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Assessment of the level of collagen type I, II and some elements and their correlation with the types of treatments for patients with rheumatoid arthritis according to disease activity

ShahlaaS. Mahmood[1*], Halla G. Mahmood[2], Mohammed H. Alosami[3]

[1]Laboratory Section, Ministry of Health , Iraq
E.mail: Shahlaa.saleem2109m@comed.uobaghdad.edu.iq
[2]Department of Clinical Biochemistry, College of Medicine, University of Baghdad, Iraq
E.mail: dr.hallaghazi@comed.uobaghdad.edu.iq
[3]Consultant Rheumatologist, Medical City-Baghdad Teaching Hospital CABM, FIBM (Rheum & Medical rehabilitation)/Iraq
E.mail: mohammadalosimi@comed.uobagdad.edu.iq

***Correspondence author:** ShahlaaS. Mahmood (Shahlaa.saleem2109m@comed.uobaghdad.edu.iq)

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease, which mainly involves the joints. RA is prevalent worldwide with increasing prevalence in elderly people. The mechanism of RA pathogenesis is still undefined, and it is interplaying between genetic susceptibility and environmental factors. Although risk factors for RA are not fully established, various studies have focused on the role of trace elements in association with RA. Patients take different types of medicines according to the activity of their disease, so the study focused on the effect of this variety of medicines according to the severity of the disease at the level of these elements. Objective This work aimed to measure serum level of Coll2-1, andsome trace elements in RA patients, study its association with disease activity and/or severity, types of treatment and to determine its ability as a biomarker of the disease status . Method: This study was conducted at Baghdad Teaching Hospital and at Ghazi Al-Hariri Hospital in the Medical City of Baghdad, Iraq, from November 2022 to March 2023. It included 130 Iraqi patients with rheumatoid arthritis. (60) patients treated with disease-modifying anti rheumatic drugs (group A) and 70 patients treated with biological treatments (group B). (98 female and 32 male). Their age range from (35− 65) years. The diagnosis of each case was identified by a clinical examination by a specialist rheumatologist and verified by a radiological and laboratory investigation. Results: The mean levels of Zinc,Copper, Selenium, Collagen type I, and Collagen type II were significantly higher in the group A compared to that in the group B($P \leq 0.05$) .No significant difference ($P \leq 0.05$) was found in the mean concentration of Iron between the two groupsAs in the case of age, sex and body mass index .this results can be considered an important parameter for early detection of rheumatoid arthritis and can also be used to determine the activity of rheumatoid arthritis.study patients according to activity of their disease revealed that patients with severe disease activity had a significantly lower levels of Iron and collagen type-II (p- value =0.001)compared to those with mild to moderate disease activity. Also according to the results Shaw by using Receiver operating characteristic (ROC) curve analysis that a constructed for Iron and collagen type-II levels as predictors for severity of rheumatoid arthritis.Conclusion: This study highlighted the importance of Coll. 2 and certain trace elements as biomarkers for following RA disease activity. Accordingly, it was concluded that the levels of iron and collagen type II could be reliable as good indicators of the severity of rheumatoid arthritis.

Keywords

disease activity; Coll2-1, Rheumatoid arthritis, Biomarkers.

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease of unknown etiology characterized by swelling, tenderness, and destruction of the affected synovial joints, leading to severe disability and premature mortality (1).

It is the most common autoimmune inflammatory arthritis in adults (2). In most patients, RA has an insidious onset. The pathogenesis of RA is not completely understood. An external trigger (e.g., cigarette smoking, infection, or trauma) that triggers an autoimmune reaction, leading to synovial hypertrophy, chronic joint inflammation along with the potential for extra-articular manifestations, is theorized to occur in genetically susceptible individuals. Synovial cell hyperplasia and endothelial cell activation are early events in the pathologic process that progresses to uncontrolled inflammation with subsequent cartilage and bone destruction (3,4). The evaluation of activity in a rheumatic disease has fundamental importance for therapeutic decisions and for the establishment of prognosis in such patients. Although traditionally, this evaluation has been performed in a purely orientation formfrom the physicians and the patients' impressions. Among different methods; the Health Assessment Questionnaire-Disability Index (HAQ-DI) of Stanford, the ACR20- 50-70 scores, the combined disease activity score-28, and (CDAI) are commonly used (4). Articular cartilage is mainly composed of water, collagen (most abundantly type II), proteoglycans (aggrecan), glycoproteins and chondrocytes. A balance between catabolic and anabolic processes normally maintains integrity of tissue; such balance allows keeping the mechanical and physiological properties of cartilage (5). Extensive work over the past decade has identified a significant number of cartilage degradation related biomarkers, including urinary C terminal telopeptides of type II collagen (uCTX-II); serum cartilage oligomeric matrix protein (COMP); serum and urine Coll2−1 and Coll2−1NO2 (6,7). Coll 2−1 is a peptide of 9 amino acids that found to be released in serum as a result of collagen type II degradation not only in osteoarthritis patients but also in rheumatoid arthritis patients (7,8).Trace elements such as selenium (Se), zinc (Zn), and copper (Cu) are cofactors for several antioxidant enzymes which prevent cellular damage caused by free radicals such as superoxide radicals and other reactive oxygen species (9). Cu2+ ions are important structural components of several enzymes such as superoxide dismutase, lysyloxidase, cytochromecoxidase, factor V, and tyrosinase but can also be involved in the generation of free radicals . Increased RA disease activity may be associated with a heightened oxidative burden and these deregulations might be due to alterations in trace element levels (9,10). Therefore, owing to the important role of immune imbalances and antioxidant depletion in RA (10,11) we aimed to assess serum concentrations of trace elementswhich have an influence on both immunological and oxidative status, in patients with RA and also investigate the association between trace element levels, collagen I , II and disease activity .

Subjects and Method: A study was included 130 Iraqi people (60) patients treated with disease-modifying antirheumatic drugs (group A) and 70 patients treated with biological treatments (group B). This study was conducted at Baghdad Teaching Hospital and at Ghazi Al-hariri Hospital in the Medical City of Bagdad, Iraq, from November 2022 to March 2023. five milliliters of blood was aspirated from each patient, divided into two partsAs a result of using more than one technique for the study. transferred into plain tube, Allows 30 minutes to clot , At 2500 rpm, the serum was then isolated by centrifugation for 10 minutes used for measurements by using ELISA technique, Flam Atomic Absorption Spectrophotometry (FAAS) and Grafite Furnace Atomic Absorption Spectrophotometry (GFAAS) to determine these parameters. The disease activity was assessed byCrohn's Disease Activity Index (CDAI) ,in this score Signs, symptoms, and history was taken 7 days ago.All statistical analysis and data reporting were performed using SPSS version 25.Difference of means that is significant according to t- test at $p < 0.05$.

Table 1: Comparison of age, gender, and BMI between the study groups

Patients Characteristics	Study Groups		P- Value*
	Group A n= 60	Group B n= 70	
Age (Years)			
< 40	12 (20.0)	11 (15.7)	0.637
40 - 49	19 (31.7)	21 (30.0)	
50 - 59	17 (28.3)	27 (38.6)	
≥ 60	12 (20.0)	11 (15.7)	
Gender			
Male	17 (28.3)	15 (21.4)	0.362
Female	43 (71.7)	55 (78.6)	
BMI (kg/m2)			
Normal	8 (13.3)	19 (27.1)	0.121
Overweight	31 (51.7)	27 (39.1)	
Obese	21 (35.0)	24 (34.8)	

*Significant difference between percentages using Pearson Chi-square test at 0.05 level

Exclusion criteria included: Age <35 years, Systemic disorders, such as diabetes, hematological diseases (coagulopathies), severe cardiovascular diseases, chronic liver and kidney disease or malignancy. Infectious disorders e.g., septic arthritis, viral arthritis, fungal arthritis and patients suffered from other rheumatic diseases such as spondyloarthropathies, systemic lupus erythematosus or dermatomyositis and others.

Study approval: The study was approved by Research Ethics Committee, Faculty of Medicine, Baghdad University, Iraq. The aim and methods of the study was explained to all participants, and an informed written consent was obtained from all participants.

Results

In this study, there was no statistically significant difference at($P \leq 0.05$) between the two studied groupsin terms of age, gender, and BMI (Table 1).

This study found a statistically significant differences in the mean concentrations of Zinc, Copper, Selenium, Collagen type I, and collagen type II between the two study groups. The mean levels of Zinc, Selenium, Copper ,Collagen type I, and Collagen type II were significantly higher in the group A compared to that in the group B (75.05 Mg/dLvs 67.48 Mg/dL, P= 0.027; 69.73 Mg/L vs 68.16 Mg/L, P= 0.001;149.1 Mg/dLvs 146.4 Mg/dL, P= 0.005, 2.43 ng/mL vs 1.97ng/mL, P= 0.005; and 4.47 ng/mL vs 3.30ng/mL, P= 0.002) respectively. No significant difference ($P \leq 0.05$) was found in the mean concentration of Iron between the two groups (Figure 1) and (Table 2).

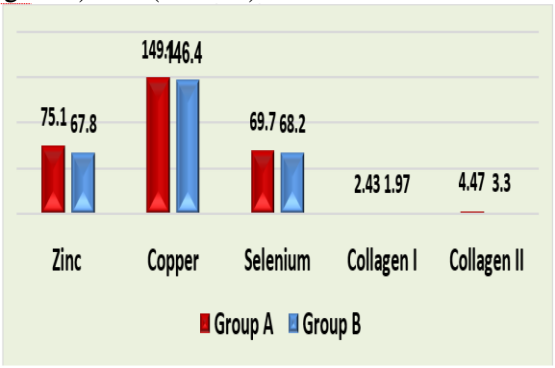

Figure 1: Mean levels of Zinc, Copper, Selenium, Collagen type I, and collagen type II in the study groups

Table 2: Comparison of biochemical parameters between the study groups

Biochemical Parameters	Study groups		P - Value*
	Group A Mean±SD	Group B Mean ± SD	
Iron (Mg/dL)	33.70 ± 11.32	34.31 ± 9.78	0.741
Zinc (Mg/dL)	75.05 ± 7.65	67.84 ± 5.85	0.027
Copper (Mg/dL)	149.1 ± 7.21	146.4 ± 9.34	0.030
Selenium (Mg/L)	69.73 ± 2.31	68.16 ± 2.97	0.001
Collagen I (ng/mL)	2.43 ± 1.13	1.97 ± 0.64	0.005
Collagen II (ng/mL)	4.47 ± 2.73	3.30 ± 0.69	0.002

*Significant difference between two independent means using Students-t-test at 0.05 level.

The comparison of biochemical parameters of study patients according to activity of their disease revealed that patients with severe disease activity had a significantly lower levels of Iron and collagen type-II compared to those with mild to moderate disease activity (28.70Mg/dLvs 37.36, P= 0.001) and (2.90 ng/mL vs 4.11ng/ml, P= 0.001) respectively. Other parameters showed no significant differences ($P \leq 0.05$) according to the disease activity (Table 3).

Table 3: Comparison of biochemical parameters according to disease activity

Biochemical Parameters	Disease Activity		P - Value
	Mild to Moderate Mean±SD	Severe Mean ± SD	
Iron (Mg/dL)	37.36 ± 9.19	28.70 ± 10.29	0.001
Zinc (Mg/dL)	71.10 ± 6.82	71.28 ± 8.81	0.448
Copper (Mg/dL)	147.6 ± 7.16	148.3 ± 10.47	0.324
Selenium (Mg/L)	68.89 ± 2.51	68.88 ± 3.21	0.494
Collagen I (ng/mL)	2.16 ± 1.04	2.22 ± 0.72	0.359
Collagen II (ng/mL)	4.11 ± 1.93	2.90 ± 0.79	0.001

The results Shaw by using Receiver operating characteristic (ROC) curve analysis that a constructed for Iron and collagen type-II levels as predictors for severity of rheumatoid arthritis. The optimal cut-off Iron value was 32.3 Mg/dL. Hence, Iron level < 32.3 Mg/dL is a predictor for severe rheumatoid arthritis, as a large significant area under the curve (AUC= 83.7%) indicating a

significant association between the lower Iron level and severe rheumatoid arthritis. This cut-off value obtained a sensitivity and specificity of 100% and 76.3% respectively, with accuracy of 83.1%. Positive predictive value and negative predictive value of Iron were 78.8% and 100% respectively (Figure 2) and (Table 4).

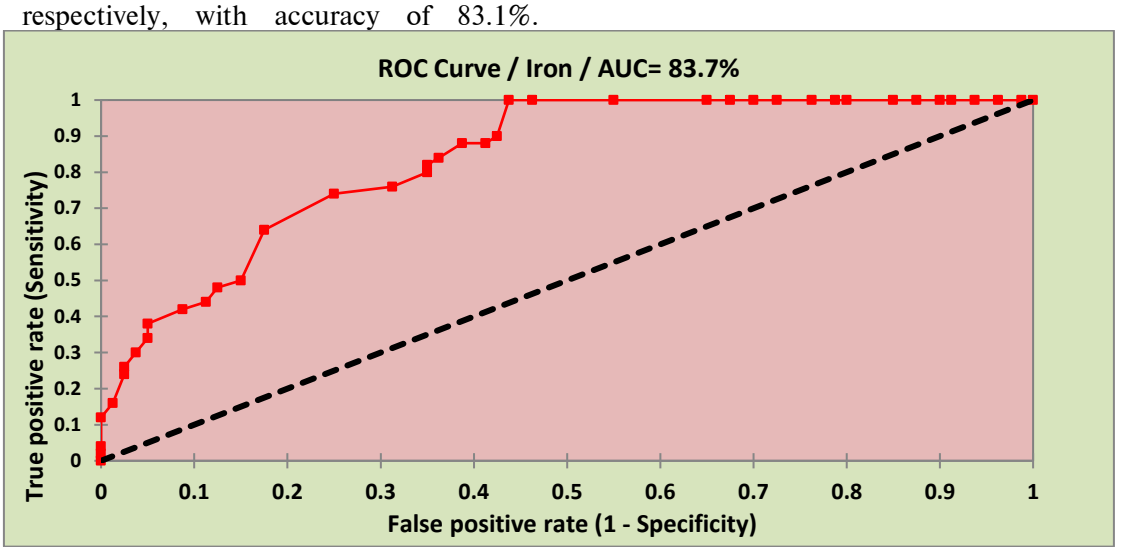

Figure 2: ROC curve of Iron in prediction of severe rheumatoid arthritis

Table 4: Diagnostic accuracy of Iron levels in prediction of severe rheumatoid arthritis

Clinical Parameter	Cut-off value	SN	SP	PPV	NPV	Accuracy
Iron (Mg/dl)	32.3	100%	76.3%	78.8%	100%	83.1%

The optimal cut-off value of collagen type-II was 3.10 ng/ml. Hence, collagen type-II level < 3.10 ng/ml is a predictor for severe rheumatoid arthritis, as a large significant area under the curve (AUC= 78.9%) indicating a significant association between the lower level of collagen type-II and severe rheumatoid arthritis. This cut-off value obtained a sensitivity and specificity of 75.4% and 91.3% respectively, with accuracy of 80.1%. Positive predictive value was 80% and negative predictive was 76.8% (Figure 3) and (Table 5).

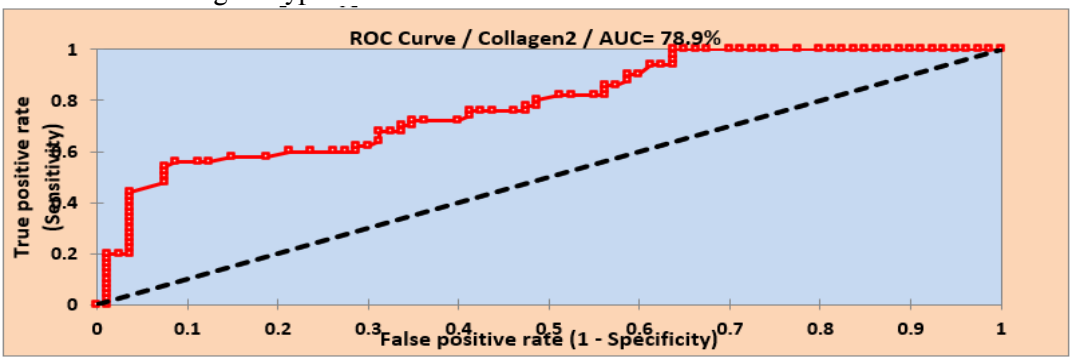

Figure 3: ROC curve of collagen type-II in prediction of severe rheumatoid arthritis

Table 5: Diagnostic accuracy of collagen type-II levels in prediction of severe rheumatoid arthritis

Clinical Parameter	Cut-off value	SN	SP	PPV	NPV	Accuracy
Collagen II (ng/mL)	3.10	75.4%	91.3%	80%	76.8%	80.1%

Discussion

This study was performed for to compare between Two groups of patients with rheumatoid arthritis. (60 patientsgroup A) treated with disease-modifying anti rheumatic drugs like Mtx. and (70 patients) treated with biological treatments like Humira ,Enbrel(group B).. as in show in table (1), there was no statistically significant difference (P ≤ 0.05) between the two

studied groups in terms of age, gender, and BMI .the authors who agreed with us ,notified that neither the age and nor the gender modified the Coll2-1 serum level in Research samples .(7,10,12).The role of trace metallic elements in chronic inflammatory states is of great interest, because many of them are co-factors in metabolic processes involving articular tissues and immune system function (10) . However, many studies showed that there was a relationship between trace elements levels and period, and types of treatment of rheumatoid arthritis diseases,but The role of Zinc and Copper in chronic inflammatory disease is of interest, because they are co-factor of important enzymes involved in collagen and bone metabolism, immune functions and antioxidant protection (9,13). An deplete of Zinc may cause anemia or reduce bone formation. Zinc also plays an important role in the catabolism of RNA by regulation RNAase activity(14). The Zinc and Copper metals prevent the formation of free radicals capable of inducing mutation and have antioxidant effects (13,15) .Results of these studies have demonstrated that both Copper and Zinc alteration can be explained by the active inflammatory process and that serum trace elements are measures of disease activity, and immune system function (11,16,17) .One hypothesis mentioned that a decreased Zinc and increase of Copper in sera of acute or chronic inflammatory processes cause an accumulation of Copper and Zinc in many body compartments and in the inflamed areas (18) but this study It is not similar to our interpretation of the lack of these elements according to our current study . Supporting the hypothesis that the development of inflammation induces an increase body requirement of Copper and Zinc ,so the level was depleted . The results obtained for Zinc concentrations in two groups of patients which are shown in table (2), show high significant difference in Zinc levels decreased according to type of treatment.We mean high significant difference in Zinc levels decreased ($p<0.05$) Concentration of Zinc is so low in patients group A as compared with those in group B . This means that chemical anti-inflammatory drugs can lead to more consumption of these elements Especially zinc, copper and selenium in the body compared to biological drugs. The result obtained in this study are similar to those published in the literature (12,13,15). Zinc deficiency is common that effect obviously apparent but not significant with disease activity as show in table (3).Colak et al. (14) reported two effect of Copper deficiency on Iron metabolism, the first occurring early was an adverse effect of Copper deficiency on Iron absorption (or mobilization), the second was inadequate erythropoiesis, even in the presence of an abundant Iron stores. Serum Copper level for group A and B are shown in table (2), and figure (1) indicates decrease serum Copper levels in the patients groups A in comparison with groups B.The decrease of Copper level was statistically significant ($p<0.001$). The results of this study indicate that Copper serum level decrease significantly during rheumatoid arthritis these results are in agreement with the reported (15,16). Selenium protects cells from oxidative damage and shown to have anti (proliferative, inflammatory, viral) and immune altering effects(11). . Dietary Selenium is essential for an optimum immune response, although the mechanisms of this requirement are not always fully understood (13,17, 21). Figure (1) represents a histogram for data of Table (2) that shows the result of Selenium determination in human sera. The mean±SD for group A is (69.73 ± 2.31) serum Selenium was highly significant differences were found ($p<0.001$), decreased in all patients group, these results proved the possible relationship between low levels of serum Selenium and rheumatoid arthritis (21,22) .Table(3) show that no effective role for disease activity on the level of trace element except Iron that effected with biological drugs and This applies to the study at the source (23)

In this work, there were highly statistically significant increase in the mean serum Coll2- 1 levels among RA patients compression ($p= 0.001$ each). And compression results show that coll. I,II and Iron level decrease with biological treatments ,this means the extent to which biological drugs affect the weakening of the protein structure of collagen. No difference was found for serum CollI level between Mild, Moderate, and sever level of activity , these results In agreement with this outcome a previous study (24,25) that reported that in RA, the serum Coll2-1 levels were found to be significantly decreased in two types of RA patients according to this study with the same range of age indicating that the rate of type II collagen degradation is increased in both group (21). In addition,. Assessing the relationship between serum Coll2 level with disease activity and severity of RA patients can be a start to understand and describe the significance of collagen type II degradation markers in predicting future progression and complications and thus taking early treatment measures to reduce those complications .A potential role of Coll2-1 in RA has been determined as its levels significantly correlated with CDAI (26). Moreover, the higher level of the disease activity of RA patients and higher levels of Coll2 indicating the possible benefit of using serum Coll2 levels in monitoring RA disease activity as we show in Ref. (27) ,this is another proof that supports the findings of the current study. In agreement with this outcome a previous study (27,28) reported that in OA and RA, the serum Coll2 levels were found to be significantly increased, indicating that the rate of

type II collagen degradation is increased in both diseases.

In fact, some few studies measured levels of collagen degradation products before and after applying different therapeutic methods to assess the effect of these therapeutic methods in slowing down the process of collagen degradation and its reflection on levels of collagen degradation products in serum, thus they further prove that Coll2-1 resulting from cartilage degradation may be used in monitoring the response to specific treatment. (24,29,30). A potential role of Coll2-1 in RA has been determined as its levels significantly correlated with disease activity. Our results are consistent with the findings of the researchers in the sources (17,31,32) that proven correlatedof coll 2 with disease activity. The results in (table 4) Shaw by using Receiver operating characteristic (ROC) curve analysis that a constructed for Iron as predictors for severity of rheumatoid arthritis. The optimal cut-off Iron value was 32.3 Mg/dL. Hence, Iron level < 32.3 Mg/dL is a predictor for severe rheumatoid arthritis, as a large significant area under the curve (AUC= 83.7%) indicating a significant association between the lower Iron level and severe rheumatoid arthritis. While the other (ROC) curve analysis show The optimal cut-off value of collagen type-II was 3.10 ng/ml. Hence, collagen type-II level < 3.10 ng/ml is a predictor for severe rheumatoid arthritis, as a large significant area under the curve (AUC= 78.9%) indicating a significant association between the lower level of collagen type-II and severe rheumatoid arthritis. This cut-off value obtained a sensitivity and specificity of 75.4% and 91.3% respectively, with accuracy of 80.1%. Positive predictive value was 80% and negative predictive was 76.8% (Figure 3) and (Table 5).

References

Lin, Y., Anzaghe, M. and Schülke, S., 2020. Update on the Pathomechanism, Diagnosis, and Treatment Options for Rheumatoid Arthritis. Cells, 9(4), p.880.

Wasserman A. (2018). Rheumatoid Arthritis: Common Questions About Diagnosis and Management. American family physician, 97(7), 455–462.

Ala S, Shokrzadeh M, Pur SA, Saeedi SS (2009) Zinc and copper plasma concentrations in rheumatoid arthritis patients from a selected population in Iran. Pakistan Journal of Biological Sciences: PJBS 12:1041.

Smolen, J. S. et al. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. Ann. Rheum. Dis. 75, 3–15 (2016)

Stejskal V, Reynolds T, Bjørklund G (2015) Increased frequency of delayed type hypersensitivity to metals in patients with connective tissue disease. Journal of Trace Elements in Medicine and Biology 31:230–236. . doi:10.1016/j.jtemb.2015.01.001

Fraser A , Fearon U, Billinghurst C, Ionescu M, Reece R, Barwick T. et al., Turnover of type II collagen and aggrecan in cartilage matrix at the onset of inflammatory arthritis in humans: relationship to mediators of systemic and local inflammation. Arthritis Rheum. Nov,2003;48(11):3085-3095.

Deberg M, Labasse A, Christgau S, Cloos P, Henriksen DB, Chapelle J-P. et al., New serum biochemical markers (Coll 2-1 and Coll 2-1 NO2) for studying oxidative-related type II collagen network degradation in patients with osteoarthritis and rheumatoid arthritis. Osteoarthritis Cartilage.2005; 13: 258-265.

Fawzy RM , Hashaad NI , Mansour AI (2017): Decrease of serum biomarker of type II Collagen degradation (Coll2-1) by intra-articular injection of an autologous plasma-rich-platelet in patients with unilateral primary knee osteoarthritis. Eur J Rheumatol. 2017,Jun;4(2):93-97.

Afridi HI, Kazi TG, Brabazon D, Naher S (2011) Association between essential trace and toxic elements in scalp hair samples of smokers rheumatoid arthritis subjects. Science of the Total Environment 15:412–413. doi:10.1016/j.scitotenv.2011.09.033

Albabawaty NS, Majid AY, , Alosami Mohammed H. , Halla G. Mahmood et al (2020): Role of high sensitivity C – Reactive Protein and some heavy metals in patients with rheumatoid arthritis, Ann Trop Med & Public Health; 23(S16): SP231604. DOI:http://doi.org/10.36295/ASRO.2020.231604

Mohammad H. Ali, Abeer J. Hassan, Enas J. Hasan .Risk Factors and Early Detection of Diabetes Mellitus in Early Rheumatoid Arthritis Women. JFacMedBagdad .2018, Vol. 60 No. 1 ;74-76 .https://doi.org/10.32007/jfacmedbagdad.60156

Omran RH, Ahmed ZA, Alrawi AA. Evaluation of Some New Cytokines in Rheumatoid Arthritis. JFacMedBagdad .2022 Oct. ;64(3):159-162. https://doi.org/10.32007/jfacmedbagdad.6431963

Siemons, L., ten Klooster, P.M., Vonkeman, H.E. et al. How age and sex affect the erythrocyte sedimentation rate and C-reactive protein in early rheumatoid arthritis. BMC MusculoskeletDisord 15, 368 (2014). https://doi.org/10.1186/1471-2474-15-368.

Colak M, Bingol NK, Ayhan O and Avci S, "Serum Copper, Zinc and Selenium levels in Rheumatoid Arthritis" Rhomatizma 2001; 16(2):66-71.

Cooles FA, Isaacs JD (2011) Pathophysiology of rheumatoid arthritis. Current Opinion in Rheumatology 23(3):233–240. doi:10.1097/ BOR.0b013e32834518a3

Oglah AA, Abid Mohammed KI, Alosami MH. A Comparative Study of Serum Amyloid A2 with Anti-cyclic Citrullinated Peptide antibody in the prognosis of a Group of Rheumatoid Arthritis Patients in Iraq. 2022.JFacMedBagdad . Oct. 17 ;64(3):153-8. https://doi.org/10.32007/jfacmedbagdad.6431947

Satish KT and Reshu M, "Assessment of mineral status (Zn, Cu, Mg and Mn) in Rheumatoid Arthritis patients in Chandigarh, India", Rheumatology Report 2009,1(5):16-20.

Hathcock JN, "Vitamins and minerals safety", 2nd ed. 2004, 35-49.

McInnes IB, Schett G (2011) The pathogenesis of rheumatoid arthritis. New England Journal of Medicine 365(23):2205–2219. doi: 10.1056/NEJMra1004965

Brig MN, Chatterje A and Shinde R, "text book medical biochemistry", 6th ed. Published by jitendarprij 2005, 178-557

Cerhan JR, Saag KG and Criswell LA. "Antioxidant Micronutrients and Risk of Rheumatoid Arthritis in Cohort of Older women" Am. J. Epidemiol 2003; 157(4): 345-354.

Sahebari, M., Rezaieyazdi, Z., &Khodashahi, M. (2019). Selenium and Autoimmune Diseases: A Review Article. Current rheumatology reviews, 15(2), 123–134.

Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-81.

Berry R and Huskisson M J. Treatment of rheumatoid arthritis. Clin Trials J.1972;4:13–5

Lohmander LS, Atley LM, Pietka TA, Eyre DR. The release of crosslinked peptides from type II collagen into human synovial fluid is increased soon after joint injury and in osteoarthritis. Arthritis Rheum.2003;48: 3130-3139

Samia M. Abdel-Monem, Amal F. Soliman, Mohammed M. Marei, SaniaKh. Elwia, Rasha M. Fawzy. Serum COLL2-1 In Rheumatoid Arthritis: Its Relation to Disease Activity and Severity. BMFJ 2022;39(1):235-246. DOI: 10.21608/bmfj.2021.90025.145

Van Riel PL and Fransen J. DAS28: a useful instrument to monitor infliximab treatment in patients with rheumatoid arthritis. Arthritis Res Ther.2005;7: 189-190.

Majhi T and Srivastava AK. " Iron Deficiency in Rheumatoid Arthritic patients especially with in the middle age", International Journal of systems Biology 2010; 2(1): 1-5.

Long FXDJS, Baocaidan G (2013) Tibetan medicine bath on the treatment of rheumatoid arthritis in the human body changes in trace element analysis. Journal of Medicine & Pharmacy of Chinese Minorities 3:007

O'Dell JR, Lemley-Gillespie S, Palmer WR, Weaver AL, Moore GF, Klassen LW (1991) Serum selenium concentrations in rheumatoid arthritis. Anna

Ferestein GS, Larry A, Cruz TF, Cheng TP, Banqueriso ML and Boyle DL "Vanadium an inhibitor of stromelysin and collagenase expression, suppresses collagen induced arthritis" J. Rheumatology 2007; 34(9): 1802-1809.

Fawzy RM , Hashaad NI , Mansour AI (2017): Decrease of serum biomarker of type II Collagen degradation (Coll2-1) by intra-articular injection of an autologous plasma-rich-platelet in patients with unilateral primary knee osteoarthritis. Eur J Rheumatol. 2017,Jun;4(2):93-97.