Antibodies of Double Stranded Deoxyribonucleic Acid and Antinuclear in Patients with Rheumatoid Arthritis: Comparison Study of Seropositive and Seronegative

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Abstract

Rheumatoid arthritis is a heterogeneous disease, which can be, based on data combining genetic risk factors and autoantibodies, sub-classified into positive and negative RA. The present study aims to establish a comparative analysis between seronegative and seropositive rheumatoid arthritis (RA), with regard to autoantibodies. A total of 151 patients with rheumatoid arthritis divided into two groups, Group A, 78 patients with seropositive RA and Group B, 73 patients with seronegative RA. The measurements included ESR, CRP, RF, anti CCP,ANA, and anti dsDNA. Highly significant differences ($p \leq 0.01$) in ESR (31.4 vs 52.8 ml/hr), CRP (10.85 vs 23.59 IU/L), RF (8.34 vs 47.67 IU/L), anti CCP (21.72 vs 41.17IU/L), ANA(0.92 vs 2.3 IU/L), and anti dsDNA(13.01 vs 29.49 IU/L) levels. Significant correlation of anti dsDNA were observed with CRP, anti CCP (and ANA in seronegative RA. Meanwhile highly significant correlation were observed with ESR, CRP, and ANA in seropositive RA. Many of RA-associated autoantibody systems have been identified and recognize post translationally modified proteins, indicating the immunogenicity of such proteins for human B cells. Among theme is anti dsDNA which may have a role of the inflammatory process of RA.

Keyword: *Rheumatoid arthritis, Anti-cyclic citrullinated peptide, antinuclear antibodies, Autoimmunity, Rheumatoid factor, Anti-double-stranded (ds) DNA.*

Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune, inflammatory disease, affecting 0.5 to 1% of adults worldwide that may cause progressive joint damage and deformity, leading to functional disability and reduced quality of life [1,2]. Rheumatoid arthritis is a heterogeneous disease, which can be, based on data combining genetic risk factors and autoantibodies, sub-classified into positive and negative RA [3]. Various biomarkers can be used to identify subjects susceptible to the disease and those with pre-clinical rheumatoid arthritis before the onset of symptoms such as rheumatoid factor and anti-citrullinated protein antibodies (anti-CCP) [4] . The presence of rheumatoid factors is used as a marker for RA. However, rheumatoid factors have modest specificity (~70%) for the disease [5]. Citrullination is one of many types of posttranslation modification undergoes protein, where an arginine amino acid is converted to a citrulline amino acid. Citrullination is a normal reaction during cell death. Apoptosis is usually accompanied with a clearance process through scavenger cells. A defect in the clearance system either in terms of efficiency or capacity may occur due to massive cell death, which may result in the accumulation and leakage of peptidyl arginine-deiminase (PAD) enzymes and the citrullinated peptide from the necrotized cell which could be recognized by the immune system, where the immunological tolerance will be avoided and the autoimmune disorders will be

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subsequently triggered [6]. The citrullination process is implicated in many human diseases and inflammations that induce autoimmunity responses against citrullinated proteins such as rheumatoid arthritis [7]. Both rheumatoid factor and anti-citrullinated protein antibodies can be correlated with a risk of developing rheumatoid arthritis and can predict more bone erosions and severe disease progression. Biomarkers such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels provide information about disease activity, while predictive biomarkers allow clinicians to assess the probability of a treatment response before starting a particular therapy particularly in the era of biological drugs[8]. Autoantibodies are proven beneficial diagnostic tools for a diversity of rheumatic and non-rheumatic autoimmune disorders. Antinuclear antibodies (ANAs) are a diverse group of autoantibodies that recognize nuclear macromolecules and their complexes. ANAs represent key biomarkers in the evaluation of rheumatic diseases [9]. It is important to highlight that certain ANAs have such high specificity that are considered predictive for certain autoimmune diseases, even before developing signs or symptoms [10]. Disease modifying antirheumatic drugs slow disease progression and can induce disease remission in some patients. Methotrexate is the first line therapy Additional disease-modifying antirheumatic drugs or biologic agents should be added if patients become intolerant to this drug [11]. Patients with rheumatoid arthritis can develop anti –double strand DNA (anti-dsDNA) antibodies, however they are usually treatment related. They are a type of *autoantibody* usually low avidity and are only detectable transiently after treatment. Anti-dsDNA specifically targets the genetic material. The presence of these antibodies can induce a lupus-like syndrome in some cases [12]. The present study aims to establish a comparative analysis between seronegative and seropositive rheumatoid arthritis (RA), with regard to autoantibodies.

Methods

A total of 151 patients with rheumatoid diagnosed according to the 1987 American College of Rheumatology (ACR) criteria [13] were conducted .They were between 25-60 years of age with duration of disease (1-5 year) and all were undergo MTX therapy .They were divided into two groups, Group A, 78 patients with seropositive RA and Group B, 73 patients with seronegative RA. ESR was measure indirectly in a sample of blood that has been placed into a tall, thin, vertical tube. Results are reported as the millimeters of plasma that are present at the top portion of the tube after one hour. Serum levels of CRP, RF and anti CCP were measured by using Nephelometry assay kits. ANA and, anti-ds DNA were measured by using coated magnetic microparticles based chemiluminescence immunoassay**.**

Statistical Analysis

Data were statistically analyzed through statistical package for social sciences software version 19.0 (SPSS 19.0, 2010, IBM Corp., NY, USA). Data were presented as mean ± standard deviation and comparisons between groups were undertaken using 2-tailed independent t-tests test which determined the significance among groups considering $P < 0.05$ statistically significant. Relationship between the variables was measured by Pearson linear correlation.

Findings

The results presented in Table 1 indicated matching in age (50.29 &47.62 year) for both groups ($p \geq 0.05$). In comparison of the results of group A (seronegative) to that of group B (seropositive) it has been observed highly significant differences ($p \leq 0.01$) in ESR (31.4 vs 52.8 ml/hr), CRP (10.85 vs 23.59 IU/L), RF (8.34 vs 47.67 IU/L), anti CCP(21.72 vs 41.17IU/L), ANA(0.92 vs 2.3 IU/L), and anti dsDNA(13.01 vs 29.49 IU/L) levels.

Table 1: Clinical Data of patientis with seropositive Rheumatoid arthritis (group A) and patientis with seronegative Rheumatoid arthritis (group B)

Parameters	Group A N=78	Group B N=73	P value
Age (year)	50.29 ±13.21	47.62 ± 14.68	0.24
ESR (mm/hr)	31.42 ± 20.15	52.85 ± 20.39	0.00
CRP (mg/dl)	10.85 ± 9.32	23.59 ± 12.06	0.00

Cont... **Table 1: Clinical Data of patientis with seropositive Rheumatoid arthritis (group A) and patientis with seronegative Rheumatoid arthritis (group B)**

RF (IU/L)	8.34 ± 5.37	47.67 ± 8.61	0.00
Anti CCP (IU/L)	21.72 ± 15.64	41.17 ± 10.88	0.00
ANA (IU/L)	0.917 ±. 0.635	2.3240 ± 1.0782	0.00
Anti dsDNA (IU/L)	13.01 ± 9.32	29.49 ± 10.26	0.00

All data are presented as the means ± SD. ESR: erythrocyte sedimentation rate; CRP C-reactive protein; RF: Rheumatoid factor; Anti CCP: anti-citrullinated protein antibodies; ANA: Antinuclear antibodies; Anti dsDNA: anti–double strand DNA. The *P* value between two groups was analyzed using the t-test.

**P* < 0.05,

***P* < 0.01.

The correlation study of anti dsDNA with diagnostic marker of rheumatoid arthritis in seronegative patients (group A) (Table2) indicated no significant correlation with ESR and RF. In contrast, a significant correlation was observed with CRP (Figure 1).Mean while highly significant correlation were observed with anti CCP(Figure 2) and ANA (Figure 3)

The correlation study of anti dsDNA with diagnostic marker of rheumatoid arthritis in seropositive patients (group B) (Taple 2) indicated no significant correlation with RF and anti CCP. In contrast, highly significant correlation were observed with ESR. , CRP and (ANA Figure 6).

Table 2: Person correlation Anti–double strand DNA with diagnosis parameters of rheumatoid arthritis

Parameter	Group A N=78	Group B N=73
ESR (mm/h)	0.19	0.372**
CRP (mg/dl)	0.245*	0.375**
RF (IU/L)	0.092	0.182
Anti CCP (IU/L)	0.56**	-0.084
ANA (IU/L)	0.36**	0.348**

**Correlation is significant at the level 0.01(2-tailed)

* Correlation is significant at the level 0.05(2-tailed)

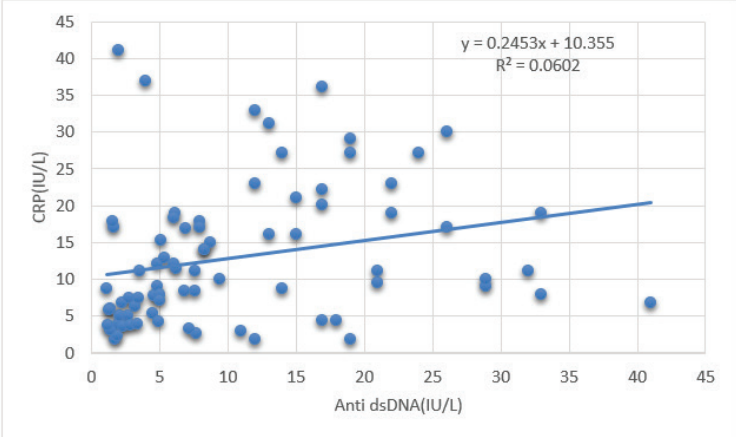

Figure 1: correlation between serum Anti ds DNA and CRP in seronegative rheumatoid arthritis patients

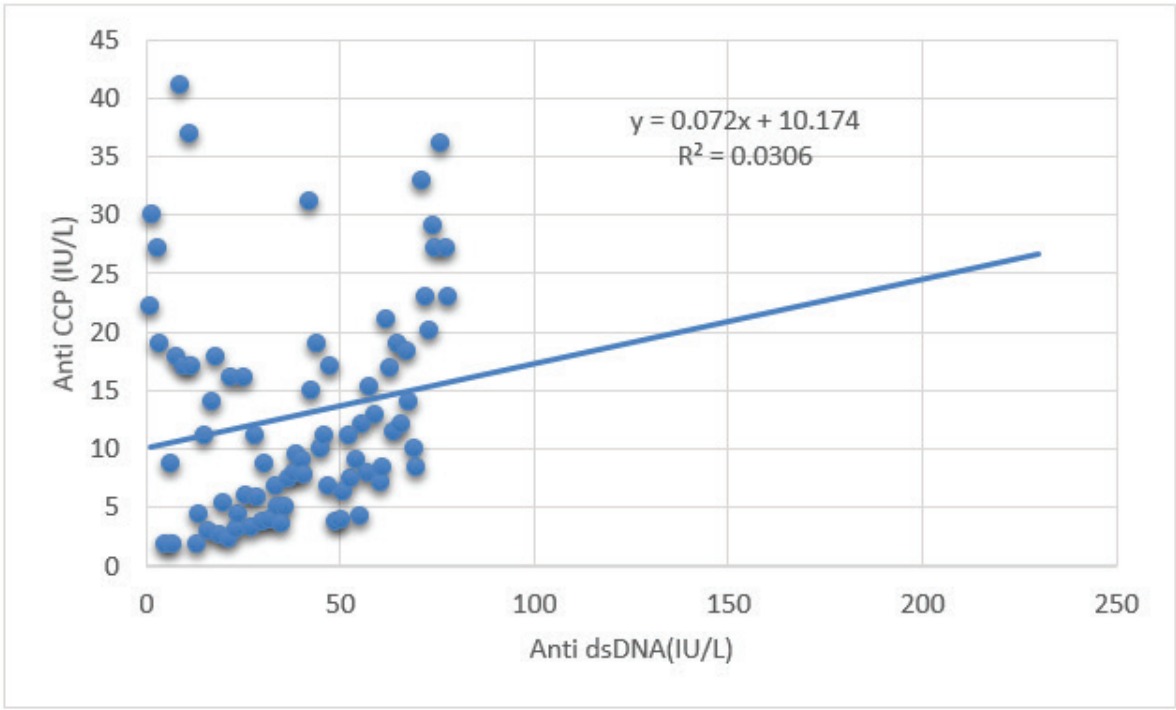

Figure 2: correlation between serum Anti ds DNA and Anti CCP in seronegative rheumatoid arthritis patients

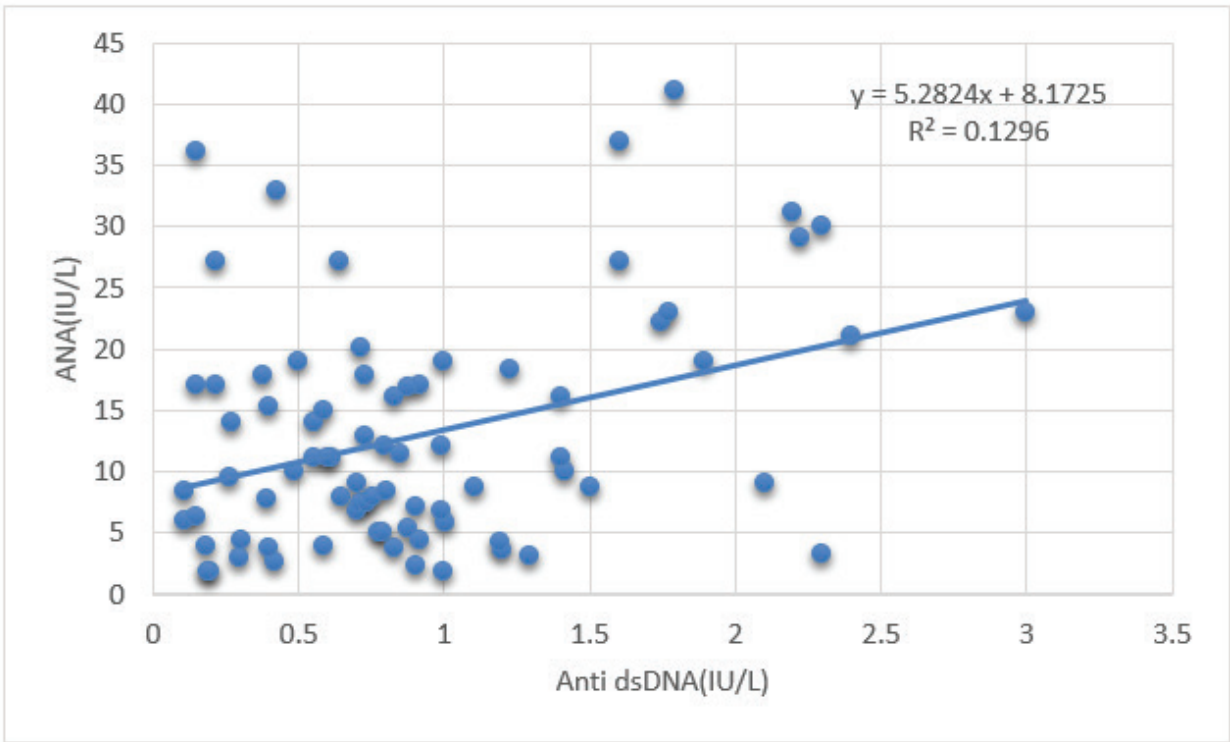

Figure 3: correlation between serum Anti ds DNA and ANA in seronegative rheumatoid arthritis patients

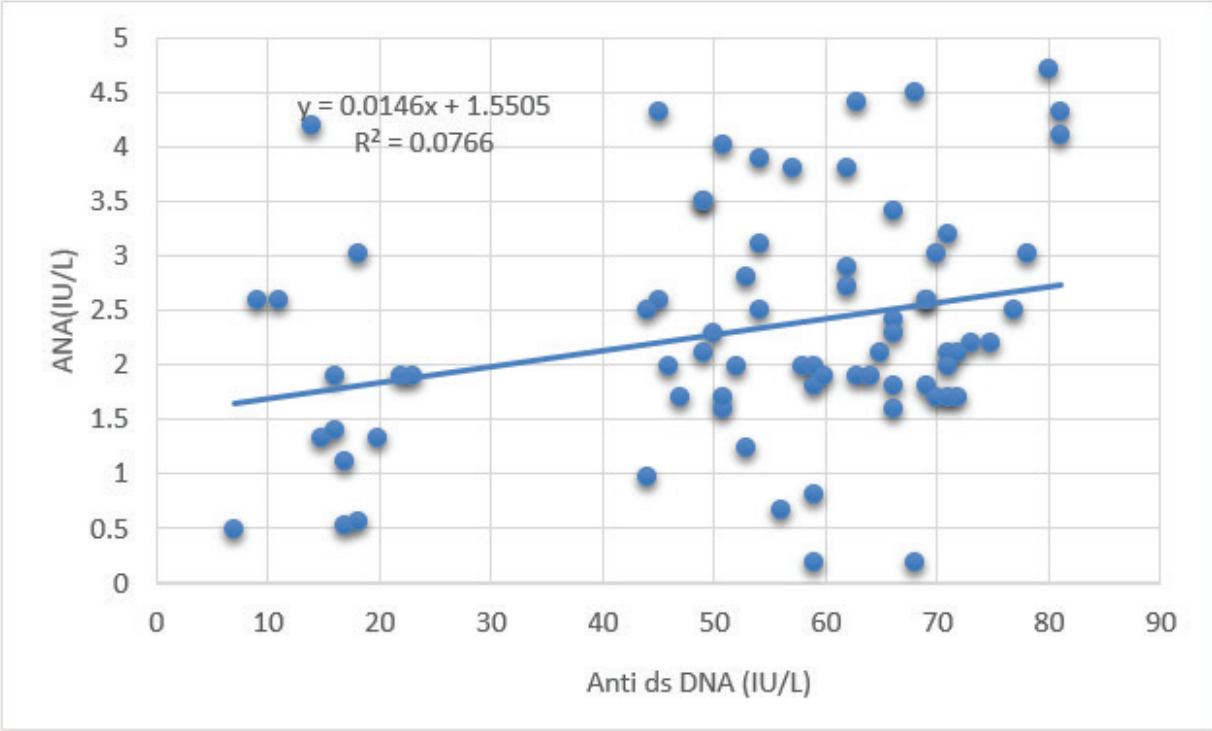

Figure 4: correlation between serum Anti ds DNA and ANA in seropositive rheumatoid arthritis patients

Discussion

The associations of RA with advancing age is consistent with prior studies [14, 15, 16]. The association of RA with age is well-known, with a peak onset among adults in their sixties [14]. The development of RA related to aging is unclear, however Weyand CM et al reported that immunosenescence occurs with aging may lead to chronic inflammation and immune-mediated tissue damage [17].In the current research there was matching in age for both studied groups which helps to eliminate differences related age in parameters results. The major role of biomarkers can be objectified by comparing the diagnostic criteria. Rheumatoid factor is the only ACR 1987 criteria biomarker for rheumatoid arthritis. Currently, the ACR/ EULAR 2010 criteria for the RA diagnosis use the rheumatoid factor (RF) and antibodies against cyclic citrullinated proteins (anti-CCP)[8] .The current research indicated significant differences in ESR and CRP levels between seronegative RA and seropositive RA. This results were agreement with Shin YS et al study who's reported that the ESR at diagnosis was 49.1 ± 36.8 mm/hr and 26.6 ± 24.3 mm/hr (p<0.01) in seropositive and seronegative groups, respectively and, the same trend was noted in CRP levels; 2.9 ± 4.1 mg/dL and 0.8 ± 1.2 mg/dL (p<0.01) [18] . In contrast Sahatçiu-Meka V, & Anton K reported that elevated average values of ERS, CRP were found in seropositive patients, but they did not present statistically significant difference with regard to sero-status [19]. Also in previous study, it has been reported that ESR was enhanced in seropositive RA patients compared to seronegative RA patients while CRP was not statistically different [20]. CRP is a sensitive index for RA disease activity and changes in CRP can predict treatment response of patients where, autoantibodies might be relevant for characterizing distinct phenotypes of RA patients [21,22]. Autoantibodies not merely provide useful information on disease outcome but also offer insights into the development of RA [23].Highly significant increase of anti-citrullinated protein, Antinuclear antibodies and Anti dsDNA in seropositive RA in comparison to that of seronegative RA which mean these test results frequently affect the course of patients' evaluations, diagnosis, and treatment. The results of correlation study showed that there were differences between seronegative RA and seropositive RA especially the relation of anti ds DNA with anti CCP

which indicated difference in the pathophysiology of the two groups. Previously it was reported that not clear whether these autoimmune responses play a pathogenetic role in the development of RA or are a consequence of the chronic inflammatory process of RA, even when the autoantibodies precede the manifestation of clinical symptoms [24,25].However the present results showed a significant correlation between anti dsDNA and ESR which suggested impossible role of the inflammatory process of RA.

Conclusion

Many of RA-associated autoantibody systems have been identified and recognize post translationally modified proteins, indicating the immunogenicity of such proteins for human B cells. Among theme is anti dsDNA which may have a role of the inflammatory process of RA

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Ethical Clearance: All patients were agree befor included in this study.

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