Association of Nuclear Factor Kappa B pathway (NF-KB) with Rheumatoid Arthritis disease and it's severity (Case-Control Study)

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Abstract

Background: Rheumatoid arthritis is an inflammatory disease that affects the joints found in the limbs. It mostly presents as recurring, persistent inflammation in the joints. When patients are advanced, the cardiac, skin, as well as other tissues and organs may have serious impediments as a result of joint abnormalities and impairments. Nuclear factor kappa B is an active transcription factor that moves from the cytoplasm to the nucleus when it is activated, NF-kB is also the most extensively researched pathophysiologic mechanism for RA. This might result in a positive auto-regulatory loop that leads to the amplification of the inflammatory process and the maintenance of chronic inflammation at local locations.Methods: The study was performed between November 2021 to April 2022. involving 70 patients with disease who fulfilled four or more of the 2010 American College of Rheumatology and European League Against Rheumatism (ACR/EULAR) classification criteria for RA and 70 individuals healthy as control groups. The (DAS28-ESR) disease activity score, Clinical activity index (CDAI), and erythrocyte sedimentation rate were measured, as well as rheumatoid factors (RF) were detected by latex agglutination in all participants. Anti-cyclic citrullinated peptide (ACCP) and NF-KB in the human serum samples were measured by enzyme-linked immunosorbent assay (ELISA). The age group of both the patients and the controls included in this study was 20-70. RA was found to be high in individuals of the age group >40 years, at a percentage of 71.4%. The mean age was 46.2±10.3 years for patients and 1.7±0.5 for controls. Results: The results clearly showed high serum NF-KB levels in patient groups with RA compared to control (P = 0.0001). NF-KB and ACCP have a weak positive correlation at (r) = 0.200. According to DAS28-ESR, there were significantly increased concentrations of NF-KB in severe patients at P values of 0.0001 in comparison to mild patients.

Conclusions: The biomarker NF-KB is a good prognostic marker for the severity of RA.

Keywords: Rheumatoid arthritis; Nuclear factor kappa B; Anti-cyclic citrullinated peptide; Rheumatoid factor.

1. Introduction

RA is a chronic and immunological illness that affects about one percent of the world's population suffers with RA. RA affects women three times as often as men (1). The skin, eyes, kidneys, heart and lungs are all ultimately affected by the disease. Deformities and bone degradation may occur as a result of joint injury caused by cartilage and bone destruction (2). As the disease progresses, the patient's quality of life, capacity to work, and lifespan are all negatively impacted, as well as the socioeconomic load on their families (3).

RA has a serious effect on a person's physical, mental, and social well-being, all of which may have a negative effect on the overall emotional well-being (4) Extra-articular manifestations and comorbidities are frequent findings in rheumatoid arthritis (RA), leading to increased morbidity and premature mortality (5).

The specific immune reactions might happen outside the articular region, especially in mucosal sites, such as in respiratory, oral and intestinal mucosa (6). However, Joint degradation that begins at the synovial membrane and involves most RA tissues is caused by the complex interaction of immune modulators (cytokines and effector cells). The creation of new blood vessels and the infiltration and/or local activation of mononuclear cells, such as macrophages and T, B, plasma, dendritic, and mast cells, are characteristics of synovitis (7).

The rigorous study of biomarkers in rheumatology emerged from the need to comprehend the processes underlying several rheumatic disorders. Discovering novel biomarkers with critical roles in various phases of development remains an issue of interest for RA. As a result, biomarkers are needed to facilitate early diagnosis and predict prognosis in RA (8).

Biomarkers are biological properties that may be objectively evaluated and serve as indications or assessments of normal or pathological processes or response to treatment. Diagnostic or prognostic markers like rheumatoid factor (RF) and measurements of disease activity like acute phase reactants are generally referred to as RA biomarkers. Biomarkers are described by the National Institutes of Health as "a property that is objectively tested and analyzed as an indication of normal biological functions, pathogenic processes, or pharmacological

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reactions to a therapeutic intervention (9).
The NF-kB family of transcription factors plays an essential role as stimuli in the cellular environment and controls the expression of vital regulatory genes such as those involved in inflammation, immunity, cell proliferation and cell death (10). It is not unexpected that NF-kB is shown to be chronically active in many inflammatory disorders, including inflammatory bowel disease, sepsis, arthritis, gastritis, asthma, atherosclerosis, and others, because NF-kB controls numerous genes implicated in inflammation (11). NF-kB proteins are capable of regulating the expression of hundreds of genes involved in physiological processes regulation such as immunity, inflammation, cell death, and proliferation (12).
Patients with rheumatoid arthritis have higher levels of cytokines, particularly TNFa, in their synovial fluid. Overproduction of TNFa may play a significant role in the development of the disease. Patients with rheumatoid arthritis have a higher concentration of macrophages in their synovium, where TNFa is generated. Suppression of NF-kB may prevent these macrophages from producing TNFa. Rheumatoid arthritis patients' synovial biopsy samples had higher NF-kB levels (13), Activated NF-κB is detected in human synovial tissues during both early and late stages of inflammation (14). Activated NF-KB members also play an important role in B cell proliferation and the generation of autoantibodies. The activation of NF-KB in dendritic cells may lead to cytokines that enhance inflammatory T cell development in the innate immune system. The severity of an illness may worsen if these cycles keep recurring (15).
The purpose of this study was to measure serum levels of NF-KB in RA Iraqi patients and to investigate correlations with the activity or severity of rheumatoid arthritis in a try to forecast the viability of using NF-KB as a disease severity indicator.

2. Materials and Methods

Patients

Participants in this research were Iraqi people (as patients) with RA who attended the Rheumatology department in Al-Sadr Medical City in Najaf, as well as healthy controls from Najaf. This research was carried out on a total of 140 participants, including 70 people with a diagnosis of rheumatoid arthritis (determined by rheumatologist doctors in line with ACR/EULAR 2010 Criteria and serological testing) and another 70 people who were considered to be healthy controls. Among the patients, there were 15 males and 55 females, ranging in age between 20 and 70 years. Among the controls, there were 10 males and 60 females, ranging in age between 20 and 70 years. Each patient and control person was questioned about their name, age, gender, diabetes, high blood pressure, smoking, RA family history, and any other items that were included in the questionnaire. The duration of RA disease in patients included in this study was a minimum of months and a maximum of 30 years, and according to the data in rheumatologist questions from RA patients, so we have done the DAS28-ESR, and CDAI for patients to classify RA patients depending on the DAS-28-ESR to mild, moderate, and severe by use of the equation in the web site (https://www.mdcalc.com/disease-activity-score-28-rheumatoid-arthritis-esr das28 esr).
Patients will be recruited to the study are carefully evaluated to confirm they meet the specific diagnostic criteria required for RA. All Patients that diagnosis by rheumatologist according to ACR/EULAR Criteria and get >or =6 score of this criteria, age between 20 –70 year, Patients excluded from the study are suffering from other autoimmune diseases, central nervous system and cardiovascular diseases, who has recently surgery or wound or acute local inflammation, patients that age older than70 year and age less than 20year.
Healthy control who were included in the study, age matching, apparently healthy people who don't suffering from joint pain or joint problem and no family history of RA but all healthy people with any inflammation and people that have ESR Titer Positive who has recently surgery or wound or acute local inflammation we will excluded them
All laboratory tests analysis was performed in the Clinical Microbiology Research Lab, College of Medicine, University of Kufa.

Material

The equipment utilized in this study, gel serum tubes 5 & 10 ml, disposable sterile syringes, 5 &10ml, disposable pipette tips, eppendorf tubes, 1.5 & 2 ml, disposable ESR tubes and disposable Glove. The instrument utilized in this study centrifuge, deep freeze, incubator, ELISA, refrigerator, micro pipettes with different sizes. The kits used in this study, nuclear factor kappa B ELISA kit (Bioassay kit), rheumatoid factor RF-latex kit, Antibodies against cyclic citrullinated peptide (Anti CCP) (IgM) kit (Bioassay kit).

Methods

We collected a sample between five and ten milliliters of venous blood. The blood sample was separated into two parts: two milliliters were collected in disposable ESR tubes for an ESR test using the Westergren method; the remaining three to seven milliliters were transferred to a sterile gel tube, centrifuged for the separation of serum after being allowed to clot at the laboratory room temperature; finally, the serum sample was divided into three aliquots in an Eppendorf tube for each patient and stored at temperatures ranging from -20 to -45 degrees C.
Prior to the start of the study, the ethical committee of the Faculty of Medicine, University of Kufa, provided its permission. Individuals' informed agreement was also gained.
The patients were clinically evaluated and diagnosed by consultant rheumatologists. Measure the serum Level of NF-KB in patients and control by using

specific enzyme-linked immunosorbent assay kit. Measure RF (Rheumatoid Factor) by agglutination test kit, ESR in serum of patients by Westergren method and serum levels of Anti-CCP antibodies in patients by ELISA kit. For RA activity, the disease activity score (DAS28) and clinical disease activity index (CDAI) will be assessed.

Statistical analysis

The SPSS program, version 20, was utilized to analyze the data. When the data was presented, Continuous variables were displayed as mean, standard deviation, and error, whereas categorical variables were shown as frequencies and percentages.

All of the research variables were significant for the Shapiro-Wilk test (p=0.001), suggesting that the variables did not follow a normal distribution, which led to comparisons between two groups being made using the Whitney U test. This method was used to compare patients and healthy subjects, as well as to compare regularity with treatment, age, gender, and response to treatment, while comparisons between three groups were made using the Kruskal-Wallis test. such as in the comparison of CDAI and DAS28-ESR. In RF comparison, the Chi square is used. In order to evaluate the degree of correlation that existed between the study's variables, Pearson correlations and scattered plots were used.

Statistical significance was regarded as having a P value must be equal to or less than 0.05.

3. Results

Table 1 explains the association of NF-KB between patients and controls. The table revealed that there was an increase significantly in the concentration of NF-KB among patients 2.5±0.2 when compared to the mean of the concentration among the control group 0.7±0.05. According to age, there is a significant increase (p 0.0001) in the serum biomarker concentration NF-KB among patients of various ages as compared to the healthy group, whereas the serum biomarker concentration NF-KB increases in patients that are aged > 40 years as compared to those aged ≤ 40 years. According to gender, there is a considerable increase significantly (p≤ 0.05) in the serum concentration of NF-KB in both male and female patients compared to control groups.

Table 2 explains the association between NF-KB and the study parameters in each patient. The results show a strong correlation for NF-KB with ESR (r) = 0.654; DAS-28ESR (r) = 0.794; and CDAI (r) = 0.706; however, weak positive with ACCP (r) = 0.200; very weak with disease duration (r) = 0.169; and age (r) = 0.086. As illustrated in the figures below (1,2,3,4,5,6).

Table 1: the NF-KB levels in patients and controls.

Variable		NF-KB patients		NF-KB Controls		P*
		No.	Mean±SE	No.	Mean±SE	
NF-KB		70	2.5±0.2	70	0.7±0.05	0.0001
Age group	≤ 40	20	2.5±0.4	20	0.5±0.08	0.0001
	>40	50	2.6±0.2	50	0.7±0.06	0.0001
Gender	Male	15	1.9±0.3	10	0.7±0.1	0.004
	Female	55	2.7±0.2	60	0.6±0.05	0.0001

Table 2: Correlation of the NF-KB in serum with the studied parameters in patients with RA.

Parameters	NF-KB	
	P	*r*
Age	0.480	0.086
Duration	0.161	0.169
Anti ccp	0.097	0.200
ESR	0.0001	0.654
DAS28-ESR	0.0001	0.794
CDAI	0.0001	0.706
* significant at p value 0.05 or less.		

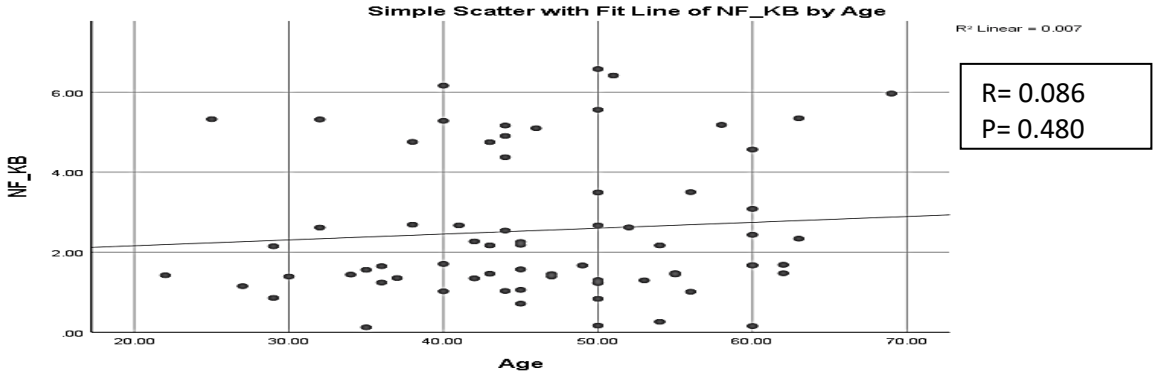

Figure (1): Correlation between NF-KB & Age.

The graph above shows a very weak positive relationship between NF-KB & age.

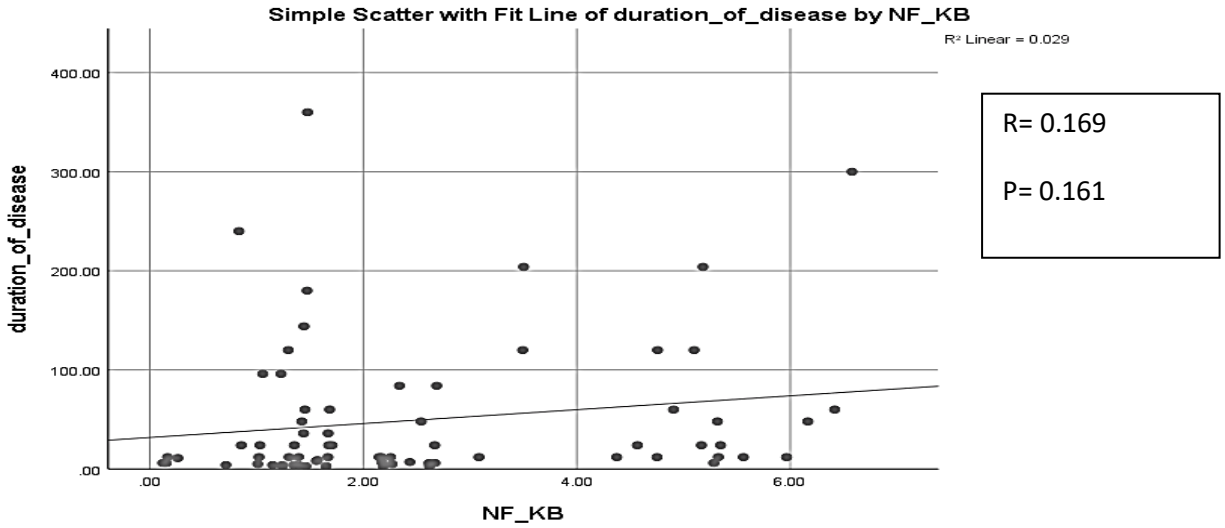

Figure (2): Correlation between NF-KB & disease duration.

The graph above shows a very weak positive association between NF-KB & illness duration.

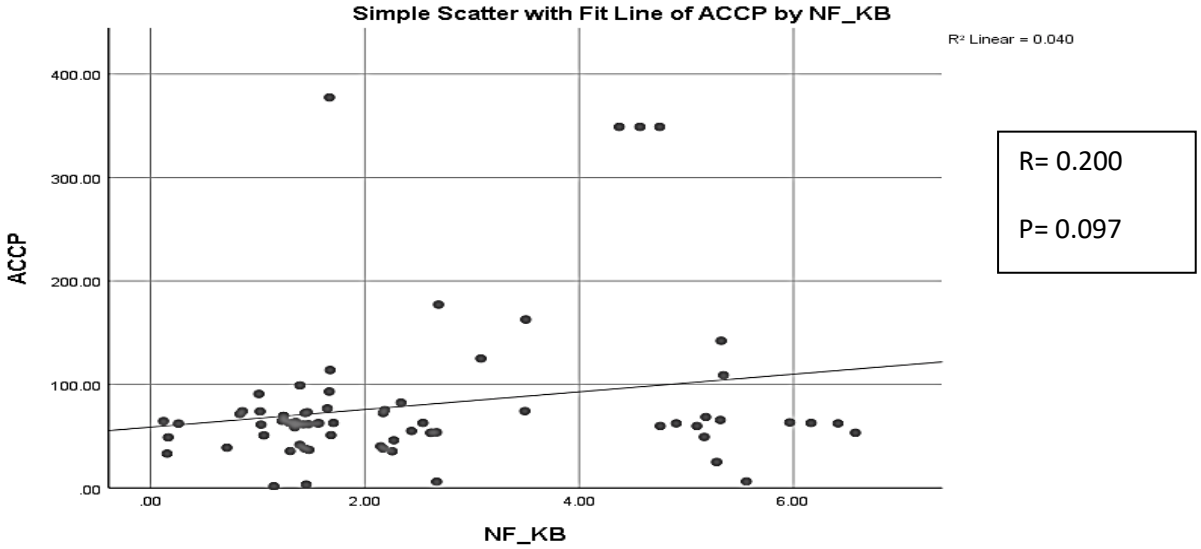

Figure (3): Correlation between NF-KB & ACCP.

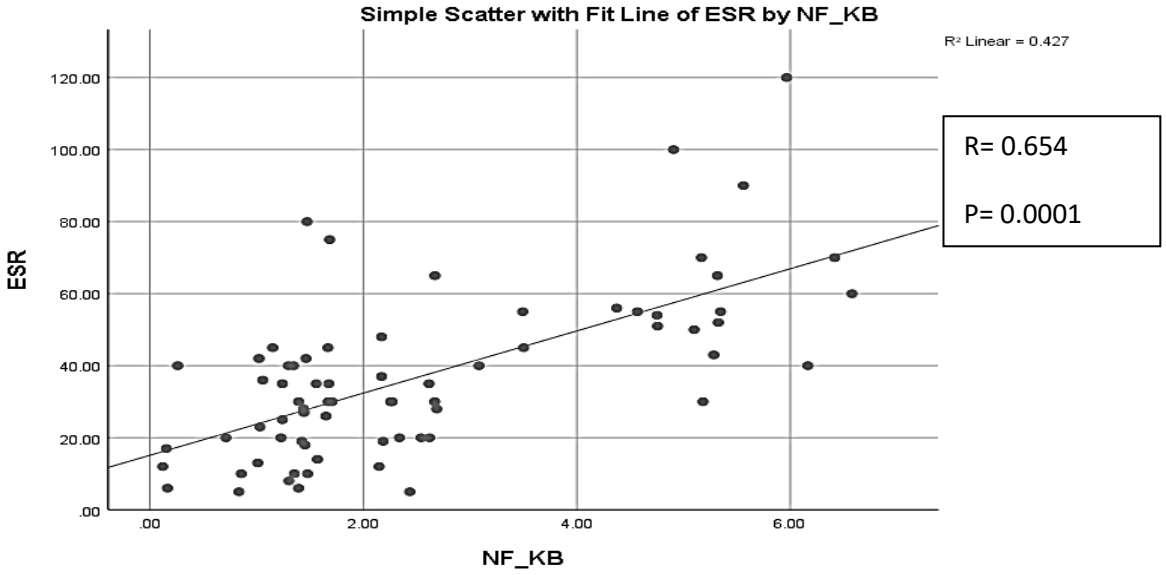

Figure (4): relation between NF-KB & ESR

The graph above shows that NF-KB and ESR have a strong positive association.

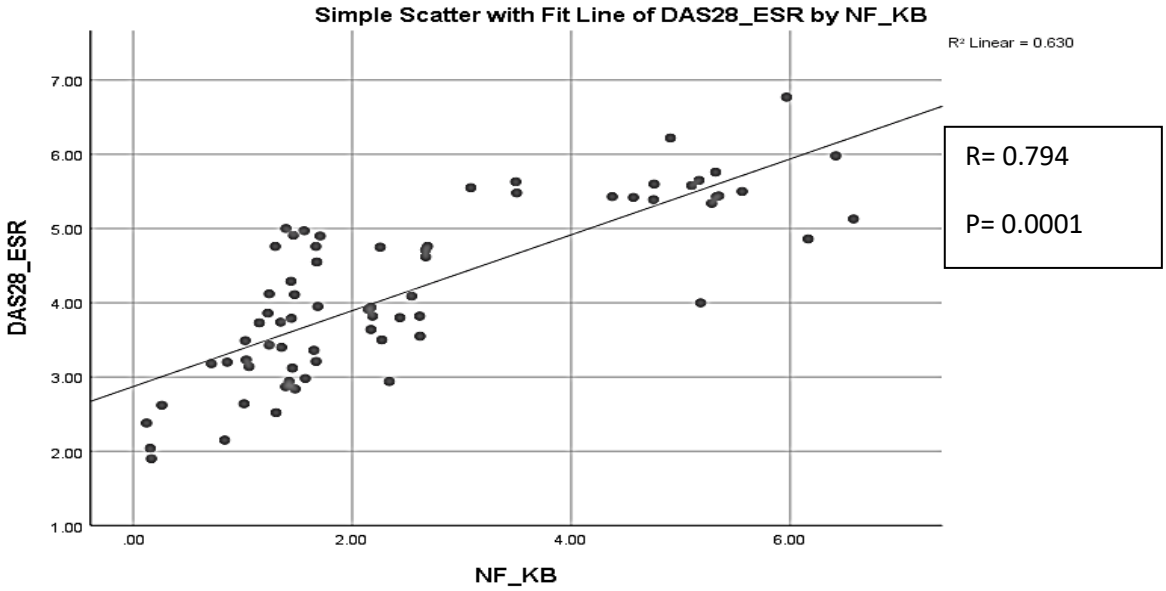

Figure (5): relation between NF-KB & DAS28-ESR.

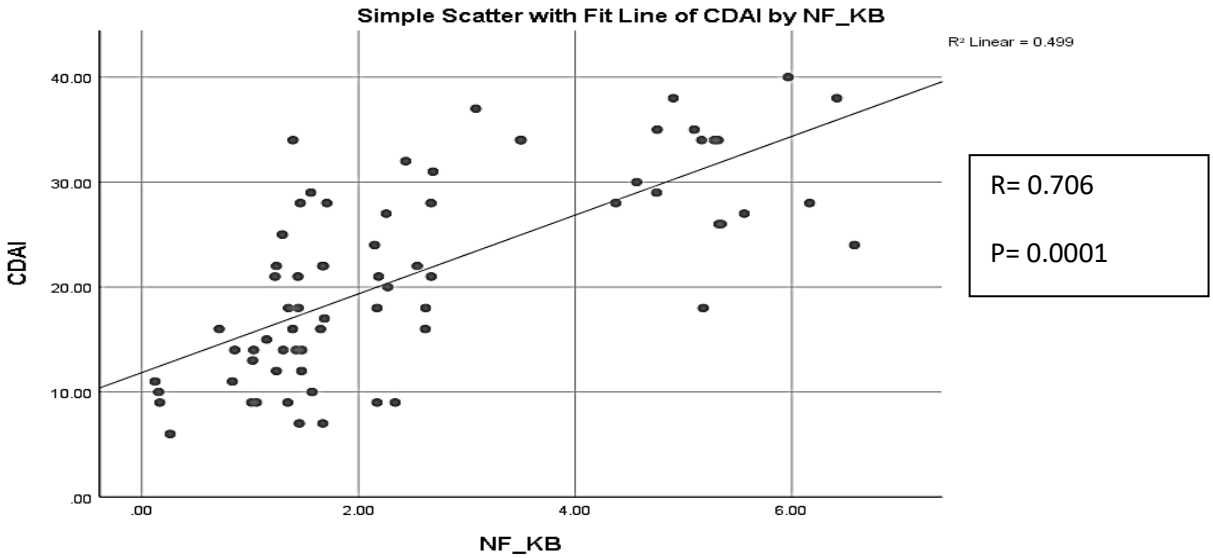

Figure (6): relation between NF-KB & CDAI.

4. Discussion

NF-kB functions as a potential regulator of inflammation, which has been identified as one of the key inflammatory processes in Autoimmune Disease (AD) like Rheumatoid Arthritis (RA), experiencing huge inflammatory cytokine production, including Interleukins-1, TNFa, and IL-6 associated with bone degradation and disease progression. NF-kB is also the most researched pathophysiological mechanism in RA, but, during the past several decades, a more recently found Nuclear factor kappa-B receptor activator ligand (RANKL) has been likewise associated with NF-kB activation and bone deterioration (16).

In the current study, at p = 0.0001, it was discovered that patient serum NF-KB concentrations were substantially greater than those of controls. Wagner *et al.* (17), found that the amount of NF-KB in patients was greater than in controls (p = 0.001), which proved this result.

Our study also found a significant variance in the NF-KB between patients and controls based on age at p = 0.0001, which was the same as what Wagner *et al.* (17) found at p = 0.001. But our research found among RA patients that NF-KB and age show a weak positive correlation (r) = 0.086. This agrees with the study by Hensvold *et al.* (18), which found a very weak positive connection between them at (r) = 0.005.

Our study also found a significant variation in levels between controls and patients based on gender at p = 0.0001, which was the same as what Boman *et al.*, (19) found at p = 0.02. increased levels of NF-KB in female patients more than in female controls.

The receptor activator of NF-kB (RANK), a kind of TNFR, is the main activator of NF-KB. Osteoprotegerin (OPG), a RANK ligand (RANKL) decoy receptor homolog, inhibits RANK by binding to RANKL and is hence crucial for controlling NF-kB activation. In this context, the result of the research by Hensvold *et al.* (18), who found a weak positive correlation between ACCP and RANKL (r = 0.22), is

consistent with our findings, which found a weak positive association between ACCP and NF-KB (r = 0.200).

The current study recorded a higher concentration of NF-KB in patients who had a poor response to treatment than in those who had a good response to treatment, with a p value = 0.0001. This is similar to the findings of Sennels *et al.* (20), who reported a higher concentration of non-response to treatment than in responses.

According to our research, NF-KB and ESR have a very strong positive association (r = 0.64, p = 0.0001). This agrees with Boman *et al.* (19) , who found a higher concentration of NF-KB ligand with ESR at p = 0.01.

The current study shows a weak positive correlation between NF-KB and illness duration (r = 0.169, p = 0.161). This is close to the study by Yang *et al.* (21), who found a very weak relationship (r = 0.093, p = 0.23) between NF-KB with disease duration.

Our study shows there is a significant positive connection between NF-KB and illness severity. DAS28-ESR at (p= 0.0001, r= 0.749), CDAI at (p= 0.0001, r= 0.706) The current result was in agreement with Salinas *et al.* (22), who discovered a strong positive relationship between NF-KB and DAS28-ESR at (p= 0.001, r= 0.703).

5. Conclusions

According to the main findings in the present study, patients have an increased concentration of NF-KB significantly in comparison to the control group. There was a significant association between NF-KB with disease activity score and CDAI among patients with RA. The biomarker NF-KB is a good prognostic marker for the severity of rheumatoid arthritis.

6. Reference

1. Maiuolo J, Muscoli C, Gliozzi M, Musolino V, Carresi C, Paone S, et al. Endothelial dysfunction and extra-articular neurological manifestations in rheumatoid arthritis. Biomolecules. 2021;11(1):1–18.
2. Saklani Kala J, Agari R, Kumar Singh A, Professor A. a Review Article on Amavata W.S.R. Rheumatoid Arthritis. 2021;08(04):734–44. Available from: www.iamj.in
3. Gautam S, Kumar U, Dada R. Yoga and its impact on chronic inflammatory autoimmune arthritis. Front Biosci - Elit. 2021;26(1).
4. Conforti A, Di Cola I, Pavlych V, Ruscitti P, Berardicurti O, Ursini F, et al. Beyond the joints, the extra-articular manifestations in rheumatoid arthritis. Autoimmun Rev [Internet]. 2021;20(2):102735. Available from: https://doi.org/10.1016/j.autrev.2020.102735
5. Figus FA, Piga M, Azzolin I, McConnell R, Iagnocco A. Rheumatoid arthritis: Extra-articular manifestations and comorbidities. Autoimmun Rev. 2021;20(4).
6. Catrina , A.I et al. Mechanisms involved in triggering rheumatoid arthritis. 2016;162–74.
7. Nogueira E, Gomes A, Preto A, Cavaco-Paulo A. Update on Therapeutic Approaches for Rheumatoid Arthritis. Curr Med Chem. 2016;23(21):2190–203.
8. Stoel B. Use of artificial intelligence in imaging in rheumatology-current status and future perspectives. RMD Open. 2020;6(1):1–10.
9. Taylor PC, Moore · Adam, Vasilescu R, Alvir J, Tarallo M. A structured literature review of the burden of illness and unmet needs in patients with rheumatoid arthritis: a current perspective. Rheumatol Int. 2016;36.
10. Zinatizadeh MR, Schock B, Chalbatani GM, Zarandi PK, Jalali SA, Miri SR. The Nuclear Factor Kappa B (NF-kB) signaling in cancer development and immune diseases. Genes Dis [Internet]. 2021;8(3):287–97. Available from: https://doi.org/10.1016/j.gendis.2020.06.005
11. Park MH, Hong JT. Roles of NF-κB in cancer and inflammatory diseases and their therapeutic approaches. Cells. 2016;5(2).
12. Giuliani C, Bucci I, Napolitano G. The role of the transcription factor Nuclear Factor-kappa B in thyroid autoimmunity and cancer. Front Endocrinol (Lausanne). 2018;9(AUG):1–8.
13. Ilchovska D (Daisy), Barrow DM. An Overview of the NF-kB mechanism of pathophysiology in rheumatoid arthritis, investigation of the NF-kB ligand RANKL and related nutritional interventions. Autoimmun Rev. 2021 Feb 1;20(2):102741.
14. Sabir JSM, El Omri A, Banaganapalli B, Al-Shaeri MA, Alkenani NA, Sabir MJ, et al. Dissecting the Role of NF-κb Protein Family and Its Regulators in Rheumatoid Arthritis Using Weighted Gene Co-Expression Network. Front Genet. 2019;10(November):1–17.
15. Nejatbakhsh Samimi L, Farhadi E, Tahmasebi MN, Jamshidi A, Sharafat Vaziri A, Mahmoudi M. NF-κB signaling in rheumatoid arthritis with focus on fibroblast-like synoviocytes. Autoimmun Highlights [Internet]. 2020;11(1). Available from: https://doi.org/10.1186/s13317-020-00135-z
16. Miraghazadeh B, Cook MC. Nuclear factor-kappaB in autoimmunity: Man and mouse. Front Immunol. 2018;9(APR).
17. Wagner L, Haefeli WE, Merle U, Lorenz HM, Hohmann N, Weiss J, et al. A nuclear factor kappa B reporter cell line used to evaluate ex vivo the net inflammatory effect of plasma samples from patients with rheumatoid arthritis, psoriasis, or COVID-19. Cytokine. 2021 Feb 1;138:155399.
18. Haj Hensvold A, Joshua V, Li W, Larkin M, Qureshi F, Israelsson L, et al. Serum RANKL levels associate with anti-citrullinated protein antibodies in early untreated rheumatoid arthritis and are modulated following methotrexate. 2015;
19. Boman A, Kokkonen H, Ärlestig L, Berglin E, Rantapää-Dahlqvist S. Receptor activator of nuclear factor kappa-B ligand (RANKL) but not sclerostin or gene polymorphisms is related to joint destruction in

early rheumatoid arthritis. Clin Rheumatol. 2017;36(5):1005–12.
20. Sennels HP, Sørensen S, Østergaard M, Knudsen LS, Hansen MS, Skjødt H, et al. Circulating levels of osteopontin, osteoprotegerin, total soluble receptor activator of nuclear factor-kappa B ligand, and high-sensitivity C-reactive protein in patients with active rheumatoid arthritis randomized to etanercept alone or in combination with methotrexate. Scand J Rheumatol. 2008;37(4):241–7.
21. Yang B, Luo L, Chen L, Niu Q, Zhang J, Xu H, et al. ETS1 polymorphism rs73013527 in relation to serum RANKL levels among patients with RA. 2021; Available from: http://dx.doi.org/10.1097/MD.0000000000024562
22. Salinas FLS, Benítez JGS, Navarro MDH, Zerón HM. NF-κB1/IKKε gene expression and clinical activity in patients with rheumatoid arthritis. Lab Med. 2018;49(1):11–7.