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Original article

The Relationship between Mannose-Binding Lectin and Diagnostic Parameters of Rheumatoid Arthritis: A Comparative Study

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Abstract
Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by bone destruction and the presence of inflammatory markers. This study aimed to assess the diagnostic significance of MBL and various RA biomarkers in patients with RA. A total of 141 participants were included in this case-control study, comprising 88 patients aged 18–72 years who were diagnosed with RA and matched with 53 healthy controls. The levels of anti-cyclic citrullinated peptide antibodies (ACCP2), rheumatoid factor (RF) IgA and IgM, and C-reactive protein (CRP) were measured using ELISA. Descriptive analysis revealed that twice as many patients with RA were females, and nine times as many had positive CRP and ESR results. Significant increases were observed in the median levels of CRP, MBL, ESR, and RF-IgM in patients with RA compared with those in the control group (P <0.05), while no difference was noted in the median RF-IgA and ACCP. It was found that females were more likely to have MBL deficiency than males (P = 0.045, OR = 3.5). Furthermore, elderly patients with RA (31–72 years old) had a higher risk of MBL deficiency than young adults (18–30 years old) (P = 0.021, OR = 8.1). Positive associations were identified between ACCP, RF-IgA, and RF-IgM levels, whereas age showed an inverse correlation with CRP levels. These data suggest that combining MBL with RF-IgM, RF-IgA, and ACCP can improve RA diagnoses and patient care.
Keywords. *Rheumatoid Arthritis, MBL, ACCP, RF-IgM, RF-IgA, Diagnosis.*

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Introduction

Rheumatoid arthritis (RA) is a chronic progressive disease characterized by joint destruction and synovial membrane proliferation [1,2]. This autoimmune disorder affects approximately 1% of the global population [3]. The initial symptoms include painful swelling of the proximal interphalangeal and metacarpophalangeal joints, accompanied by morning stiffness. Inflammation spreads from small to large joints such as the elbow, knee, and ankle [3,4]. RA occurs more frequently in females, with a 3:1 ratio, suggesting the involvement of sex hormones. The disease typically begins between the ages of 30 and 50 [5].

RA susceptibility is associated with mannose-binding lectin (MBL) deficiency [6]. MBL, a protein that plays a crucial role in activating the complement system and is a key component of innate immunity, is significantly involved in this autoimmune disorder [7]. The lectin pathway, one of the three pathways of the complement system, is triggered by the pattern recognition receptors MBL, ficolin, and collectin [8]. Evidence suggests that mutations in MBL2 or MBL deficiency are risk factors for autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis [9-11]. Low MBL levels are linked to the early development of RA and poor prognosis [12].

The diagnosis of RA relies on clinical manifestations, as patients may not exhibit typical symptoms meeting the ACR 1987 classification criteria [13]. RA is associated with multiple autoantibodies [1,14]. Rheumatoid factors are autoantibodies of various isotypes, including IgM and IgA [15]. Autoantibody serology, including rheumatoid factors, antibodies against citrullinated peptide (ACCP2), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR), is crucial for the diagnosis of RA [16]. CRP and ESR are nonspecific inflammatory markers that are commonly elevated in rheumatic diseases such as RA and SLE [17].

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Research on MBL in RA, a topic of considerable interest and importance, has yielded inconsistent findings. Some studies report elevated MBL levels in patients with RA, while others associate lower MBL levels with the early onset and progression of the disease. To clarify this discrepancy, this study aimed to investigate the relationship between serum MBL levels and nonspecific and specific diagnostic parameters for RA in patients with the disease.

Methods

This case-control study was conducted at The National Ribat University Hospital NRUH Clinic in Khartoum State, Sudan, and was approved by the local committee. After obtaining informed consent, 88 patients with early diagnosis of RA (less than 3 months) were included, as confirmed by a clinical rheumatologist, according to the ACR criteria. The study's strength was enhanced by the selection of 53 healthy participants who were matched by sex and age and served as the control group. Exclusion criteria included patients with chronic diseases, such as renal failure, liver diseases, chronic inflammation, severe illness, and pregnant women. Clinical data and study variables were collected using a structured questionnaire for both patients and controls. Blood samples (6 mL) were withdrawn from each participant via venipuncture. Serum was extracted by centrifugation at 3000 rpm and stored at -70°C until use.

Estimation of RA biomarkers

Serum MBL levels were quantitatively determined using a sandwich ELISA method (R&D Systems, Minneapolis, USA). In parallel, the levels of ACCP2 and RF, including both IgA and IgM isotypes, were assessed using an indirect ELISA technique, employing the cutting-edge 1-2 P machine analyzer from Euroimmune, Germany. To measure inflammation, hs-CRP levels were quantified using an automated clinical chemistry analyzer, specifically the A15 version 4.1.1 (Bio System, Barcelona, Spain). Additionally, the ESR was meticulously evaluated using the trisodium citrate Westergren tube method, which ensured a thorough understanding of the inflammatory status reflected in the results.

Statistical analysis

Statistical analyses were performed using SPSS software (version 21, SPSS Inc., Chicago, IL, USA), and figures were generated using GraphPad Prism version 8. Demographic variables are presented as frequencies and percentages with adjusted cutoff values. A chi-square test was employed to identify the risk factors associated with severe MBL deficiency compared to MBL sufficiency. For data that were not normally distributed, the Mann-Whitney test was used to compare medians between the case and control groups. Pearson's correlation coefficient was used to correlate the numerical study variables. The results are expressed as percentages (%), medians, and odds ratios (OR) with 95% confidence intervals (CI). Statistical significance was set at a p-value of ≤ 0.05.

Results

Descriptive analyses indicated that 25% of the 88 patients with RA were young adults (18–30 years) and 75% were elderly (31–72 years). Furthermore, 67% of the patients were female, and 33% were male. The residential distribution was as follows: 54.5% of the population lived in rural areas, and 45.5% resided in urban areas. CRP was positive in 91% of the patients, while an ESR of less than 30 mm/h was noted in 96.6% of the patients (Table 1).

Table 1. Baseline characteristics of rheumatoid arthritis patients.

Characteristics	Classes	Frequency (%)
Age (Years)	18 – 30 years	22(25.0%)
	31 – 72 years	66(75.0%)
Sex	Males	29(33.0%)
	Females	59(67.0%)
Residence	Rural	48(54.5%)
	Urban	40(45.5%)
Duration	≤ 2 months	44(50.0%)
	> 2 months	44(50.0%)
MBL ng/ml	Deficient	20(22.7%)
	Sufficient	68(77.3%)

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CRP ng/L	Negative	08(9.00%)
	Positive	80(91.0%)
ACCP2 RU/mL	Negative	36(40.9%)
	Positive	52(59.1%)
RF-IgA RU/mL	Negative	46(52.3%)
	Positive	42(47.7%)
RF-IgM RU/mL	Negative	38(43.2%)
	Positive	50(56.8%)
ESR mm/h	≤30	03(3.40%)
	>30	85(96.6%)

The Mann-Whitney U test, used due to the non-normal distribution of the data as revealed by the Shapiro-Wilk test, highlighted significant differences in biomarker levels. The results showed substantial increases in the median levels of CRP (M=14.5, P=0.001), MBL (M=1834, P=0.004), ESR (M=66.5, P=0.001), and RF-IgM (M=25.5, P=0.001) in patients with RA compared to those in the control group, which exhibited CRP (M=2.79), MBL (M=1029), ESR (M=25.3), and RF-IgM (M=9.50), respectively. Importantly, no significant difference was observed in the median IgA and ACCP2 levels between the RA and control groups (Figure 1).

A chi-square analysis revealed a significant association between sex, age group, and MBL deficiency, with females being more likely to have MBL deficiency than males (P = 0.045, OR = 3.5). Additionally, elderly patients with RA (31 – 72 years old) were at a higher risk of MBL deficiency than young adults (18 – 30 years old) (P = 0.021, OR = 8.1). Furthermore, no associations were detected between positive RF-IgM, positive RF-IgA, positive ACCP2, and the duration of RA per month (P > 0.05). The results are presented in (Table 2).

Table 2. Percentages and adjusted odds ratios (OR) at 95% confidence intervals (CI) of sex, age, duration, and RA parameter status for associations of MBL-deficient with MBL-sufficient in RA patients.

Characteristics	MBL Deficient Frequency (%)	MBL Sufficient Frequency (%)	P-value	OR	95% CI Lower-Upper
Sex					
Females	17(28.81%)	42(71.19%)	0.045	3.5	1.03 – 12.05
Males	3(10.34%)	26(89.66%)			
Age					
18 – 30 years	1(4.55%)	21(95.45%)	0.021	8.1	1.39 – 88.7
31 – 72 years	19(28.78%)	47(71.21%)			
Duration					
≤ 2 months	8(18.18%	36(81.82%)	0.308	0.59	0.59 – 4.81
> 2 months	12(27.27%)	32(72.73%)			
RF-IgM					
Positive	11(22.0%)	39(78.0%)	0.851	0.90	0.35 – 2.41
Negative	9(23.68%)	29(76.32%)			
RF-IgA					
Positive	8(19.05%)	34(80.95%)	0.431	0.66	0.23 – 1.89
Negative	12(26.09%)	34(73.91%)			
ACCP2					
Positive	10(19.23%)	42(80.77%)	0.346	0.61	0.24 – 1.59
Negative	10(27.78%)	26(72.22%)			

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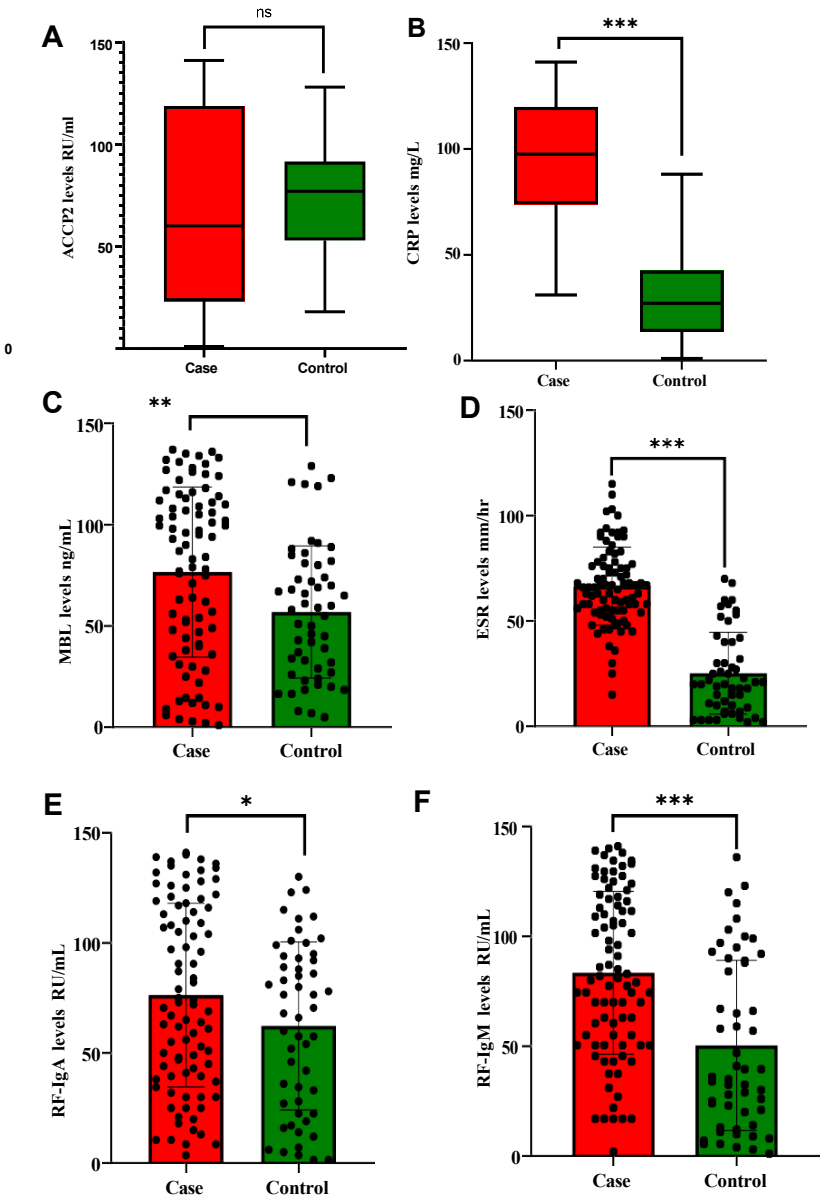

Figure 1. Shows the comparison of A: ACCP2, B: hsCRP, C: MBL, D: ESR, E: RF-IgA, and F: RF-IgG levels between the RA and control groups.

Pearson's correlation coefficient analysis revealed a significant inverse relationship between age and CRP levels, with a correlation coefficient of $R^2 = -0.281$ and a statistically significant p- value of 0.004 (as illustrated in Figure 2).

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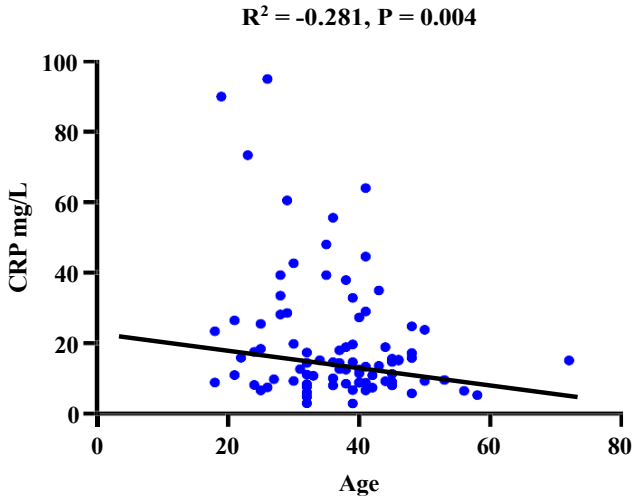

Figure 2. Dot plot illustrating the negative correlation between age and hsCRP levels in patients with RA, with R² indicating the correlation coefficient.

Additionally, the analysis identified several proportional relationships between ACCP2 levels and RF-IgM, RF-IgA levels, and ESR, all with p-values less than 0.05 (see Figure 3).

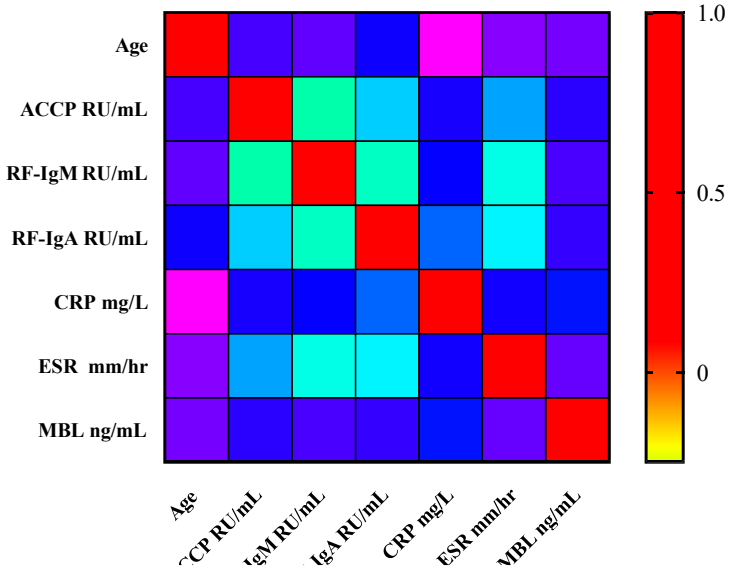

Figure 3. The correlations between age, ACCP, RF-IgM, RF-IgA, hsCRP, ESR, and MBL levels in patients with RA. Red and violet indicate positive and negative correlations, respectively. The results are expressed as R² (correlation coefficient) and p-values.

Discussion

The demographic data revealed that the prevalence of RA in females was twice as high as that in males, and individuals aged 31 years and older were four times more likely to have RA than their younger counterparts, highlighting a significant prevalence among females and older patients. These findings are of utmost importance in understanding the prevalence of RA. Additionally, the number of rural residents was nearly equal to that of urban residents. Previous studies have corroborated these findings, showing a higher prevalence of RA in females aged > 35 years [18, 19, 20]. Other studies have indicated that autoimmune diseases such as RA are more common in women than in men, suggesting that sex hormones may play a role in the pathogenesis [3,4]. Conversely, another study reported that RA was nine times more prevalent in females than in males [2]. These variations may be due to hormonal, genetic, lifestyle, or environmental factors. Furthermore, in Sudan, the use of steroid cosmetics may increase the risk of RA in women.

The results of our study are significant, indicating substantial increases in the median levels of CRP, MBL, ESR, RF-IgA, and RF-IgM in patients with RA compared with those in the control group. These findings suggest that ESR

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and CRP levels alone are insufficient for diagnosing this condition. However, their diagnostic value is enhanced when assessed alongside the RF profile, which includes RF-IgM and IgA components associated with the onset of RA. Our study's findings align with previous research, indicating that ESR and acute-phase proteins, such as CRP, are associated with various inflammatory diseases [21]. Furthermore, RF-IgM levels increase before RA is diagnosed, followed by an increase in RF-IgA levels [22].

A previous study has demonstrated elevated MBL levels in patients with RA. Conversely, another study found that a high MBL genotype was associated with increased MBL levels in RF- negative RA patients [23], indicating the potential diagnostic value of MBL in the early onset of RA. However, our study found that MBL levels significantly increased. These conflicting findings suggest that the role of MBL in RA is complex and may vary depending on the specific patient population or disease subtype. Previous studies have reported similar contradictions, with some expecting acute-phase protein and MBL expression to increase in RA [24]. In contrast, others have noted lower serum MBL levels in patients with RA compared to controls [25]. This contradiction is further highlighted by a survey that found similar mean serum MBL levels in patients and controls [26]. There was no notable difference in median ACCP2 levels between the RA and control groups. While ACCP levels are significantly elevated in patients with RA, previous research has suggested that RF-IgM and IgA serve as more effective markers for diagnosing RA than ACCP. This is because some healthy controls exhibit lower ACCP levels [27], which underscores the diagnostic value of ACCP in RA. However, it is essential to note that ACCP levels can still aid in diagnosing RA, particularly when combined with other markers, such as RF-IgM and IgA.

Chi-square analysis was used to examine the risk factors associated with MBL deficiency. This study revealed that females and older patients with RA are at a heightened risk of MBL deficiency. These findings underscore the higher prevalence of RA among females and the elderly, highlighting the influence of steroid hormones and the age-related risk of autoimmune diseases. Given that MBL deficiency is associated with infectious and autoimmune diseases, females and the elderly are more susceptible to RA. Conversely, no associations were found between RF-IgM, RF-IgG, ACCP, and MBL deficiency. This is attributed to the possibility that in RA patients, the mutant MBL gene may either overexpress or down regulate the MBL protein. These findings align with those of previous studies, which reported that 11% of patients with RA and 6% of controls had undetectable MBL [11, 28].

Pearson's correlation was used to assess the relationship between parametric data. The results indicated proportional associations between ACCP and RF-IgA and RF-IgM levels, suggesting that elevated RF-IgM levels influence the generation of RF-IgA and ACCP. A previous study revealed that in autoimmune diseases, RF-IgM is typically followed by the production of RF-IgA and RF-IgG. Conversely, an inverse relationship was observed between age and CRP levels, implying that the production of acute-phase proteins decreases with advancing age in patients with RA [29]. These findings shed light on the disease process and help us understand the role of age in the production of acute-phase proteins.

However, the study is the first to explore the MBL and RF indicators in Sudanese patients with RA. It has limitations, notably its hospital-based case control design and difficulties in gathering control subjects, which were excluded based on ESR and hsCRP levels. Therefore, prospective studies are required to validate these findings.

Conclusions

This study indicates that the risk of RA is 2-fold higher in women than in men. Patients are 9- fold more likely to have positive CRP and ESR levels. CRP, MBL, ESR, and RF-IgM levels were higher in patients with RA compared to the control group, whereas no difference was observed in RF-IgA and ACCP levels. Females are more vulnerable to MBL deficiency than males. Furthermore, elderly patients with RA are at a higher risk of MBL deficiency than are young adults. RF-IgA and ACCP levels were positively correlated with RF-IgM levels, whereas age was inversely associated with CRP levels. Integrating MBL with RF-IgM, RF-IgA, and ACCP2 could enhance the diagnosis and management of RA. This study underscores the need for further prospective research to investigate the genetic variations in MBL expression in Sudanese patients with RA.

Ethical Considerations

This study was approved by the Faculty of Medical Laboratory Sciences at National Ribat University, Khartoum. Verbal consent was obtained from all participants prior to sample collection, and the data were used anonymously.

Conflicts of interest
The authors declare that they have no conflicts of interest.

Data availability
The data underlying the findings can be obtained from the corresponding author.

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Author contributions
S.A.E. S.B.M and A.O.M. conceptualized the study and drafted the manuscript. S.B.M., S.A.E., A.A.A., and A.M.A were responsible for the data collection and study design. A.O.M., A.E.A and A.M.I. supervised statistical analyses. All authors have read, edited, and revised the manuscript accordingly.

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