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Effects of Arthritis on Hematological and Immune Markers in Rats

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Abstract

Background: In last fifty years, much has been written about the abnormalities in hematological and immune markers associated with different types of arthritis as part to investigate and if possible to treat the disease or it common complication(s).

Aim: To detect the effect of arthritis on different hematological parameters as well as on some immune markers.

Methodology: A total 40 adult male rats were selected, prepared, and divided randomly into two groups; an experimental arthritic group (30 rats) subjected for single intradermal injection of complete Freund's adjuvant (CFA) at a dose of 0.1 ml into the right hind paw in addition to a negative healthy control group (10 rats). After 3 weeks, all rats were anesthetized by chloroform and subjected for direct collection of blood. Hematological parameters were measured using of automated haemolyser; while level of immune markers were identified by ELISA.

Results: In arthritic rats, there were significant decreases in RBCs, HCT, Hb, MCH, WBCs and neutrophils; while, there were significant increases in PLTs, lymphocytes, monocytes, basophils and eosinophils. However, insignificant differences were recorded between values of MCV and MCHC of both arthritic and healthy groups. For immune markers, the findings of RF and TNF- were revealed a significant elevation in rats of arthritic group when compared to those of healthy group.

Conclusions: Arthritis can cause a significant abnormality in blood parameters as well as in immune markers. Hence, furthermore studies are necessary to estimate the effect of arthritis on other immune markers and all body organs. Also, this study assumed that the using of PLT parameters as markers in arthritic patients or other inflammatory diseases may provide valuable information and better explain the disease pathogenesis or activity since the early diagnosis of arthritis is difficult..

Keywords: Joint inflammation, Complete Freund's adjuvant, Platelets, Complete blood count, Rheumatoid factor, Tumor necrosis factor, Iraq.

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Introduction

Arthritis is derived from the Greek word for "joint disease" is defined as an acute or chronic inflammation of joint (s), often with pain and structural damage (Weyand and Goronzy, 2021). Arthritis can be ranged from autoimmune processes (rheumatic arthritis, psoriatic arthritis, ankylosing spondylitis), inflammation caused by crystal deposits (gout, pseudogout, alkaline phosphatase), and infections (lytic arthritis). In addition, the disease may be related to other diseases such as scleroderma, myositis, tuberculosis, and celiac disease (Chimenti *et al*., 2015; Senthelal *et al*., 2020). The disease can affect all population, but patients with immunosuppression, aging, diabetes, artificial joints, and intravenous drug use are at higher risk (Montgomery *et al*., 2017; Spyridakis *et al*., 2019).

Rheumatoid arthritis (RA) is an inflammatory disease manifested by infiltration of inflammatory cells to synovial tissues and inflammatory process that may cause a joint deformity and severe joint pain (Zhang and Lee, 2018). Severe fatigue is the almost severe and more pronounced signs of RA when compared to normal fatigue seen in people who have not been diagnosed with rheumatoid arthritis (Santos *et al*., 2019; Pope, 2020). Factors associated with increased anxiety in RA patients include medical problems and psychological and social aspects of life, lack of social interaction, lack of lifelong activities, loss of control of prognosis, and the greater financial burden of RA (Kwiatkowska *et al*., 2018; Chancay *et al*., 2019; Fenton *et al*., 2019). The financial burden of serious symptoms and illness suggests an integrated approach (Deane and Holers, 2021; Edilova *et al*., 2021). The environment is involved in the production of anti-citrullinated protein antigens (ACPA) in RA, and epigenetic regulation links the environment with genes. Gene-environment interactions affect the responsiveness of autoantibodies to the citrullinated antigen of RA (Guo *et al*., 2018). Rheumatoid arthritis in adults is generally divided into two types: serum-positive RA or serum-negative RA (Surmont and Diamond, 2015; Derksen *et al*., 2017).

Recent studies have shown an increasing of arthritis that could be found many months prior to appearance of clinical signs, and RA may not be recognized by the clinical phenotype. It showed that continued subclinical inflammatory processes in a joint can permanently damage the cartilage and bone (Suurmond *et al*., 2015). Many studies have attempted to define RA diagnostic methods in clinical practice to better identify the true onset of the disease (Thomas *et al*., 2008). There is no single diagnostic test for patients in the early stages of rheumatoid arthritis, and evaluation requires a combination of clinical features and laboratory tests (Heidari, 2011). Therefore, this study was aimed to induce arthritis in animal model (rat) and to detect alteration in hematological and some immune markers; rheumatoid factor (RF) and tumor-necrosis factor-alpha (TNF-α).

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Materials and methods

Ethical approval

This study was approved by the license of the Scientific Committee of the College of Science in the University of Wasit (Wasit, Iraq).

Animals and samples

Totally, 40 adult male rats were purchased and subjected initially for 1 week preparation. Then, the study rats were divided into two groups; an experimental arthritic group involved 30 rats and negative control group involved 10 rats. Rats of experimental groups were intradermal injected with a single dose (0.1 ml) of CFA (Sigma-Aldrich, USA) into the right hind paw (Yamagishi *et al*., 2012), but not for rats of negative group. After 3 weeks, all study rats were anesthetized by chloroform (CDH, India) and blood was collected directly from the heat of all study rats into an EDTA-vacutainer labeled tubes.

Hematology

The hematological parameters including red blood corpuscles (RBCs), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLTs), white blood cells (WBCs), lymphocytes, monocytes, neutrophils, basophils and eosinophils were estimated as soon as possible using the automated Mythic 18 Vet Haemolyser (Orphèe / Switzerland). Then, all blood samples were centrifuged at 5000 rpm for 10 minutes and the obtained plasma were kept into 1.5 ml Eppendorf tubes for estimation of immune markers.

Measurement of immune markers

Indirect and sandwich enzyme-linked immunosorbent assay (ELISA) kits were used to detect the concentration of RF and TNF-α, respectively, in plasma of study animals. Following the manufacturers' instructions of both kits (SunLong Biotech, China), the kit contents and samples were prepared, processed and the optical density (OD) was read at a wavelength of 450 nm. For RF, the test effectiveness and critical value (CUT OFF) was determined; while for TNF-α, the concentrations of tested samples were identified using the Standard Curve based on the ODs and concentrations of the Standard solution.

Statistical analysis

All data were analysed using the *t-test* in the GraphPad Prism Software at a significant differences of $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***), and $P<0.0001$ (****), (Al-Abedi *et al*., 2022; Gharban *et al*., 2022). Values of hematological and immune markers were represented at mean ± standard error mean (M ± SEM).

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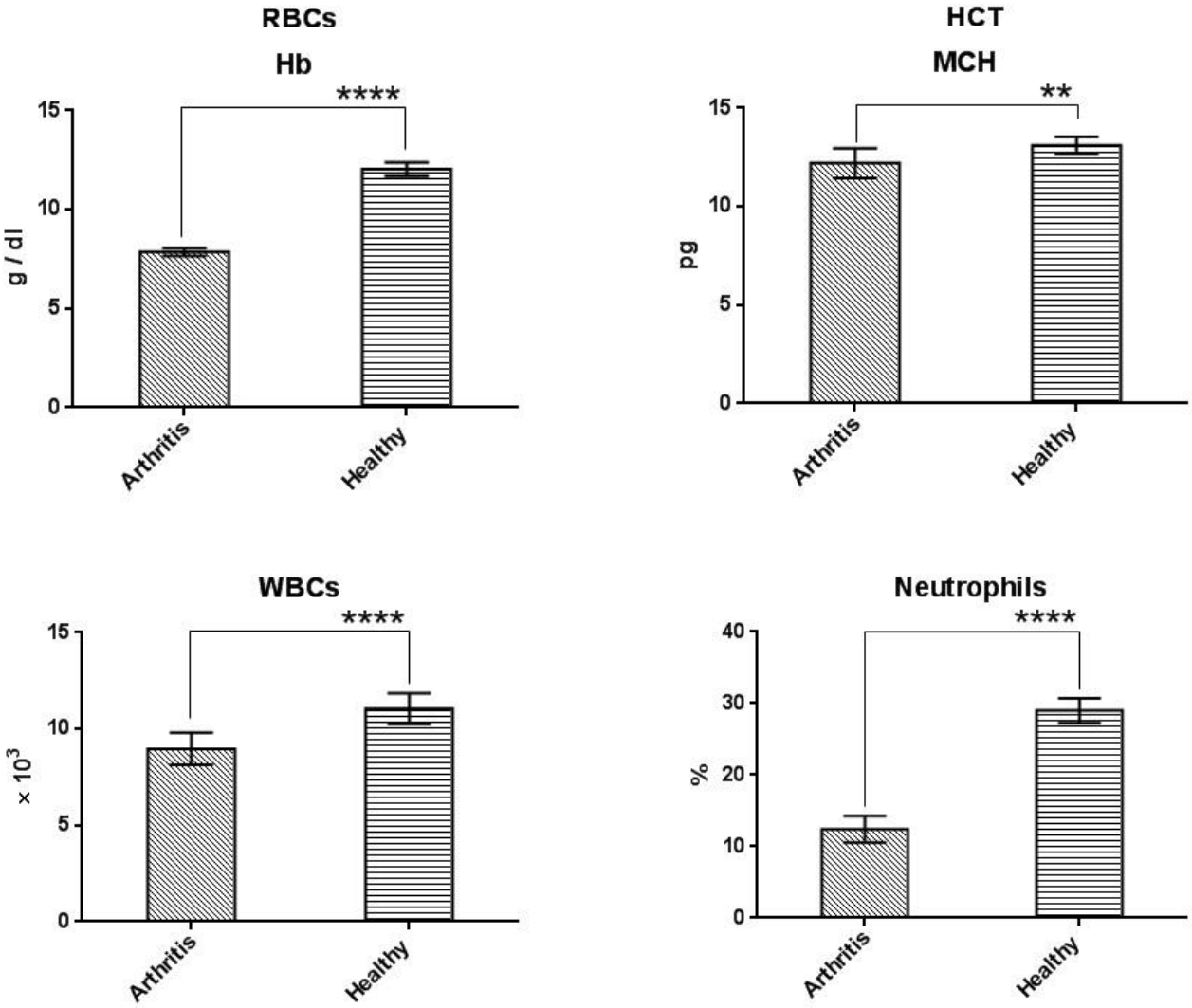
Results

Hematology

In arthritic rats, significant decreases (P<0.05) in values of RBCs (6.92 ± 0.24 × 10^6), HCT (28.23 ± 2.65%), Hb (7.86 ± 0.2 g/dl), MCH (12.21 ± 0.76 pg), WBCs (8.97 ± 0.83 × 10^3) and neutrophils (12.42 ± 1.83%) were showed in comparison with those of healthy control group; 9.45 ± 0.13 × 10^6, 40.02 ± 2.93%, 12.04 ± 0.34 g/dl, 13.12 ± 0.42 pg, 8.97 ± 0.83 × 10^3 and 29 ± 1.7, respectively. While, significant increases (P<0.05) in values of PLTs (436 ± 42.57 × 10^3), lymphocytes (78.57 ± 4.92%), monocytes (4.5 ± 0.36%), basophils (1 ± 0.05%) and eosinophils (2.5 ± 0.09%) were reported in arthritic rats when compared to those of healthy rats (281.96 ± 28.1 × 10^3, 63.57 ± 3.11%, 3 ± 0.28%, 0.5 ± 0.02% and 1.5 ± 0.05%, respectively). Nonetheless, insignificant differences (P>0.05) were recorded between values MCV and MCHC of arthritis (40.85 ± 3.71 fl and 27.12 ± 2.3 g/dl, respectively) and healthy (42.53 ± 3.24 fl and 30.85 ± 2.91 g/dl, respectively) groups (Figure 1).



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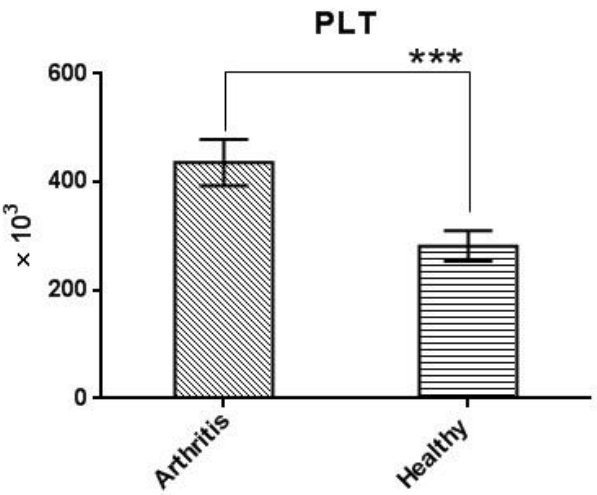

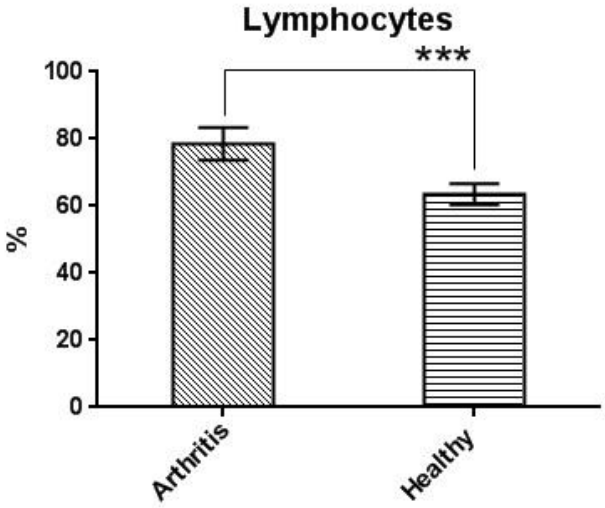

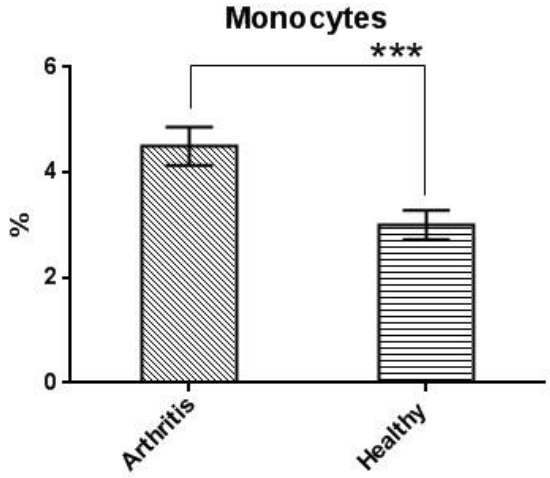

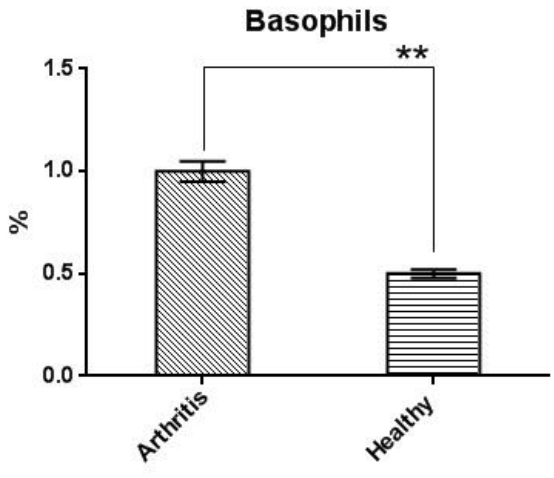

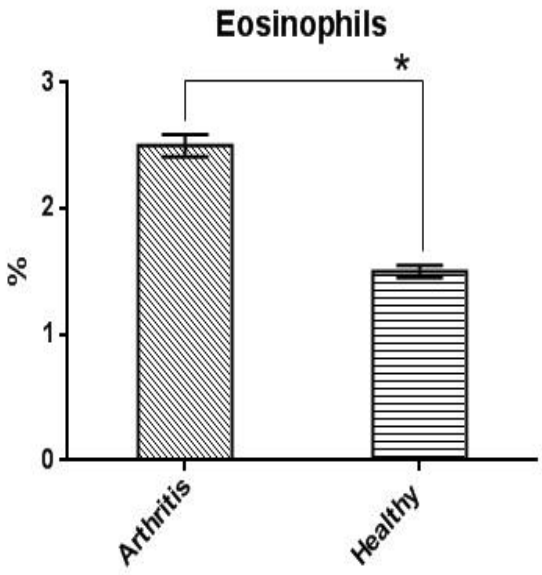

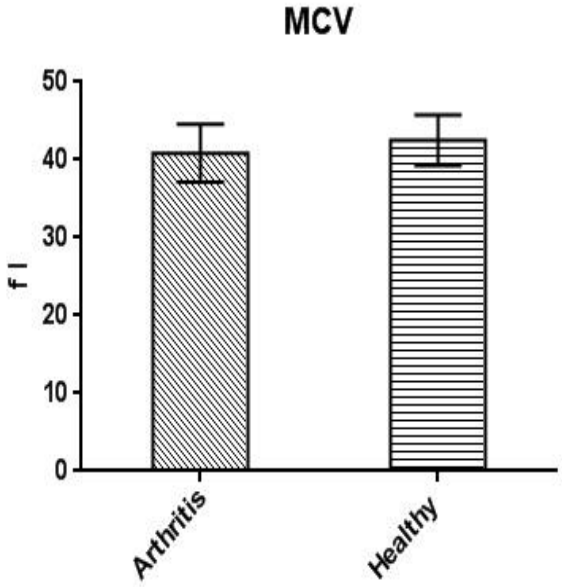

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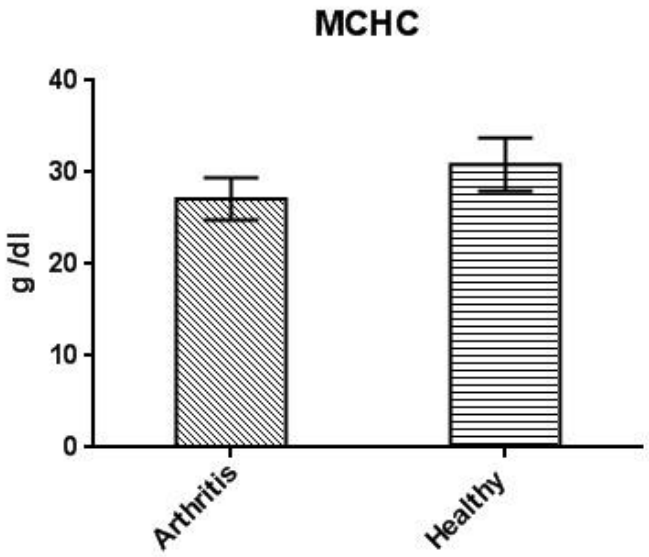

Figure (1): Results of hematology in rats of arthritic and healthy groups

Immune markers

The findings of RF and TNF- were revealed a significant elevation ($P<0.05$) in values of arthritic (395.41 ± 21.76 and 127.56 ± 6.48 ng/L, respectively) group when compared to those of healthy group; 198.65 ± 19.2 and 52.43 ± 3.27 ng/L, respectively (Figure 2).

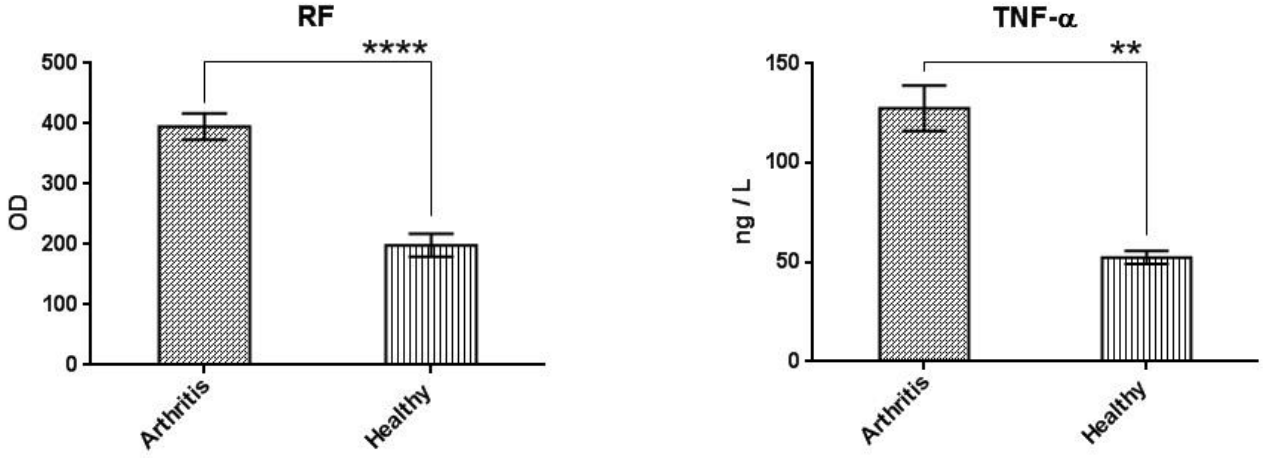

Figure (2): Results of immune markers in rats of arthritic and healthy groups

Discussion

Studying of hematology is one of the most sensitive parameters for assessing the effect of disease in the body as well as to estimate the efficacy and toxicity of different therapies (Mukinda and Eagles, 2020). In this study, CFA-induced arthritis may have toxic effects on blood cells such as hemolysis, decreased in Hb production, and increased immunomodulatory activity that affects the immune response. Bowman (2002) mentioned that anemia may occur classically in arthritis as a result of

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reduced serum iron and transferrin saturation as well as due to cytokine driven apoptosis of erythroid progenitors, erythroid progenitor and cytokine mediated inhibition of iron utilization. Hematological parameters like those related to PLTs functions can almost activated during the pathogenesis of rheumatoid arthritis. Probable presence of direct associations for PLTs concentration with the clinical activity of disease was estimated by limited researchers. In one study, the authors have observed unexpectedly that PLT plays multifactorial roles in developing of inflammation since there is an elevation in inflammatory activity of thrombocytes throughout development of arthritis (Boilard *et al*., 2012). Tekeoğlu *et al*. (2016) confirmed that the level of such activity has direct proportional to PLTs count.

At present, serum biomarkers used in the diagnosis of established arthritis are RF and TNF. RF is a protein produced by the immune system to attack the tissues of people with autoimmune diseases such as rheumatoid arthritis (RA), (Valesini *et al*., 2015; Illescas-Montes *et al*., 2019). The increasing concentration of RF in arthritic rats, observed in current study, was similar to findings demonstrated by several studies (Ahmed *et al*., 2015; Gupta *et al*., 2020; El-Tanbouly and Abdelrahman, 2022). Early studies for etiology of arthritis were demonstrated the activity of RF with immune complexes related to synovitis and vasculitis (Lipsky *et al*, 1989; Vollertsen and Conn, 1990; Lotz *et al*., 1992). Subsequent studies identified the role of T-cell responses, cytokines and chemokines to arthritis severity (Kokkonen *et al*., 2010; Diani *et al*., 2015). Currently, many studies have been underscored for great roles of humoral immunity during the etiopathogenesis of arthritis (Jasemi *et al*., 2021; Jyssum *et al*., 2022). In arthritic patients, RFs induction could represent an indicator for severity of diseases with a potential activation of B cells (Kordtabar *et al*., 2019). Recent clinical trials have used depletion of B cell to support an idea that "humoral immunity produced by RF plays an important role in the disease process" (Jasemi *et al*., 2021; Jang *et al*., 2022; Kim *et al*., 2022). In arthritis, inflammation and joint destruction cause T-cells to migrate to the synovial environment. Therefore, the recent treatment option for this degenerative disease is to control the symptoms and drug damage that affects several regions in immune system (Farrugia and Baron, 2016). One of the advanced immune therapies is based on the using of TNF-α inhibitors. There is strong evidence that TNF-α released by PBMCs to contribute in development of the immune system against infections (Abbasi *et al*., 2019; Zamri and De Vries, 2020). The high levels of TNF-α observed in patients were active and widespread; suggesting an involvement of TNF-α in mechanisms that regulate T cell inhibitory activity (Luo *et al*., 2022). However, the increasing level of TNF-α observed in arthritic rats of this study is similar with that showed by other studies (Findeisen, 2019; Liang, 2021; Jang, 2021).

Conclusion

This study concluded that arthritis causes a significant abnormality in blood parameters and the targeted immune markers which could be due to inflammatory process in affected joints. However, the author does not exclude the possibility of developing a physiological malignancy in a body as a result of the disease which affect in turn on blood contents. Hence, furthermore studies are necessary to estimate the effect of arthritis on other immune markers and all body organs. Also, this study assumed that the using of PLT as a marker among the patients of arthritis or other inflammation may provide valuable information and better explain the disease pathogenesis or activity.

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