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Salahaddin University-Erbil

Immunopathogenesis of Adult Rheumatoid Arthritis Patients

Submitted to the Department of (Biology) in partial fulfillment of the requirements for the degree of **BSc.** in **Biology**

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CERTIFICATE

This research project has been written under my supervision and has been submitted for the award of the **BSc.** degree in **Biology** with my approval as a supervisor.

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Date: 1/4/2024

DEDICATION

This research paper is dedicated to our parents who gives their outmost support, and never-ending inspiration throughout the study. They are the one who provide the resources that needed in the making of this study. It also dedicated the teachers who are behind in making this research possible through guiding the research to complete this study.

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ABSTRACT

Autoimmune diseases such as rheumatoid arthritis (RA) are the consequence of a persistent imbalance between pro- and anti-inflammatory immune mechanisms, leading to chronic inflammation. Because the prevalence of RA has increased in our country. The objective of this study was to determine whether the C-reactive protein (CRP), Erythrocyte Sedimentation Rate (ESR), Rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibody and Antinuclear Antibody (ANA) are elevated in RA patients and to investigate the relationship between these markers and disease activity in RA, and to minimize the severity of the disease by using medicinal plants (Herbal plants in our country). The sample collected from patient in different age, gender, at four hospitals (Rizgary Hospital, Republic teaching Hospital, Ashti Hospital, Khalifan Hospital) in Erbil and Soran city, the study involved 46 rheumatoid arthritis patients aged 21-71 years. Within this group, there were 15 male participants and 31 female participants, highlighting a notable gender disparity. Specifically, the distribution of patients according to gender unveiled that 32% were females, whereas 14% were males. Samples were diagnosis was based on clinical examination and laboratory investigations. Blood samples were collected from each patient and sera separated and stored at -20C for further investigation. Serum was also collected from apparently healthy, age and gender matched individual's control. Laboratory parameters including CRP, ESR, RF, anti-CCP antibody and ANA test were done. The results indicated that disease activity is more severe in the female than the male, this gender disproportion is further emphasized by the calculated female-to-male ratio of 3:1, underscoring the higher prevalence of RA among females compared to males. This discrepancy is probably due to differences in lifestyle and physical activity between the studied populations. The majority of patients included were in the age group 20-70 years. We found that RA patients had significantly higher levels of serum CRP (p<0.001), ESR (p<0.001), RF (p<0.001), anti-CCP antibody (p<0.001) and ANA test (p<0.001), as compared to healthy controls. These results demonstrate that RA disease more prone in

female, RA patients have high levels of inflammatory markers. These findings suggest a possible role of these markers in the pathogenesis of RA. Moreover, these biomarkers can be used as markers of disease activity in the diagnosis and treatment of RA.
Keywords: Rheumatoid arthritis, autoimmune disease, CRP, ESR, RF, anti-CCP antibody, ANA test

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RF: (Rheumatoid factor)

Anti-CCP test: anti-cyclic citrullinated peptide test

ANA test: Antinuclear Antibody test

ESR test: Erythrocyte Sedimentation Rate test

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease affecting the joints. Among the features that characterize RA are swollen joints, mononuclear cell infiltration and synovial cell proliferation leading eventually to cartilage and bone destruction. One of the prominent features of RA is the presence of rheumatoid factor which is a family of autoantibodies directed against the FC (fragment crystalilizable region) part of IgG molecule. Rheumatoid factors (RF) exist as IgG, IgM and IgA isotypes. The first person to describe and classify this debilitating disease was the French doctor Augustin Jacob Landré-Beauvais in 1880. Landré-Beauvais recorded the important manifestations of the disease with "asthenic gout," indicating that the condition occurred well in women (Landré-Beauvais, 2001). Other than in RA patients, RF isotypes may be present in other autoimmune diseases such as Primary Sjogren's syndrome, mixed connective tissue disease, systemic lupus erythematosus and systemic sclerosis (Ingegnoli et al., 2013). RF is a chronic rheumatic disease that affects 0·5–1% of the global population (Frazzei et al., 2023). It mainly causes pain in multiple joints of the body, but is usually also associated with cardiovascular disease, pulmonary damage, and severe disability (Finckh et al., 2022).

The pathogenesis of RA is characterised by the complex interaction of genetic predisposition, epigenetic regulation and environmental factors. Although several mechanisms underlying the disease are not yet completely defined, many advances have been achieved in the last year. Due to multiple genome-wide associations studies (GWAS) and progress in genomic sequencing technologies, several loci linked to an increased risk of RA have been discovered. Among them, both the human leukocyte antigen (HLA) and the non-HLA genes were found to be deeply involved (Saevarsdottir et al., 2022). It can also affect extra-articular organs (e.g., heart, lungs, eyes, blood vessels) and reduce life span (Widdifield et al., 2018).

Additionally, autoantibodies to RF and citrullinated protein are often present. Risk factors for RA include smoking, gender (females show higher incidence), obesity, old age, and genetics with genetic and epigenetic factors comprising ~30% of risk (Smolen et al., 2020). With prevalence increasing with age; the disease commonly comes up between the ages of 40 and 50 in individuals with the condition three to five times more in women than in men (Radu and Bungau, 2021). Repeated and symmetrical multiple micro arthritis is the primary clinical manifestation of the disease, occurring in the hand, wrist, foot, knee, and other joints. In the early stages of the disease, redness, swelling, heat, pain, and joint dysfunction are common (Kolarz et al., 2018). In the late stages of the condition, different degrees of rigidity and deformity of joints are seen and, finally, drive several degrees of bone corrosion and skeletal muscle atrophy, synovitis invasion of articular cartilage, sub-cartilage bone erosion, and damage to ligaments and tendons (Coutant and Miossec, 2020).

The cause of RA remains unknown, but it is generally considered related to environmental and genetic factors. The mechanisms of action include the joints attacking by body's immune system by mistake, which causes joint capsule inflammation and thickening, and promotes damage to bones and cartilage at these sites. In the clinical, RA diagnosis is based on the patient's physical manifestations and symptoms (Cush, 2022a). X-rays and laboratory tests can assist in the diagnosis or exclusion of some similar disorders, viz. lupus erythematosus, psoriatic arthritis, and fibromyalgia (Cush, 2022b).Drug-target therapy has played a crucial role in RA, leading to development of biological drugs such as interleukin-1β (IL-1β) inhibitors, tumor necrosis factor-α (TNF-α) inhibitors and interleukin-6 (IL-6) inhibitors. In spite of advancement in drug development, usage of these target-specific medicines was limited due to deleterious side effects (vulnerable to severe infection and over activation of immune effect in vivo) (Burmester et al., 2020). *Matricaria chamomilla*, also known as chamomile, has been used for centuries to treat joint pain (Pirouzpanah et al., 2017). The dried flower part of the

plant has historically been used in the treatment of rheumatic pain and inflammation (Pirouzpanah et al., 2017). The activity of *Zingiber officinale* as an anti-inflammatory agent was investigated (Thomson et al., 2002) Evidences reported that consumption of ginger aids in relieving pain of joints associated with rheumatoid arthritis (Thomson et al., 2002).

The aimed of this study is to know the lifestyle changes, affect people's health (increased Rheumatoid arthritis), to determine the cause and risk factors in our country that increased this disease, to minimize the severity of the disease by using medicinal plants (Herbal plants in our country), to determine the relationship between the decrease in mineral level and the incidence of Rheumatoid arthritis.

2. METHODOLOGY & RESEARCH DESIGN

2.1 Study design and sampling

A case-control study was conducted from the RA patients regularly attending major Hospitals in Kurdistan region; (Rizgary Hospital, Republic teaching Hospital, Ashti Hospital, Khalifan Hospital) and outpatient's rheumatology clinics in Erbil and Soran city during the period from 4th November 2023 to 28th February 2024.

2.2 Inclusion and exclusion criteria

All patients aged between $\geq$ 20 to $\leq$ 70 years, of both genders, who signed the consent form and were willing to participate are included. Young and teenage individuals, or individuals with a history of disability, psychological and mental diseases, malignant tumors, incomplete data, who refused to sign the consent, and were unwilling to participate were excluded from the study.

2.3 Sample size

This study included 46 RA patients (31 females and 15 males) attending rheumatology department of the Hospitals and outpatient's rheumatology clinics in Erbil and Soran city during the period from November 2023 to February 2024.

2.4 Questionnaire

A trained team was recruited to interview the respondents using a semi-structured questionnaire. However, two specialists took the full clinical history and examined all patients physically. The questionnaire recorded information about the sociodemographic factors, chronic disease, body mass index (BMI), employment, and physical activity.

2.5 Doctor-Diagnosed arthritis

In this study, the definition of doctor-diagnosed arthritis decided on answering "yes" to the standard question, "Have you ever been informed by a doctor or other health professional that you have some form of arthritis, rheumatoid arthritis, gout, lupus, or fibromyalgia?"

2.6 Rheumatoid Arthritis

Blood samples were collected from patient and sera were separated and stored at -20 °C who has self-reported "Doctor-Diagnosed Arthritis" undergone further laboratory tests including the CRP, ESR , RF, anti-CCP antibody and ANA test were done according to (Mathkhor et al., 2021) . Normal ranges of laboratory parameters were described as follows: CRP (normal 0–5), ESR (normal 25 mm/h by Wintergreen method), RF (normal <5 by nephelometry method), anti-CCP (normal <20 by ELISA method). The rheumatoid factor (RA) was measured according to the criteria of the "American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) guideline" issued in 2010. Serum was also collected and tested from apparently healthy, age and gender matched individual's control.

2.7 Dependent variable

In this study, the dependent variable was the presence of arthritis or not, depending on the answer of patients to the question "Have you ever been informed by a doctor or other health professional that you have some form of arthritis, rheumatoid arthritis, gout, lupus, or fibromyalgia?" Independent variables The age of respondents was categorized into six age groups that involved 20-30, 30-40,40-50, 50-60, and <70. The gender was either male or female. The education was reported in three levels that included the elementary education and below middle and high school, and high education. Patients

were answered whether they had a history of chronic diseases such as diabetes mellitus, hypertension, or ischemic heart disease. The response of employment was currently employed or not including retired and housewives.

Statistical Analysis

Continuous and categorical variables were expressed as mean ± standard deviation (SD) and proportions. Quantitative variables were summarized as mean values and a 95% confidence interval (CI). If appropriate, the differences between groups were analyzed by chi-square test or Fisher's exact test for categorical data. All statistical analyses were performed using SPSS 16.0 software. p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

The study consisted of 46 patients aged from 21 to 71 years with established RA. Among those patients were 15 males and 31 females, highlighting a notable gender disparity. Specifically, the distribution of patients according to gender unveiled that 32% were females, whereas 14% were males, accentuating a substantial predominance of female patients within the cohort. This gender disproportion is further emphasized by the calculated female-to-male ratio of 2.28:1, underscoring the higher prevalence of RA among females compared to males. Notably, the observed disparity between female and male RA patients was deemed highly significant, a finding that is graphically depicted in Figure 1, emphasizing the pronounced discrepancy in disease prevalence between the two genders within the studied population. The analysis of age distribution within the patient cohort revealed that the highest proportions were concentrated within the age brackets of 40-50 years and 50-60 years, comprising 12% and 14% of the total population, respectively (Figure 2).

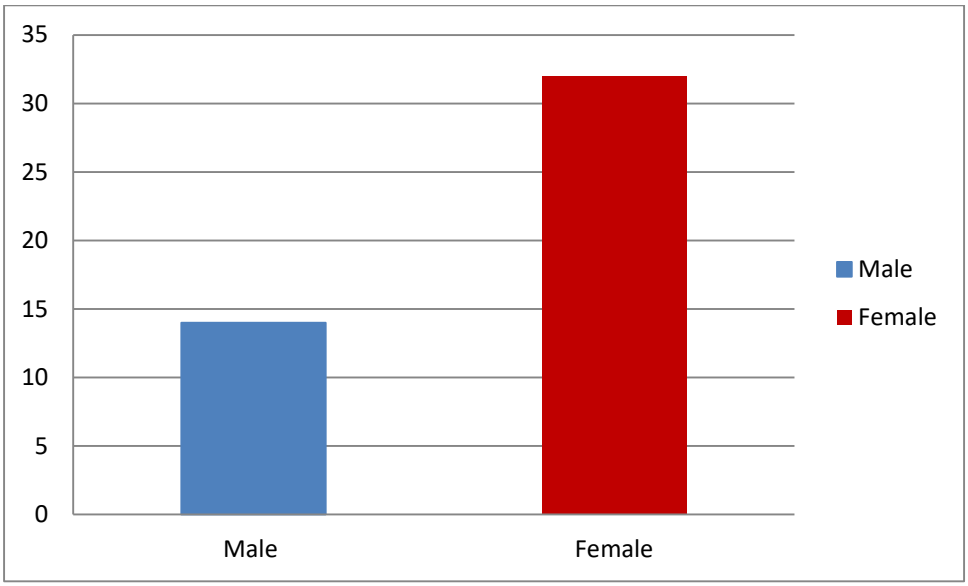

Figure 1: Gender distribution of Rheumatoid Arthritis patients

The majority of patients (12% and 14%) were in the age groups 40-50 and 50-60 respectively as shown in Figure 2.

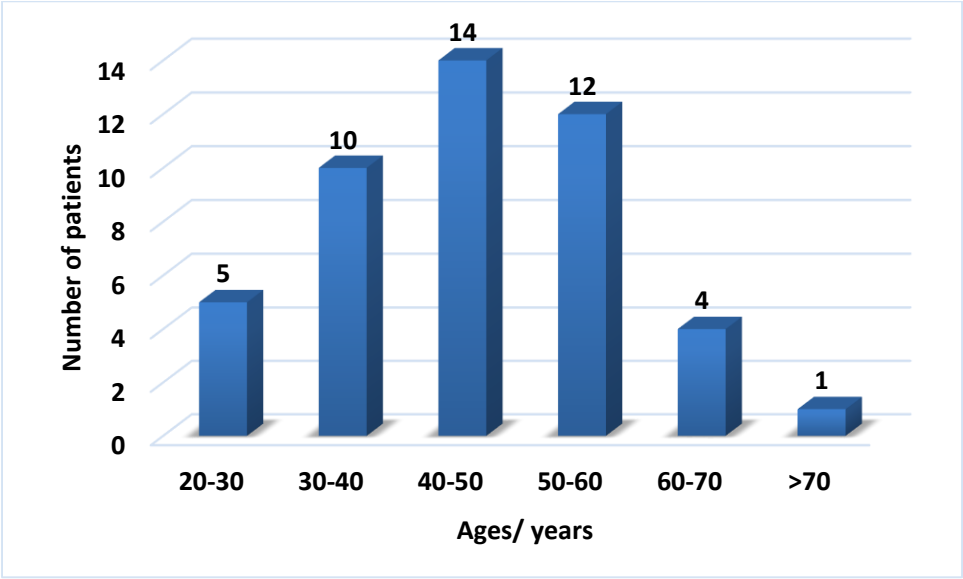

Figure 2: Age wise distribution of Rheumatoid Arthritis patients

As shown in figure 3 that 26% of the participants exhibited CRP positivity, indicative of an inflammatory response. Conversely, 20% of the individuals were classified as healthy, displaying normal levels of CRP.

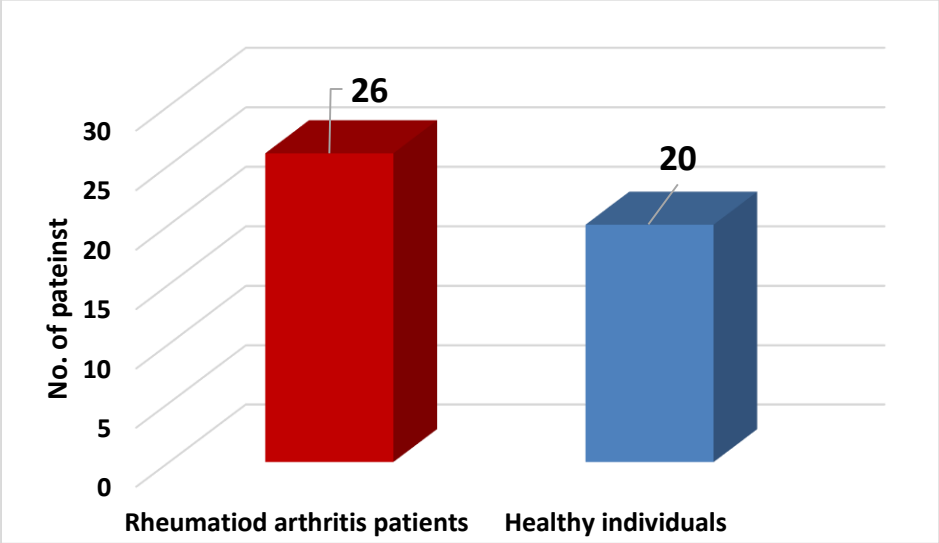

Figure 3: CRP levels in Rheumatoid arthritis patients and Healthy individuals

The ESR at diagnosis was 49.1 ± 36.8 mm/hr and 26.6 ± 24.3 mm/hr ($p<0.01$) in seropositive and seronegative groups, respectively Figure 4.

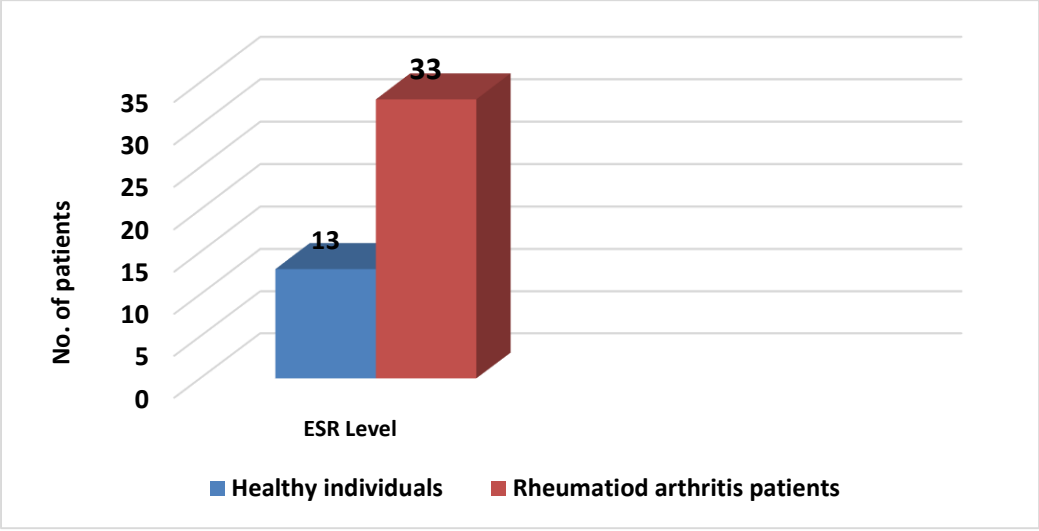

Figure 4: Distribution ESR levels in Rheumatoid arthritis patients and Healthy individuals

The analysis of anti-cyclic citrullinated peptide (anti-CCP) levels revealed that 30% of the patients tested positive for anti-CCP, while 16% tested negative (Figure 5).

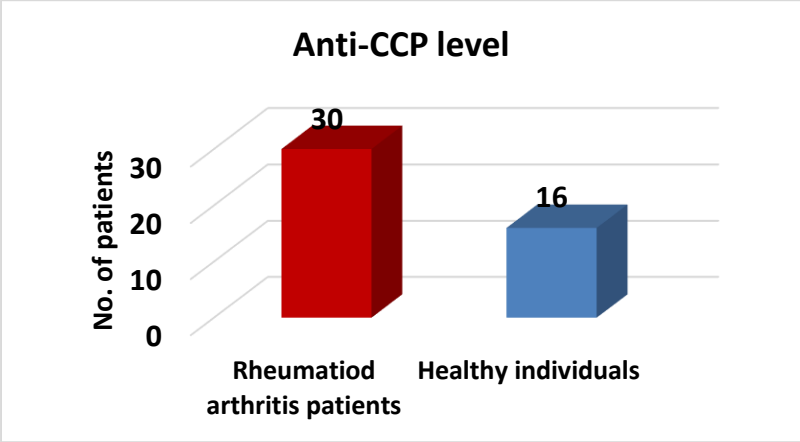

Figure 5: Distribution of Anti-CCP levels in Rheumatoid arthritis patients and Healthy individuals

Rheumatoid factor (RF) status among the patient cohort revealed that 38% of the individuals tested positive for RF, while 8% tested negative (figure 6).

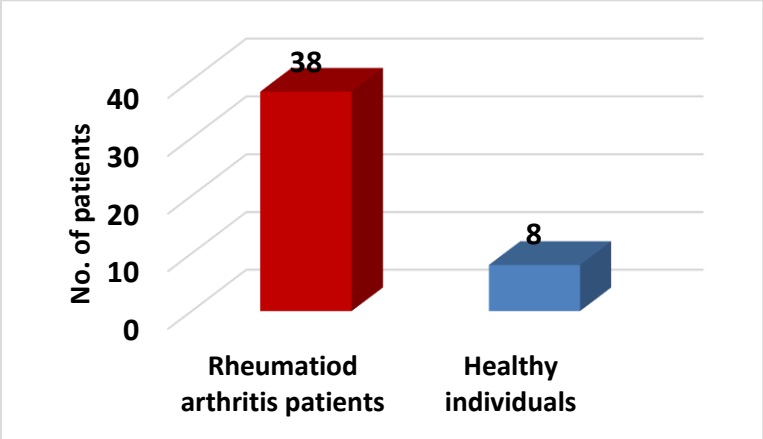

Figure 6: percentage of RF test in Rheumatoid arthritis patients

Figure 7 illustrated that 67% of individuals exhibited normal antinuclear antibodies (ANA) levels, while 33% displayed abnormal ANA levels.

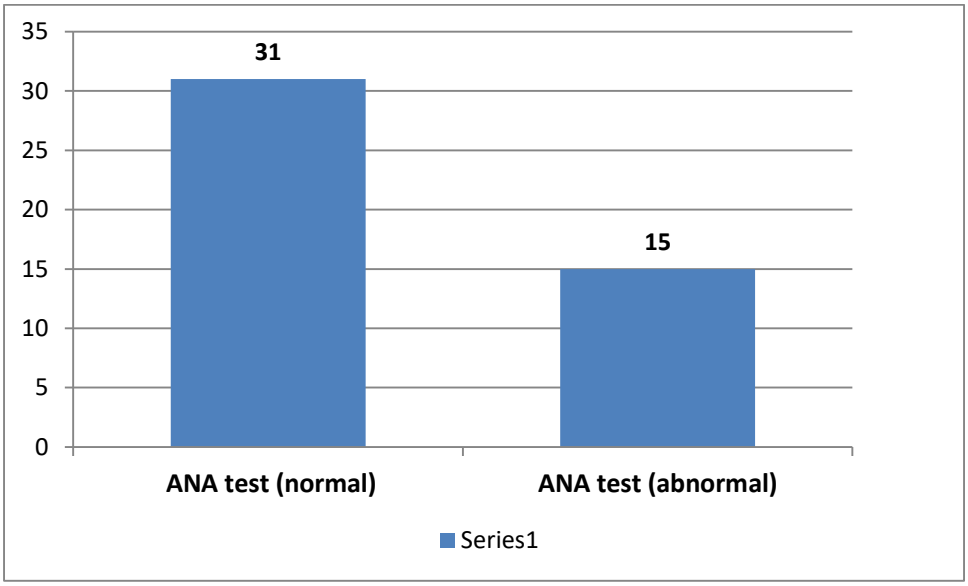

Figure 7: Percentage of ANA test in Rheumatoid arthritis patients

Discussion

Rheumatoid arthritis is a chronic autoimmune disease affecting the joints. It is characterized by a progressive symmetric inflammation of affected joints resulting in cartilage destruction, bone erosion, and disability (Aletaha and Smolen, 2018). Prognosis is influenced by clinical factors, and at initial diagnosis. Also, ESR,CRP, and RF at diagnosis are laboratory factors that affect the prognosis of RA (Bordean et al., 2023). The manifestation of symptoms in patients with RA is influenced by various genetic factors and epigenetic changes, as well as environmental factors including smoking, obesity, and alterations in the oral and intestinal microbiome. This results in a highly diverse patient population. These factors contribute to cellular immune proliferation and the formation of autoantibodies, which perpetuate the inflammatory cascade of the synovial membrane.(Buch et al., 2021).

Our result shows a differences in the prevalence of RA was found between female (32%) and male (14%) patients included in the study and the highest proportions were in the age 40-50 years and 50-60 years, comprising 12% and 14% respectively . This result is in agreement with a study of (Hussein et al., 2018) revealed that 82% of patients were females and 18% were males. (Dessie et al., 2021) reported that the total RA patients, 9 (12.4%) and 64 (87.6%) were males and females, respectively. This discrepancy is probably due to differences in life style and physical activity between the studied populations. Hormonal and genetic factors may contribute to sexual variation in the prevalence of RA(Favalli et al., 2019).

While international literature shows that the disorder is three times more common in females, in this study case-control it was found that this figure was 3:1 times more common in women than in men. More research is needed in order to explain this difference (Duraj et al., 2013). As similar were also found in the age of patients as this cohort was

younger than those reported in Albania (53.2 vs. 62.4 years old), but same than those reported in Greece (46.8 years old) (Anagnostopoulos et al., 2010), while the progress time of the disease is also slightly higher than the figure reported by Kirchhoff et al. (9.6 years old in Colombia vs. 8.0 years old in Germany)(Kirchhoff et al., 2011). No available data was found on comorbidities that accompanied the patient with RA. However, association with osteoporosis, high blood pressure and dyslipidemia are not surprising because there have already been reports across the country of patients with these morbidities and the use of anti-inflammatory drugs (Machado et al., 2008).

Serum rheumatoid factor is important in the diagnosis and prognosis of RA. Reported that RA patient with high ESR and CRP have serious radiological changes including bony erosion, regardless of RF Status. In our study the level of CRP and ESR were measured and utilized to measure disease activity status in RA patients. 26% of the participants exhibited CRP positivity, indicative of an inflammatory response and 33% of the individuals exhibited ESR positivity. Disease activity evaluation using C-reactive protein was supported by previous data (Roodenrijs et al., 2018). Although measurement of C-reactive protein is non-specific, it is utilized to evaluate disease activity status in RA patients (He et al., 2020). In general, CRP plays an important role in host defence mechanisms against infectious agents and in the inflammatory response (Sproston and Ashworth, 2018). CRP binding to immunoglobulin Fc gamma receptors (FcgR) promotes the production of proinflammatory cytokines leading to an amplification loop of inflammation (Newling et al., 2019). It is produced predominantly by hepatocytes in response to stimulation by IL-6, (Choy and Rose-John, 2017) but CRP has also been reported to be expressed by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes (Sproston and Ashworth, 2018).

As figure 3 that 26% of the participants exhibited CRP positivity, indicative of an inflammatory response. Conversely, 20% of the individuals were classified as healthy, displaying normal levels of CRP. Our findings revealed that 33% of the individuals exhibited ESR positivity, while 20% of the individuals were classified as healthy, displaying normal levels of ESR (figure4). The analysis of anti-CCP, RF and ANA levels revealed that 30% of the patients tested positive for anti-CCP, 38% of the individuals tested positive for RF, and 67% of individuals exhibited normal antinuclear antibodies (ANA). Serum markers like anti-CCP and RF have been often used to favour the diagnosis of RA (Chou et al., 2007). Anti-CCP antibodies should be tested at the earliest for RA because of their high specificity in RA. These antibodies are associated with more active, erosive, and severe form of RA (Gr et al., 2022).

4. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the results of the present study indicate that the levels of inflammatory markers hs-CRP, IL-6, IL-10 and TNF-¿ are significantly more elevated in RA patients than in healthy controls, and correlated with the disease activity. DAS28 correlates positively with hs-CRP, IL-6 and TNF-¿ and negatively with anti-inflammatory cytokine IL-10. These findings suggest that inflammatory markers may be involved in the pathogenesis of RA, and that levels of these markers reflect the activity of the disease.

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