation by FLSs. Thus, RA patients who were treated by MTX exhibited a decrease in blood levels of MMP-3 [31, 32]. Supporting our findings, a study by Shiozawa et al. was conducted to find the effect of MTX monotherapy on the disease activity level and evaluated the MMP-3 serum level in RA patients through follow-up of RA patients for three years. It was discovered that the MTX treatment reduces the level of disease activity to the remission stage and the level of MMP-3 was decreased during the follow-up period [31]. On the other hand, in the extracellular space of chondrocytes, Gal-3 stimulates the synthesis of enzymes MMP-3 and ADAMTS5 and the two are responsible for cartilage proteoglycan degradation [33, 34]. Thus, there is a positive correlation between the Gal-3 and MMP-3 as we found in this study. Therefore, the reducing levels of Gal-3 in the MTX therapy group, in turn, affects decreasing the level of MMP-3.

The patients receiving the biologic ETA monotherapy showed higher MMP-3 serum concentration in this study when looking at the normal values of healthy control. On the contrary, a study by Catrina et al. evaluated the level of MMP-3 by monitoring the duration of the effect of the ETA medication. It showed a significant reduction in levels of MMP-3 in RA

patients [35].
Following our findings, the level of MMP-3 in combination treatment was (2.86 ± 1.54) and it insignificantly decreased when compared to its level in ETA monotherapy which was (3.3 ± 1.4). Also, it was insignificantly elevated in comparison to MTX monotherapy which was (2.1 ± 0.99) as seen in Figure (1.b). Although this variation of MMP-3 level within the three groups was statistically non-significant it confirms the antirheumatic effect of MTX treatment. This nonsignificant result of the combined treatment group compared to groups of monotherapies may be due to the interference of the effects of the two medications simultaneously, one of which has a reducing effect of the MMP-3, and the other may reduce the effect of the first medication (MTX) or maintains of its effect on the MMP-3 marker.
Finally, the non-significant difference between the control group and Un-T group of patients may be attributed to those patients who were newly diagnosed with RA disease with a duration < 1 year or may be too small a sample size of the group. Nevertheless, there was an apparent rise in MMP-3 concentration in Un-T group in comparison to normal levels of the control group as seen in Figure (1.b).
Notably, Gal-3 induces MMP-3 in the extracellular environment of chondrocytes and other destructive cytokines which are fundamental for proteoglycan damage in cartilage [8], [34]. Therefore, there was a positive association between Gal-3 and MMP-3 as we found. This study demonstrated that this relationship was affected by the mechanism of the received DMARDs treatments but the exact mechanism of effect is still unknown and needs more investigations and evidence to prove. It remains strong and statistically significant across all patient groups. It differed in the association strength, which was strong in healthy status and became stronger in patients who were never addressed then showed a moderate relationship in RA patients receiving the three different treatments. Nevertheless, these significant correlations across all groups suggest a consistent and preserved association (as indicated by high r values) between Gal-3 and MMP-3, regardless of treatment or control status. The reduction in Gal-3 and MMP-3 levels caused by the mechanism of MTX possibly slightly influences the relationship between those markers without disruption of their association, indicating that these biomarkers may be linked through related pathological pathways in RA disease.

The accurate evaluation of RA disease activity is necessary because the low activity or remission state reflects the improved treatment outcomes for the patient. Our results showed that ETA and combined treatment groups attained a lower percentage of patients having high disease activity levels. Patients who received MTX therapy have a higher ratio of severe activity levels and a low ratio of patients with mild activity as listed in Table 4. Although, the MTX monotherapy achieves a lower concentration of Gal-3 and MMP-3 in serum, However, this treatment fails to achieve the target low disease activity threshold in RA patients. Such discrepancy could be probably due to many other proinflammatory cytokines or interleukins like TNFα, IL-1, IL-12, IL-6, IL-8, IL-17, IL-21, and granulocyte-macrophage colony-stimulating factor (GM-CSF)" and other else that implicated in the pathogenesis of RA disease and related to disease activity [32],[33], [36]. Those proinflammatory variables are related to many genetic and environmental or ROS-related oxidative stress. All above collectively increases the activity of disease. Therefore, early treatment is required and necessary for the controlling of the disease. Gal-3 and MMP-3 were shown and proven they have beneficial characteristics to monitor the efficacy of the received MTX therapy better than to observe the RA disease activity.

Conclusion

The study concludes that MTX monotherapy at the recommended dose exhibits anti-inflammatory effects by reducing serum levels of Gal-3 and MMP-3. ETA monotherapy

effectively lowers serum Gal-3 to normal levels but does not significantly affect MMP-3. When mixing ETA with MTX as a combined therapy the reducing effect of MTX on both Gal-3 and MMP-3 was observed but was statistically insignificant. MTX drug did not attain the desired low level of RA disease but the ETA treatment attained a higher percentage of patients with mild and lower patients ratio with severe disease activity and reduced the activity of the disease. Both Gal-3 and MMP-3 have beneficial features to observe the efficacy of the received MTX therapy more than observation of the disease activity. Further, the reduction in Gal-3 and MMP-3 levels caused by the mechanism of MTX slightly can influence the relationship between those markers in treatment groups without disrupting their association and remain significant within the overall groups of study.

Ethical Clearance

Ethical approval was obtained by the ethical committee at College of Science for Women / University of Baghdad.

Interest Discrepancies

The authors declare that they have no conflicts of interest.

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