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ORIGINAL ARTICLE

Receptor Activator of Nuclear Factor Kappa-B Ligand and Presepsin as Crucial Biomarkers in Women with Rheumatoid Arthritis

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ABSTRACT

Background: *Rheumatoid arthritis (RA) is a long-term autoimmune disorder that primarily targets the joints. It causes persistent inflammation, leading to pain, swelling, and gradual joint deterioration.* ***Objective:*** *This study investigates the immunological role of Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) and presepsin in the blood of women with rheumatoid arthritis.* ***Methodology:*** *A case-control study was conducted at AL-Najaf Medical City, Iraq from October 2023 to February 2025. This study enrolled 60 healthy participants as a control group, 30 early RA women, 30 moderate RA Women, and 30 severe RA Women; all Women were diagnosed by the physician. RANKL and presepsin have been measured in women' blood using the ELISA technique.* ***Results:*** *The blood levels of RANKL (42.12±2.432 pg/ml) and presepsin (211.7±7.937 pg/ml) in all women were significantly higher than those in the control group (19.93 ± 1.416 pg/ml and 182.8 ± 14.51 pg/ml, respectively), with P-values of < 0.0001 and 0.0339. RANKL levels showed a significant increase (P < 0.05) in women with moderate and severe RA, while presepsin levels were significantly elevated (P < 0.0241) in women with severe RA compared to healthy controls. Additionally, a positive correlation was observed between the two markers in women with moderate and severe RA.* ***Conclusion:*** *RANKL and presepsin may be viable markers for diagnosing rheumatoid arthritis, and their raised levels may indicate disease severity. In addition, these biomarkers may be useful in the treatment of rheumatoid arthritis among women.*

INTRODUCTION

Rheumatoid arthritis is a complex and debilitating autoimmune disease that attacks the joint structures of the body. Severe inflammation, pain, and damage to areas of the body are caused as a result of this[1]. The disease sends the body's immunity into overdrive, both innate and adaptive immunity in this case. This results in the proliferation of bad immune cells and the release of inflammatory cytokines[2]. These have damaging effects on the tissues at the joints[3]. The disease usually affects joints. The wrist, hands, feet, spine, knees, and jaw are most commonly affected[4]. The disease inflammation of the synovial membranes that surround the joints when there is no or little use of the limbs. This results in deformities, loss of function and ultimate death[5]. It frequently causes tiredness, fever, and loss of appetite[6]. Research done on this issue supports the role of immune modulators and signaling pathways in the development of this disease[7]. RANKL is a member of the tumor necrosis factor superfamily that plays a central role in the regulation of bone metabolism[8].

Recent studies have highlighted the potential role of RANKL in rheumatoid arthritis progression[9]. To be more specific, it has been implicated in the regulation of osteoclast differentiation and activation, processes which mediate bone and cartilage destruction[10]. Recent research has highlighted the potential role of RANKL in enhancing the evolution of rheumatoid arthritis. RANKL plays a role in controlling osteoclast differentiation and activation[11] .

A promising biomarker is presepsin that is also known as soluble cluster of differentiation 14 subtypes (sCD14-ST)[12]. Scientists proved the release of this protein into the blood during bacterial infection response[13]. Many researches showed its diagnostic and prognostic role of sepsis. A study showed results yielded strong diagnostic accuracy for presepsin[14]. The purpose of this research is to determine the levels of RANKL and presepsin in an early, moderate, and severe stage of rheumatoid arthritis in women. By studying their immune role, it will be determined whether they can serve as biomarkers to severity check. Also, a feasibility study of these markers as alternative chemical treatment options will be studied (marker for success of treatment).

METHODOLOGY

Participants in the study

A case-control investigation was conducted in the Rheumatology and Arthritis Department of Al-Najaf Hospital, Iraq, during the period from October 1, 2023 to February 1, 2025. A total of 150 participants, male or female, were recruited from 18 to 60 years of age. There were a total of 90 women with diagnosis of rheumatoid arthritis, allocated equally into three subgroups of early stage RA, moderate RA and severe RA. A control group of 60 healthy subjects was included (without any clinical conditions, or underlying diseases). To ensure the accuracy and reliability of the data, all diagnoses of RA were confirmed by specialized physicians before they were included in this study.

Ethical Considerations

The study received approval from both the Institutional Ethics Committee of the College of Medicine at the University of Kufa, Faculty of Science (7624/ 13/12/2023) and the Scientific Research Committee of the Najaf Health Department.

Measurement of blood levels of RANKL and presepsin

This test was conducted according to the manufacturing company instructions (Bioassay Technology Laboratory. Shanghai, China). Five ml of blood sample were collected from all the individuals and 2 ml of serum for each individual has been obtained by centrifugation at 8000 rpm/10 min. This serum concentration has been used for the measurement of RANKL and presepsin by the enzyme-linked immunosorbent assay (ELISA)[15,16].

Statistical analysis

The mean and standard error (SE) were calculated for each value using GraphPad Prism. A p-value of less than 0.05 was considered statistically significant in the analysis[17,18].

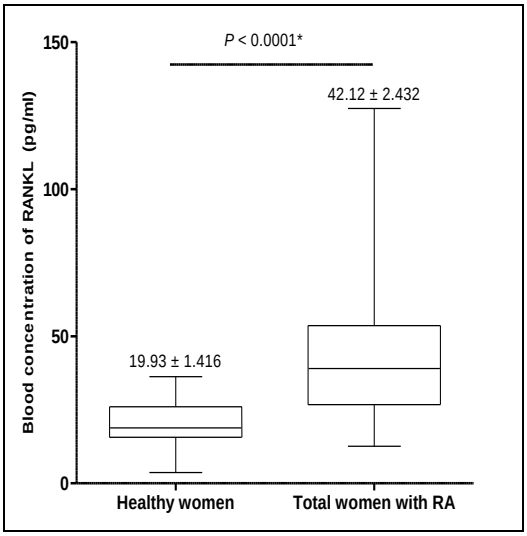

Fig. 1: RANKL blood levels in total women with RA compared to the control group

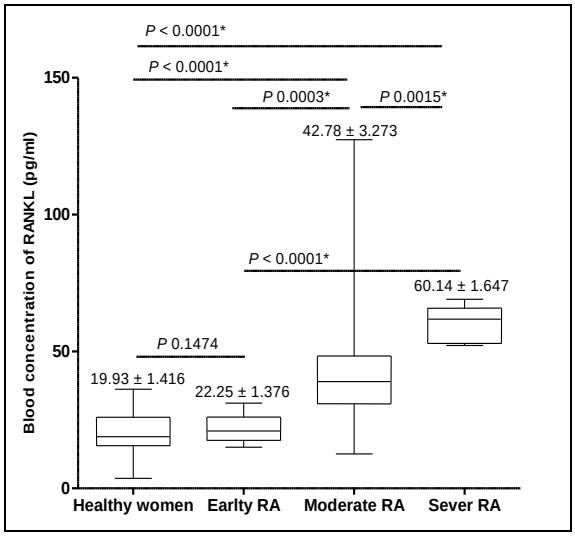

Fig. 2: RANKL blood levels in women with early, moderate, sever RA compared to the control group

RESULTS

RANKL

The study revealed a notable increase in serum RANKL levels in women with rheumatoid arthritis (42.12 ± 2.432 pg/ml) when compared to healthy controls (19.93 ± 1.416 pg/ml, $p < 0.0001$) (Figure 1). However, women with early-stage RA (22.25 ± 1.376 pg/ml) did not show a statistically significant increase in RANKL levels compared to the control group ($P = 0.1474$). In contrast, women with moderate (22.25 ± 1.376 pg/ml) and severe RA (60.14 ± 1.647 pg/ml) exhibited significantly higher RANKL levels compared to controls ($p < 0.0001$). Additionally, women with severe RA had significantly elevated RANKL levels compared to those with early and moderate RA ($P < 0.05$) (Figure 2).

Presepsin

The study found a significant increase in blood presepsin levels among women with rheumatoid arthritis (RA) (211.7 ± 7.937 pg/ml) compared to healthy controls (182.8 ± 14.51 pg/ml, P = 0.0339) (Figure 3). However, women with early-stage RA (194.7 ± 7.739 pg/ml) and moderate-stage RA (209.9 ± 10.96 pg/ml) did not exhibit a statistically significant difference in presepsin levels compared to the control group (P = 0.2850, P = 0.0680, respectively). In contrast, women with severe RA (233.7 ± 20.79 pg/ml) had significantly higher presepsin levels than healthy controls (P = 0.0241). Furthermore, presepsin levels in women with severe RA were significantly elevated

compared to those with early-stage RA (P = 0.0448) (Figure 4).

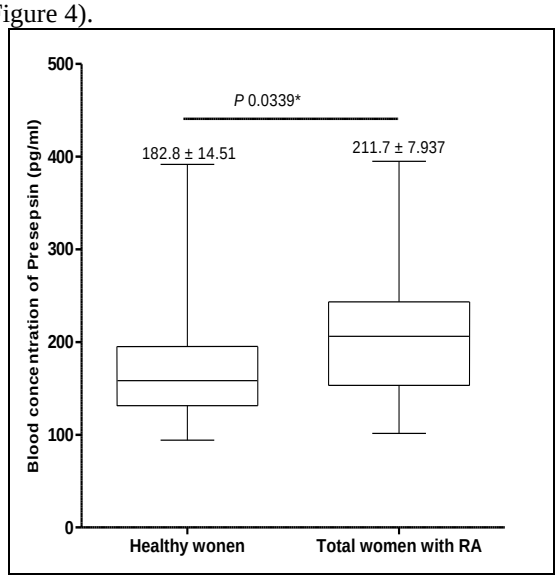

Fig. 3: Presepsin blood levels in total women with RA compared to the control group

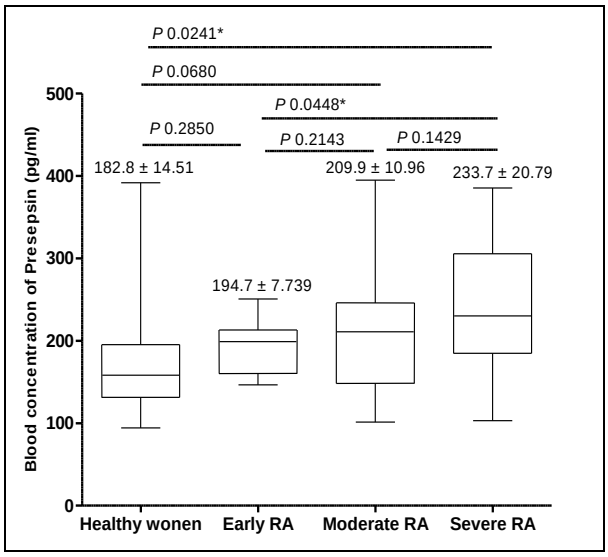

Fig. 4: Presepsin blood levels in women with early, moderate, sever RA compared to the control group

Correlation

The results indicated a positive relationship between RANKL and presepsin levels in the blood of women with early-stage RA, though the correlation was not statistically significant (P = 0.5337, Figure 5). A similar positive correlation was also observed in women with moderate RA (P = 0.9871, Figure 6) and severe RA (P = 0.8135, Figure 7).

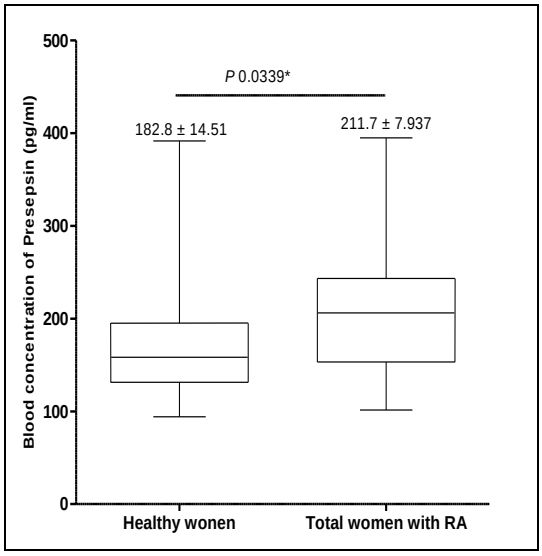

Fig. 5: Presepsin blood levels in total women with RA compared to the control group

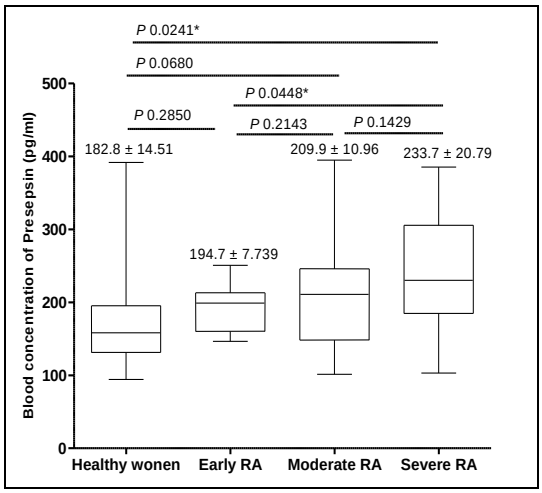

Fig. 6: Presepsin blood levels in women with early, moderate, sever RA compared to the control group

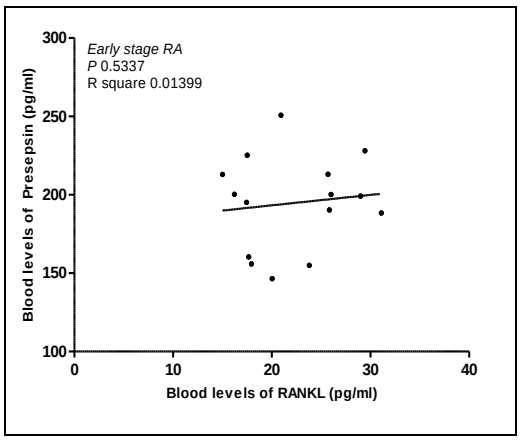

Fig. 7: Correlation between serum levels of RANKL and Presepsin in women with early RA

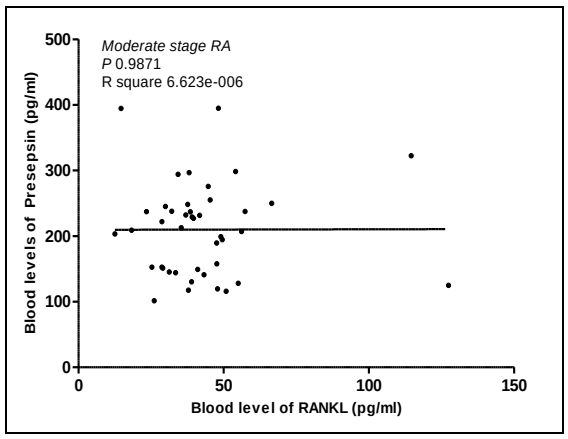

Fig. 8: Correlation between serum levels of RANKL and Presepsin in women with moderate RA

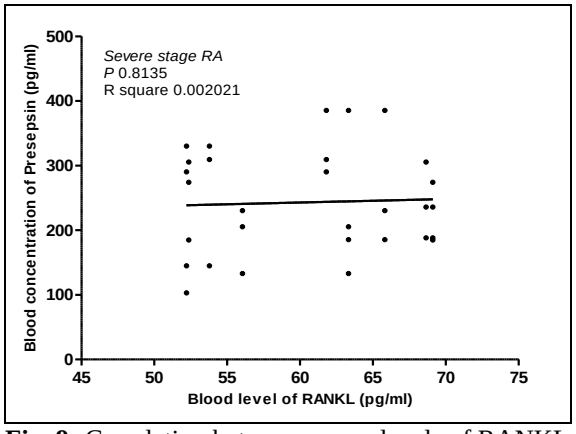

Fig. 9: Correlation between serum levels of RANKL and Presepsin in women with severe RA

DISCUSSION

Serum RANKL levels were higher in RA women compared to controls. Also, it reached higher significantly levels, in moderate to severe cases. The highest levels of RANKL were observed for severe RA, notably linking RANKL with disease severity (Figure 1and 2). NF-κB signaling pathway is known to play a pivotal role in the development and pathogenesis of autoimmune diseases, such as rheumatoid arthritis (RA)[19]. It is a master regulator of the inflammatory response, modulating the expression of cytokines and chemokines that drive RA pathogenesis. Targeting NF-κB as possible therapeutical strategy is being investigated for RA and other inflammatory diseases[20]. Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1)[21], with a variety of stimuli leading to activation of the NF-κB family of transcription factors Upon activation the NF-κB translocates to the nucleus and induces the transcription of genes encoding cytokines, chemokines, and adhesion molecules, all important mediators of inflammation. Thus, this pathway has a key role in preserving the typical inflammatory milieu in RA[22].

Overall, NF-κB is chronically activated in RA synovial tissue, resulting in persistent local inflammation and progressive joint destruction. Increases in the concentrations of TNF-α and IL-1 in the synovial fluid will additionally stimulate NF-κB, forming a positive feedback loop to further propagate inflammation and tissue destruction[23].

NF-κB also regulates expression of the receptor activator of nuclear factor kappa-B ligand (RANKL), a major mediator of osteoclast differentiation and activation[24]. Elevated RANKL levels promote bone resorption, which is a characteristic feature of joint destruction in RA. Indeed, animal models have shown that inhibiting NF-κB or RANKL decreases joint destruction, suggesting they might be of therapeutic interest[25]. As two NF-κB subunits (p65 and p50) are involved in RA pathogenesis, NF-κB is still a very attractive target for therapeutic interruption. Strategies that would block activation of NF-κB or its downstream actions may reduce inflammation and prevent joint destruction, possibly paving the way for new studies of RA management[26].

Our study reported elevated blood presepsin levels in women with RA, especially in severe cases. While early and moderate RA showed no significant difference from healthy controls. Severe RA had significantly higher presepsin levels, with a marked increase compared to early-stage RA.

Presepsin levels have been shown to be increased in RA patients where it may reflect immune activation and participation of the monocyte-macrophage system in the course of the disease[27]. These findings point to the potential utility of presepsin as a mechanistic focus in RA disease pathogenesis and a biomarker of disease activity or treatment response[28]. Moreover, patients with RA are intrinsically at an increased risk of infections because of underlying immune system dysfunction as well as by virtue of the use of immunosuppression. As a well-known marker for bacterial infections, presepsin may distinguish RA-related inflammation from systemic responses triggered by infection, thus assisting with more accurate clinical decision-making[29]. However, the role of presepsin in RA remains to be clarifying even it may be a potential biomarker. However, more studies would be needed before it can be firmly established as a reliable biomarker of disease activity, risk of infection and/or response to treatment[30]. Further, unaware of the RA mechanism of immune response, a detailed predicted role of presepsin may provide additional information about the immune-regulatory mechanisms and help us improve the management of this disease.

The investigators found that blood levels of RANKL correlate positively with those of presepsin in women who have early RA. However, this association was

non-significant. A similar trend for positive correlation was also observed for women with moderate RA (Figure 5, 6 and 7).

Recent studies highlight a potential correlation between serum levels of RANKL and presepsin, particularly in chronic inflammatory diseases like rheumatoid arthritis (RA). RANKL is a key regulator of bone metabolism, promoting osteoclast differentiation and bone resorption, leading to joint destruction in RA[31]. In contrast, presepsin is an emerging biomarker of immune activation, associated with systemic inflammation and immune dysregulation. Both markers are influenced by inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1), suggesting a possible biological connection between them[32].

In severe RA, research indicates that both RANKL and presepsin levels are significantly elevated;, implying that as disease severity progresses, systemic inflammation also intensifies[33]. This relationship underscores the interplay between immune system activation and bone degradation, which are central to RA pathogenesis. Elevated RANKL levels contribute to joint destruction, while increased presepsin levels may reflect heightened inflammatory activity, making their combined assessment valuable for understanding disease dynamics[34].

Identifying a correlation between these biomarkers could offer a more comprehensive approach to assessing RA severity. Patients with higher levels of both RANKL and presepsin may be at greater risk for severe joint damage and systemic complications. This insight could improve risk stratification and disease monitoring, potentially guiding treatment decisions[35].

Finally: further research is needed to validate the clinical utility of presepsin as a complementary marker to RANKL. If confirmed, monitoring both biomarkers could enhance early detection of disease progression and improve personalized treatment strategies for RA patients.

CONCLUSION

RANKL and presepsin are potential key indicators for diagnosing rheumatoid arthritis, as their elevated levels may provide valuable insights into disease progression and severity. Moreover, these biomarkers may not only aid in diagnosis but also have potential implications for treatment strategies, particularly in women with RA. Understanding the interplay between RANKL, presepsin, and inflammatory cytokines could open new avenues for targeted therapies aimed at reducing joint damage and controlling immune responses more effectively.

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Conflicts of interest
The authors hereby declare that they have no conflicts of interest to disclose in relation to this study. They confirm that there are no financial, personal, or professional relationships or affiliations that could have influenced the objectivity or impartiality of the research. All findings and conclusions presented are based solely on the results of the study and are free from external biases or conflicts.

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