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# Significance of antinuclear antibody in Indian patients with rheumatoid arthritis

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***Abstract*---**This study aimed to understand the differences in disease duration, serological parameters, extra Articular Manifestation and treatment of Indian RA patients depending upon antinuclear antibody (ANA) positivity. This study was planned as a cross-sectional observational study, wherein 184 patients 18 years and above diagnosed as RA and tested for Antinuclear Antibody, who reported for consultation at rheumatology department of Medanta –The Medicity hospital from August 2020 to February 2022 were enrolled in this study. Patient's demographic data, present complains, extra –articular features, seronegative features, associated medical conditions like CAD, HTN, DM, Tuberculosis, hypothyroidism etc, family history current and previous was collected on the day of consultation with physician. Cross tables were generated and Chi square test was used for testing of association for comparison between groups. The study included 184 patients of RA. ANA positivity rate was 45.1% (n=83). The majority of the patients were female- 155(84.2%). There was no significant association was observed between the study groups in

terms of gender, mean age, mean age of onset, and median disease duration. ANA positivity was observed significantly higher in patients with pre-existing comorbidity – hypothyroid (ANA+ vs ANA-: 56.6% vs 38.6%; p = 0.015*), and it was also observed significantly higher in patients who had sicca symptoms in extra-articular manifestations (ANA+ vs ANA-: 51.8% vs 36.6%; p = 0.039*). No association was observed between the groups with respect to deformities, erosions, and treatment. ANA positivity was observed significantly higher in patients with pre-existing comorbidity – hypothyroid and it was also observed significantly higher in patients who had sicca symptoms in extra-articular manifestations. No association was observed between the groups with respect to deformities, erosions, and treatment.Further, ANA positivity rate was also higher in patients who were RF positive as compared to those patients who were RF negative. In terms of diagnostics, there were no differences in RF or CCP seropositivity. These findings could indicate a difference in clinical presentation or perception of patients with RA who are ANA-positive. It is recommended to conduct studies for assessment of long term outcomes in ANA positive patients.

***Keywords*---**significance antinuclear antibody, Indian patients, rheumatoid arthritis.

## Introduction

It is known that antinuclear antibody (ANA) testing has been an established as an assessment tool for diagnosis of autoimmune disorders such as systemic lupus erythematosus (SLE). Also, ANA are the most commonly evaluated nonspecific autoantibodies in clinical practice and it has been observed that ANA positivity found to be appeared well before clinical presentation of the autoimmune disorders. Further, ANA testing is a decisive mechanism for assessing autoimmune disorders, such as SLE, scleroderma and polymyositis/dermatomyositis. However, current practice for classification of Rheumatoid arthritis (RA) does not include ANA positivity and ANA testing is performed in many patients who are ultimately diagnosed with RA.

Reported data from Dallas Regional Autoimmune Disease Registry suggests that the ANA positivity rate is about 20% to 30% of the healthy general population (1). Variety of methodology have been used to estimate ANA prevalence in selected populations, further, the prevalence of ANAs in United States (US) in healthy individuals is about 3-15% and production of these autoantibodies is strongly age-dependent which, further, increases to 10-37% in healthy persons over the age of 65 (2). Also, ANA is more prevalent among females as compare to males (17.8% vs. 9.6%, $P < 0.001$) having female to male ratio peaking at 40–49 years of age (3). A magnitude of the ANA-positive population thought to represent the preclinical stage of autoimmune disorders based on investigations that autoantibodies are usually produced prior to clinical manifestations of disease (4). In recent years, the incidence of autoimmune disorders has seen a rise in India.

However, the prevalence in India is still incomprehensible since only few studies on respective diseaseshave been initiated.

Additionally, increase in prevalence of ANA positivity during 1988 and 2012 in the United States suggests that there is an increase in autoimmune pathology (5, Dinse et al). Furthermore, reported data suggests that patients with ANA positivity have increased all-cause mortality rate and cardiovascular events (6). However, characteristics associated with ANA production remains unknown and what drives the observed differences is indistinct which suggests that there is a gap in the understanding of the role a positive ANA has in the management of RA. Therefore, objective of this study is to develop differences in the clinical course and significance, treatment and outcomes of patients with RA who are ANA positive.

Also, in 2005 observational study data suggests that patients with ANA-positive Rheumatoid Arthritis (RA) who were treated with tumor necrosis factor (TNF) inhibitors were more likely to have certain autoimmune complications, including a drug-induced lupus, than those not treated with TNF inhibitors (7). However, a single cohort study published in 2014 of 454 patients with RA treated with a TNF inhibitor did not show any clear association between ANA positivity and the lupus-like complication. In that 2014 study, some links were made between the development of ANA positivity during treatment and secondary treatment nonresponse (8). This study aimed to understand the differences in disease duration, serological parameters, extra Articular Manifestation and treatment of Indian RA patients depending upon antinuclear antibody (ANA) positivity.

**Methods**

This study was planned as a cross-sectional observational study, wherein 184 patients 18 years and above diagnosed as RA and tested for ANA ,who reported for consultation at rheumatology department of Medanta –The Medicity hospital from August 2020 to February 2022 were enrolled in this study. All patients with RA that fulfilledthe American College of Rheumatology classification criteria (1987) and/or the American College of Rheumatology/European League against Rheumatism criteria were enrolled in this study. The clinical profile of the patients studied appears in Table –I. Patients were excluded with presence of disease markers of HIV 1 or 2, Hepatitis B or C viruses and VDRL; pregnant females, history of intake/administration of any investigational treatment within the last 12 weeks in a clinical study prior to day of consultation.. This study was initiated after approval of Medanta Institutional Ethics committee (MIEC). Total of 184patients were enrolled for the study, out of which,were tested for ANA positivity, based on their clinical manifestations. Medical records were mainly accessed from the Medanta's patient records i.e., eHIS. The study was conducted in accordance with the IEC approved protocol, Site-SOPs, ICH GCP guidelines, ICMR guidelines and the principles of Declaration of Helsinki and applicable regulatory requirements.

**Data Collection**

Data of confirmed RA patients was collected on day of consultation. The data comprised of : a. Demographic data b. age of Onset; c. Disease duration , d. RF & CCP status, e. ANA status, f. Radiology erosions, g. Treatment status; & h. extra articular manifestation such as nodules, eye, sicca, cardiac, ILD, vasculitis, neuropathy, Fibromyalgia, Osteoporosis and malignancy; deformities & erosion ; &i. current and previous **L**aboratory parameters were also recorded at the time of consultation, Treatments with DMARDs, Biologicals& JAK inhibitors. Data related to patient's treatment with methotrexate (MTX), leflunomide, sulfasalazine and hydroxychloroquine (HCQs) was also collected.

**Statistical Method**

The analysis included profiling of patients on different demographic, past history, clinical & laboratory findings. Quantitative parameters were expressed as mean and standard deviation. Categorical data were expressed as absolute number and percentage. Independent Student t – test was used for testing of mean difference between two independent groups for normally distributed data whereas Mann-Whitney U test was used for non-normally distributed data. Cross tables were generated and Chi square test was used for testing of associations. p-value less than 0.05 was considered statistically significant. All analysis were performed using SPSS software version 24.0

Table 1:Patient Characteristics with respect to study group

| | ANA Positive (n = 83) | ANA Negative (n = 101) | Total (n = 184) | p-value |
|---|---|---|---|---|
| Gender, n (%) | | | | |
| Female | 70 (84.3%) | 85 (84.2%) | 155 (84.2%) | 0.974 |
| Male | 13 (15.7%) | 16 (15.8%) | 29 (15.8%) | |
| Age (Years) | | | | |
| Mean ± SD | 55.0 ± 12.1 | 54.9 ± 10.9 | 54.9 ± 11.4 | 0.960 |
| Age of Onset (Years), n (%) | | | | |
| ≤ 35 | 18 (21.7%) | 20 (19.8%) | 38 (20.7%) | 0.867 |
| 35 – 60 | 58 (69.9%) | 74 (73.3%) | 132 (71.7%) | |
| > 60 | 7 (8.4%) | 7 (6.9%) | 14 (7.6%) | |
| Mean ± SD | 45.6 ± 11.5 | 45.3 ± 12.5 | 45.4 ± 12.0 | 0.872 |
| Disease Duration (Years), n (%) | | | | |
| ≤ 2 | 17 (20.5%) | 18 (17.8%) | 35 (19%) | 0.912 |
| 2 – 5 | 13 (15.7%) | 19 (18.8%) | 32 (17.4%) | |
| 5 – 10 | 20 (24.1%) | 26 (25.7%) | 46 (25%) | |
| > 10 | 33 (39.8%) | 38 (37.6%) | 71 (38.6%) | |
| Median (IQR) | 8.0 (4.0 - 13.2) | 9.0 (4.0 - 14.0) | 8.5 (4.0 - 14.0) | 0.829 |
| Serology, n (%) | | | | |
| Seropositive | 71 (85.5%) | 86 (85.1%) | 157 (85.3%) | 0.940 |
| Seronegative | 12 (14.5%) | 15 (14.9%) | 27 (14.7%) | |

| | | | | |
|---|---|---|---|---|
| Rheumatoid Factor, n (%) | | | | |
| Positive | 60 (72.3%) | 71 (70.3%) | 131 (71.2%) | 0.767 |
| Negative | 23 (27.7%) | 30 (29.7%) | 53 (28.8%) | |
| CCP, n (%) | | | | |
| Positive | 53 (63.9%) | 65 (64.4%) | 118 (64.1%) | 0.944 |
| Negative | 30 (36.1%) | 36 (35.6%) | 66 (35.9%) | |
| Comorbidities, n (%) | | | | |
| Hypertension | 40 (48.2%) | 54 (53.5%) | 94 (51.1%) | 0.476 |
| Hypothyroid | 47 (56.6%) | 39 (38.6%) | 86 (46.7%) | 0.015* |
| Diabetes M | 21 (25.3%) | 30 (29.7%) | 51 (27.7%) | 0.507 |
| CAD | 13 (15.7%) | 9 (8.9%) | 22 (12%) | 0.160 |
| H/O TB | 7 (8.4%) | 5 (5%) | 12 (6.5%) | 0.341 |
| Chronic Kidney Disease | 1 (1.2%) | 2 (2%) | 3 (1.6%) | 0.679 |
| Extra Articular Manifestation, n (%) | | | | |
| Sicca Symptoms | 43 (51.8%) | 37 (36.6%) | 80 (43.5%) | 0.039* |
| ILD | 12 (14.5%) | 19 (18.8%) | 31 (16.8%) | 0.432 |
| Fibromyalgia | 10 (12%) | 8 (7.9%) | 18 (9.8%) | 0.348 |
| Nodules | 6 (7.2%) | 4 (4%) | 10 (5.4%) | 0.330 |
| Osteoporosis | 3 (3.6%) | 6 (5.9%) | 9 (4.9%) | 0.467 |
| Neuropathy | 5 (6%) | 2 (2%) | 7 (3.8%) | 0.154 |
| Malignancy | 0 (0%) | 2 (2%) | 2 (1.1%) | - |
| Vasculitis | 1 (1.2%) | 0 (0%) | 1 (0.5%) | - |
| Das 28 ESR, n (%) | | | | |
| Remission | 15 (21.7%) | 14 (18.2%) | 29 (19.9%) | 0.874 |
| Low Disease Activity | 17 (24.6%) | 23 (29.9%) | 40 (27.4%) | |
| Moderate Disease Activity | 27 (39.1%) | 28 (36.4%) | 55 (37.7%) | |
| High Disease Activity | 10 (14.5%) | 12 (15.6%) | 22 (15.1%) | |
| Deformities, n (%) | | | | |
| Yes | 24 (28.9%) | 25 (24.8%) | 49 (26.6%) | 0.525 |
| Nodules | 59 (71.1%) | 76 (75.2%) | 135 (73.4%) | |
| Erosions, n (%) | | | | |
| Yes | 15 (18.1%) | 9 (8.9%) | 24 (13%) | 0.066 |
| No | 68 (81.9%) | 92 (91.1%) | 160 (87%) | |
| Treatment, n (%) | | | | |
| Methotrexate | 72 (86.7%) | 85 (84.2%) | 157 (85.3%) | 0.621 |
| Lefno | 57 (68.7%) | 72 (71.3%) | 129 (70.1%) | 0.700 |
| HCQS | 41 (49.4%) | 50 (49.5%) | 91 (49.5%) | 0.988 |
| Saaz | 17 (20.5%) | 16 (15.8%) | 33 (17.9%) | 0.414 |
| Tofacitinib | 5 (6%) | 9 (8.9%) | 14 (7.6%) | 0.462 |
| Rutaximab | 0 (0%) | 4 (4%) | 4 (2.2%) | - |
| TNF Inhibitor | 2 (2.4%) | 0 (0%) | 2 (1.1%) | - |
| Tocilizumab | 0 (0%) | 1 (1%) | 1 (0.5%) | - |

*p<0.05; statistically significant

Abbreviations: ANA, antinuclear antibody; RF, rheumatoid factor; CCP, anti-cyclic citrullinated peptide; HTN, hypertension; DM, diabetes mellitus; CKD, chronic kidney disease; TB, tuberculosis; SD, standard deviation; IQR- interquartile range;

## Results

The study included 184 patients of RA. ANA positivity rate was 45.1% (n=83). The majority of the patients were female- 155(84.2%). There was no significant association was observed between the study groups in terms of gender, mean age, mean age of onset, and median disease duration. ANA positivity was observed significantly higher in patients with pre-existing comorbidity – hypothyroid (ANA+ vs ANA-: 56.6% vs 38.6%; p = 0.015*), and it was also observed significantly higher in patients who had sicca symptoms in extra-articular manifestations (ANA+ vs ANA-: 51.8% vs 36.6%; p = 0.039*). No association was observed between the groups with respect to deformities, erosions, and treatment.

## Discussion

45.1% of patients were found ANA positive which is slightly higher than the result in previous study (9). It may be due to the reason that not all enrolled patients were tested for ANA. They were chosen based on their clinical features. In another study (10), the ANA positivity was found to be 60%. Patients who developed RA before 35 years of age, have higher chances of ANA positivity as compared to patients with delayed onset. Patients who developed RA at or before the age of 35 years were slightly more prone to be ANA positive. The maximum number of ANA negative patients were found to be those who developed RA in the age 35-60 years. 7% of patients who developed RA after 60 years of age were ANA positive, suggesting a relation between ANA positivity and late onset RA. In all categories of disease duration, number of ANA negative patients were higher, which is contrary to the findings of previous study (9) that suggested a correlation between ANA positivity and prolonged duration of disease. Gender of patient was found to be having no direct relation with ANA positivity which is contrary to the findings of previous studies (11)(12) that noted significantly higher percentage of ANA positivity in female patients. Disease duration was found to be higher in ANA negative patients in all age groups which is contrary to the previous study (9). Presence of comorbidities gave mixed results and were insufficient to establish any direct or indirect relationship with ANA positivity. Presence of nodules was also found to be unrelated to ANA positivity. Presence of sicca symptoms was significantly higher in ANA positive patients. ANA positivity was not found to have any effect on development of osteoporosis. Extra articular manifestations like nodules, ILD, rheumatoid nodules showed no significant difference in ANA positive and ANA negative patients, suggesting no clear or direct correlation, which is in accordance with previous study findings (13). Results also suggest that ANA positive patients were more likely to be seropositive.

## Conclusion

There were no differences in RF and/or CCP seropositivity group. Importantly, patients who had hypothyroid and Sicca symptoms in extra-articular manifestations were observed significantly higher in ANA positive patients. These findings could indicate a difference in clinical presentation or perception of patients with RA who are ANA-positive. It is recommended to conduct studies for assessment of long term outcomes in ANA positive patients.

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