RESEARCH ARTICLE

Study of Correlation Between TLR-2 Serum Level, *Streptococcus pyogenes,* and Development of Rheumatoid Arthritis

Hind F. Al-Hammami, Mahdi H. AL-Ammar*

Department of Biology, Faculty of Science, University of Kufa, Baghdad, Iraq.

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ABSTRACT

Objective: The study's main goal is to evaluate streptococcal serology and investigate the involvement of Toll-like receptor 2 (TLR-2) in the development of Rheumatoid arthritis.

Methods: A total of 35 RA patients admitted to hospital from November 2018 untill end of January 2019 were enrolled, including 4 males and 31 females with age ranged 30 to 69 years and 15 healthy participants from the Medical Examination Center of hospital were chosen as the control group with same age. The two groups were examined by anti-streptolysin O (ASO) test to confirm the bacterial infection, immunologic parameters of arthritis; rheumatoid factor (RF), C reactive protein (CRP) were measured and Cell surface. toll-like receptors (TLR-2) level were measured by enzyme linked immunosorbent assay (ELISA).

Results: Elevated ASO levels have also been found in patients suffering from acute rheumatoid arthritis and other streptococcal infection. The finding of this study revealed that (30) (85.7%) of rheumatoid arthritis (RA) patient serum samples was positive for this test, positive results may indicate an acute streptococcal infection compared with control blood samples.The level of TLR-2 in serum of RA patients was significantly higher than that in the control group.

Conclusion: *Streptococcus pyogenes* could be important role in the etiopathogenesis of RA, these observations support the notion of a potential role for activation through TLR-2 in the inflammation and joint destruction of RA.

Keywords: Antistreptolysin O, Rheumatoid arthritis, Rheumatoid factor.

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INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common inflammatory autoimmune diseases. It is characterized by persistent synovitis, systemic inflammation and production of autoantibodies.[1] Antibodies against infectious microbes were detected in the sera of early RA patients and the levels of these antibodies correlated with the disease activity of RA. For example, elevated levels of IgM and IgA antibodies to *P. mirabilis* were found in RF-positive early RA patients.[2] *Streptococcus pyogenes*, *Salmonella, mycobacterium*, and enterobacterium, that induction of arthritis by infection in animal models.[3] The relationship between the microbiome and RA has been suspected for many years and supported by pre-clinical studies. For example, arthritis susceptibility and severity in a variety of rodent strains is decreased when maintained germ-free or in environments with a restricted bacterial flora.[4] These data suggest that bacteria provide complex adjuvant functions that enhance autoimmunity, either directly (e.g., bacterial cell walls or lipopolysaccharides) or by critically altering the immunoregulatory mucosal environment.[5] The TLR constitute one of the groups of receptors in the immune system involved in inflammatory responses of various cell types to microbial antigens.[6] The clinical relevance of TLR activation in RA is supported by enhanced expression of TLRs 2, 3, 4, and 7 in the synovial lining and elevated TLR-2 expression in CD16 peripheral blood monocytes and synovial macrophages from RA patients.[7] The aim of this study to evaluate the role of *S. pyogenes* in Rheumatoid arthritis among patients with disease.

METHODOLOGY

Subjects and Samples Collection

A total of 35 RA patients admitted to hospital from November 2018 untill end of January 2019 were enrolled, including 4 males and 31 females from 30 to 69 years old and 15 healthy participants from the Medical Examination Center of hospital were chosen as the control group with same age. The two groups were examined by ASO test to confirm the bacterial infection. Tstatistical difference between the two groups

*Author for Correspondence: mahdi.alammar@uokufa.edu.iq

in gender at ($p > 0.05$). About 5 mL of blood samples were taken from each patient in a sterile test tube (plain tube) and allowed to clot at room temperature up to 1-hour. The sera were separated by centrifugation for 10 minutes at 2500 r.p.m, (then, serum was divided into two Eppendorf tubes, one tube for TLR-2 ELISA assay and another tube for RF test, ASO test, and CRP assay procedure).

Rheumatoid Arthritis Detection

- *Rheumatoid Factor (RF) Test:* RF-Latex Test is a rapid agglutination procedure, based on a modification of the Singer method, developed for the direct detection and the semi-quantitation on a slide of RF in serum. The assay is performed by testing a suspension of latex particles coated with human gamma globulin against unknown serums. The presence or absence of a visible agglutination indicates an RF concentration equal to or greater than 8 IU/mL to detect the RF in the samples tested.
- *Anti-streptolysin O (ASO) Test:* After the recommended techniques, latex particles in the reagent will agglutinate (clump) in the presence of ASO antibodies with a concentration equal to or greater than 200 IU/mL. The negative agglutination generally indicates the absence of anti-streptolysin O antibodies.
- *C-reactive Protein (CRP) Test:* When used by the recommended techniques, latex particles in the reagent will agglutinate (clump) in the presence of CRP with the content of CRP in the sample ≥ 6 mg/L, normal levels are <6 mg/L. No agglutination generally indicates the absence of CRP.

Determination of Serum TLR-2

According to the manufacturers' guidelines, the serum TLR-2 levels measured by commercial ELISA kits (USA, Elabscinece).

Statistical Analysis

Table 1: General Information and Clinical Features of RA Patients and Controls.

Parameters / *Groups*	*Patients n=35*	*Control n=15*	*Sig.*
Age (year)	51.80 ± 1.55	53.07 ± 3.25	0.692
Gender Female n(%) Male n(%)	31(88.6%) * 4 (11.4%)	10 (66.7%) 5 (33.3%)	0.001 0.73
CRP n(%) N P	0 (0.0%) 35 (100%)	15 (100%) 0 (0.0%)	–
RF n(%) N P	0 (0.0%) 35 (100%)	15 (100%) 0 (0.0%)	–
ASO n(%) P	5 (14.3%) * 30 (85.7%)	15 (100%) 0 (0.0%)	0.021*
TLR-2	4.6 ± 0.58 *	0.6 ± 0.122	<0.0001

CRP: C-reactive Protein, SD: Standard Deviation
Values are expressed as No. or mean ± SD.

Statistical analysis was carried out using Student's t-test by statistical packages for social science software (SPSS). Values are expressed as mean ± SD, and values of $p < 0.05$ were considered statistically significant.

RESULTS

The Clinical Profile of RA Patients

The clinical features of the 35 RA patients and 15 healthy controls were listed in Table 1. Mean age of the disease was 51.80 ± 1.55 years, the mean age of healthy control was 53.07 ± 3.25 years. There were no statistical differences between the RA patients and controls regarding age and $p > 0.05$. The finding obtained from this study were illustrated in the Table 1 that shows the gender distribution among patients revealed that the majority of patients with RA were females (88.6%) with a significant difference at ($p = 0.001$) compared with control of females were (66.7%) while in males were 11.4% in RA patients (females at more risk than males) compared with control of males were (33.3%) with non-significant at ($p = 0.73$). As shown in Table 1, the percentages of RF, CRP, and ASO were significantly higher in RA group than in the control group. The study shows 35 (100%) samples of patients are positive results, with RF test and CRP- test by the degree of agglutination visible macroscopically, positive results of RF test this indicate to found rheumatoid factors in patients serum. In comparison, all control blood samples 15 (100%) gave a negative result for this test, the result of ASO test in this study that 30 (85.7%) of RA patients serum samples was positive for this test, positive results may indicate an acute streptococcal infection compared with control blood samples 15 (100%)were gave a negative result for this test.

Association of Serum Levels of TLR-2 and RA Patients

The mean in patients with RA and the healthy control group has been demonstrated in Table 1. The mean serum level of TLR-2 in patients with RA (4.6 ± 0.58 pg/mL) was significantly higher than that in the control group (0.62 ± 0.122 pg/mL), $p < 0.001$.

DISCUSSION

Rheumatoid arthritis (RA) is a long-lasting autoimmune disorder that primarily affects joints. It typically results in warm, swollen, and painful joints. Pain and stiffness often worsen following rest. The wrist and hands are often involved with the same joints on both sides of the body. The disease may also affect other parts of the body. This may result in a low red blood cell count, inflammation around the lungs, and inflammation around the heart. Fever and low energy may also be present. Often, symptoms come on gradually over weeks to months.[8]

This study showed this disease occurred in all ages but the highest frequency of patient age infected with RA (50–59) years from another group; this study agree with Muhammed *et al.*[9] found the disease affects all ages, but the rate of infection

increased with age and the severity of the disease with age between 40 to 60 years. These results showed the females more than males in study groups infected with RA.[10] they found the cohort of RA patients were predominantly female, which is similar to findings from other parts of the world, including the United States of America. The dysregulation of the oestrogen level might explain why women are more likely to develop RA than men. In contrast, androgens may play a suppressive role in the development of the disease.[11] Rheumatoid factor is a specific autoantibody directed towards the IgG molecule.[12] The IgM rheumatoid factors (IgM-RF) are the major RF species found in RA, and they can be detected in 60–80% of established cases of RA and 50–60% of RA patients in the early stages of the disease.[13] In this study, elevated presence of anti-streptolysin O antibodies in a patient's sera may give evidence of recent infection in group A, and this result agrees with a study by Uckay *et al*.,[14] which showed that infections caused by beta-hemolytic *streptococci,* streptolysin O is one of the two hemolytic exotoxins liberated from the bacteria. This stimulates the production of ASO antibodies in the human serum. The results of this study agree with the results of 15. In this study, the percentage of positive streptococcal tests was found to be 25–30.3% with ASO levels >600–800 IU/mL in patients with RA while in high levels of CRP in serum of RA patients compared with control group and elevated in erythrocyte sedimentation rate of RA patients. This result relates with results by Tishler et al.[16]

They showed one potential marker is CRP. Like the erythrocyte sedimentation rate becomes elevated during clinical RA, another potential marker for increased risk of RA may be CRP, since CRP is a sensitive marker of systemic inflammation and is elevated in patients with RA. This study shows TLR-2 is highly level in serum of RA patients as similar to previous research [17] that TLRs are highly expressed in patients with RA, with increased expression of TLR2 and TLR4 on peripheral blood monocytes from patients with RA has been demonstrated. Another recent study data demonstrated the increased expression of TLR2 and TLR4 on RA synovial fluid macrophages compared with control in vitro differentiated macrophages,also employing normal macrophages, the expression of TLR2 correlated with activation by the TLR2 ligand peptidoglycan.[18] There are links between TLR-2 and the streptococcal infection of RA that matched with study by Fieber and ovarik 19which showed that TLRs recognize specific pathogen-associated molecular patterns (PAMPs) common to different pathogen species. *S. pyogenes*, also called Group A Streptococcus.[20] That examine the role of TLR ligands in experimental arthritis, streptococcal cell wall induced arthritis was examined. In conclusion, These data suggest that bacteria provide complex adjuvant functions that enhance autoimmunity by elevated ASO levels among patients who have acute rheumatoid arthritis and other streptococcal infections. These findings represent that the *S. pyogenes* may have an important role in the development of RA.

CONCLUSION

S. pyogenes could be important role in the etiopathogenesis of RA, these observations support the notion of a potential role for activation through TLR-2 in the inflammation and joint destruction of RA.

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