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# Pattern and Frequency of Anti-nuclear Antibody Positivity in Paediatric Rheumatic Diseases

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**Abstract**

***Background:*** *Anti-nuclear antibodies (ANAs) are specific antibodies directed against a variety of nuclear antigens detected in the serum of patients with many rheumatic and non-rheumatic diseases.These antibodies are not only involved in the pathogenesis, but also constitute the basis for diagnosis and treatment of paediatric rheumatic diseases. The objective of the study was to identify the patterns and frequency of ANA positivity in Paediatric Rheumatic Diseases.*

***Methodology:*** *It was a retrospective study. Fourteen hundred and sixty eight records of paediaric rheumatology patients were analyzed. Statistical analysis were done to observe the frequency and association of different patterns of ANA in Juvenile idiopathic arthritis and systemic lupus erythematosus patients.*

***Results:*** *Among the 1468 patients of PRDs, frequency of JIA cases was the highest (65 %) followed by SLE, Scleroderma, juvenile dermatomyositis, and others. Among the 261 PRD patients ANA positivity was 65%. ANA positivity was 100%, 92%, 40% and 31.5% in Mixed connective tissue disease, SLE, JDM and Scleroderma patients respectively. Homogenous staining pattern was found in 59% and speckled pattern in 22.9%. There was significant association between ANA positivity and uveitis in oligoarticular JIA patients. Significant association was also found between homogeneous patterns of ANA and renal involvement in SLE patients.*

***Conclusion:*** *ANA positivity was highest in MCTD cases followed by SLE cases. Majority of SLE cases had homogeneous pattern of ANA.Staining patterns of ANA had significant association with the clinical manifestations in SLE and JIA cases.*

***Keywords:*** *anti-nuclear antibody, Immunofluorescence, paediatric rheumatic diseases, SLE, JIA.*

## Introduction

Paediatric rheumatic disease (PRDs) is a group of disorder characterized by chronic inflammation of unknown cause, affecting the musculoskeletal system, blood vessels and other tissues. Juvenile idiopathic arthritis (JIA), systemic lupus erythematosus (SLE), juvenile dermatomyositis (JDM), systemic sclerosis, childhood vasculitis and non- inflammatory disorders are some of the common PRDs.[1] Auto-antibodies are useful markers for the diagnosis and monitoring of disease activities of various PRDs.[2] Prevention of morbidities and complications are possible, if PRDs are diagnosed early. Early recognition and diagnosis as well as timely intervention can improve the outcome of PRDs.[3]

Presence of auto-antibody to a variety of cellular component is a frequent finding in rheumatic diseases. Among them anti-nuclear antibody (ANA) is the most important one. ANAs are antibodies directed against various nuclear components. It is usually detected by indirect immunofluorescence (IF) method, utilizing mono-layers of Hep-2 cells, which have further increased the sensitivity of the test.[3] ANA

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is usually present in 10% in normal populations, 98% in SLE children, 70-80% inoligoarticular JIA cases, 60-80% children with scleroderma and 60% children with juvenile dermatomyositis (JDM).[4] Presence of ANA is associated with increased risk of uveitis in oligoarticular JIA patients.[5]

On the basis of their distributions, IF-ANA staining patterns can be subdivided into homogenous/ chromosomal (H-ANA), centromeric (C-ANA), speckled or extra-chromosomal (S-ANA), nucleolar (N-ANA), nuclear membrane, nuclear dot and other defined patterns[6]. Staining patterns are usually associated with different systemic manifestations and laboratory measures of SLE patients.[7]Different studies are done to see the clinical significance of ANA in SLE in our country and abroad[3,8], but no study has been done on ANA and PRDs. The objectives of the current study was to evaluate the ANA positivity and its staining patterns in different PRDs. Association between ANA staining patterns with the systemic involvement in SLE and association of ANA with uveitis among oligo-articular JIA patients were also assessed in this study.

**Materials and Methods:**

It was a retrospective study conducted in the Paediatric Rheumatology follow up clinic of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. Medical records from the Paediatric Rheumatology Clinic and inpatient Department of Paediatrics from July 2007 to July 2016 were analyzed. All the patients fulfilling the ILAR classification criteria of oligo articular Juvenile Idiopathic Arthritis (JIA)[9], revised ACR classification criteria 1997 for SLE[10], EULAR- PReS classification criteria of childhood vasculitis[11], Bohan A, Peter JB classification criteria for Juvenile Dermatomyositis (JDM) [12], preliminary criteria for the classification of Systemic Sclerosis (SS)[13] and classification criteria for Mixed connective tissue disease (MCTD)[14] were enrolled in this study.

Fourteen hundred and sixty eight records of registered PRD patients were analyzed in this study.Among 1468 patients 261 had their ANAtest done. Data were collected in a predesigned questionnaire which included baseline socio-demographic and clinical characteristics, ANA positivity and patterns of positivity.

Indirect Immuno-fluorescence method using HEp-2 cell line was the standard approach for detecting ANAs.[7] Positive autoantibody titers at a dilution equal to or greater than 1:160 were usually considered as clinically significant. [15-17]

Statistical analysis was done to assess the frequency of positivity, association of different patterns with different systemic involvement.

**Results:**

A total number of 1468 patients were enrolled as PRDs during the study period. Table I shows the distribution of paediatric rheumatological diseases in our settings. JIA (65%) found the bulk of the patients followed by SLE (9.8%) and others. Among the JIA patients, enthesitis related arthritis (34%) and systemic JIA (22%) cases were the majority. Among all the PRDs, mean age at the onset of disease was 8.5 years, male female ratio was 1:1.9 and the mean disease duration was 1.2 months to 9.4 months.

**Table I**

*Distribution of Paediatric Rheumatological Diseases (n=1468)*

| Types of PRDs | Number (n) | Percentage |
|---|---|---|
| SLE | 147 | 9.8 % |
| JIA | 976 | 65.0% |
| Enthesitis related arthritis | 510 | 34.0% |
| Systemic JIA | 330 | 22.0% |
| Oligo persistent | 65 | 4.4 % |
| Oligo extended | 07 | 0.4 % |
| Poly negative | 50 | 3.4 % |
| Poly positive | 14 | 0.8 % |
| JDM | 15 | 1.0 % |
| Systemic sclerosis | 13 | 0.9 % |
| Localized scleroderma | 06 | 0.4 % |
| PAN | 06 | 0.4 % |
| MCTD | 02 | 0.1 % |
| Others | 303 | 22.4% |
| Total | 1468 | 100% |

ANA test were done for 261 PRDs caseswhich included oligoarticularJIA,SLE,JDM ,SS , PAN and MCTD patients. Among them ANA positivity was 65 %. ANA positivity was 100% in MCTD cases followed by 92% and 27% in SLE and oligo JIA patients respectively (Table II).

**Table II**
*Distribution of ANA positivity among Paediatric Rheumatic Diseases (n=261)*

| Types of PRDs | Number | ANA positive cases | |
|---|---|---|---|
| | | n | % |
| SLE | 147 | 136 | 91 |
| Oligo persistent JIA | 65 | 15 | 23 |
| Oligo extended JIA | 07 | 04 | 57.1 |
| JDM | 15 | 06 | 40 |
| Systemic sclerosis | 13 | 04 | 30.7 |
| Localized scleroderma | 06 | 02 | 33.3 |
| PAN | 06 | 01 | 16.6 |
| MCTD | 02 | 02 | 100 |
| Total | 261 | 170 | 65.1 |

Table III shows the patterns of ANA in different PRDs. Almost all types of staining patterns were found in SLE and oligoarticular JIA cases. Homogenous staining pattern was the highest.Association of ANA positivity and uveitis among oligoarticular JIA patients was present in this study.

Among SLE cases, homogeneous ANA pattern (69.3%) was associated with different systemic involvements including renal, musculoskeletal, haematological and neuropsychiatric manifestations, whereas speckled pattern(8.8%) was associated with renal involvement only (Table IV).

**Table III**
*Different Patterns of ANA in Paediatric Rheumatological Diseases*

| PRDs | ANA Patterns, n (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Types | Number of ANA positive(%) | Homo-genous | Fine speckeled | Coarse speckled | Centro-mere | Neo-cleolar | Cyto-plasmic |
| SLE (147) | 136(92%) | 102 (69.3) | 13 (8.8) | 12 (8.1) | 00 (00 ) | 02(1.4) | 07 (4.8) |
| JIA (72) | | | | | | | |
| Oligo persistent JIA(65) | 15(23%) | 06 (9.2) | 06 (9.2) | 01 (1.5 ) | 01 (1.5) | 01(1.5) | 00 (00 ) |
| Oligo extended JIA(7) | 04 (57%) | 04(100 ) | 00 (00 ) | 00 (00 ) | 00 (00) | 00 (00) | 00 (00 ) |
| JDM (15) | 06 (40%) | 03 (20) | 01 (6.7) | 02 (13.3) | 00 (00 ) | 00 (00) | 00 (00 ) |
| Systemic sclerosis (13) | 04(30.7%) | 02(15.3) | 00 (00 ) | 02 (15.3) | 00 (00 ) | 00 (00) | 00 (00 ) |
| Localized scleroderma(6) | 02(33%) | 00 (00 ) | 02 (33.3) | 00 (00 ) | 00 (00 ) | 00 (00) | 00 (00 ) |
| PAN (6) | 01(16.6%) | 01(16.7) | 00 (00 ) | 00 (00 ) | 00 (00 ) | 00 (00) | 00 (00 ) |
| MCTD (2) | 02(100%) | 00 (00 ) | 00 (00 ) | 02 (100) | 00 (00 ) | 00 (00) | 00 (00 ) |

**Table IV**
*ANA Patterns and SLE (n=147)*

| Systems involved | | Patterns of ANA, n (%) | | P |
|---|---|---|---|---|
| | | Homogenous | FineSpeckled & others pattern | value |
| Renal | 87 | 53 (60.5%) | 34(41%) | 0.001 |
| Musculoskeletal | 17 | 15 (88.2%) | 00(00) | |
| Hematological | 18 | 17 (94.4%) | 00(00) | |
| Neuropsychiatric | 21 | 14 (66.6%) | 00(00) | |
| Endocrine | 02 | 02(100%) | 00(00) | |
| Hepatic | 01 | 01(100%) | 00(00) | |

**Discussion:**

ANA are specific antibodies directed against a variety of nuclear antigens which have been detected in the patients with many rheumatic and non–rheumatic diseases and also in normal individuals.[8,18]Many studies were conducted in different countries for detection of ANA in rheumatic disease including JIA, SLE ,SS, JDM and MCTD.[11-13,19,20] This was the first study in our country where frequency of the positivity was assessed in different PRDs and patterns of ANAs were correlated with different systemic manifestations.

Y see et al in a similar study found that among 170 PRD patients majority were SLE(51.8%) followed by JIA(28.8%) and JDM (10%). Majority were female (M:F-1: 2.2) in their series.[21] But in the present study, JIA cases was the highest followed by SLE. It is well established that PRDs are female predominating disease but in our study males were found as the main bulk excepting SLE where girls were predominant. This might be due to the socio-cultural background where male children are given priority and more care. A retrospective study done in USAshowed that among 500 patients, 113 (22.6%) patients were ANA positive. The highest number of patients had JIA (61%), followed by SLE (22%) in that study.[19]

Our study showed that 61.8 % patients were ANA positive. MCTD cases were 100% ANA positive with coarse speckled pattern. High-titer speckled pattern ANA is usually found in MCTD.[22]In this study, we had 2 MCTD cases with coarse speckled staining patterns.

Sharmin et al in their study found six staining patterns of ANA positivity in SLE patients. Speckled pattern (43.7%) followed by peripheral (34.3%) and homogenous pattern (21.8%) were present in their study.[15] Gonzalez and Rothdfield reported that the homogenous and peripheral patterns were found most frequently in SLE patients and only a few had speckled pattern.[23] Present study found homogenous (46.3%), speckled (17.6%), nucleolar (1.1%) and cytoplasmic (2.6%) staining pattern.Most of the literature reportedpresence of homogenous pattern in the majority of SLE cases.[7,8,24] The present study findings are consistent with them. Mararina et al showed that ANA was positive in 91.5% of SLE patients in their series which is similar to our study. They also found homogenous and speckled patterns in the majority (63.5% and 34.7% cases respectively).[24] A Swedish study found that homogenous ANA pattern was significantly associated with 'immunological disorder' (the 10$^{th}$ ACR-82 criterion) whereas speckled ANA pattern was inversely associated with arthritis, 'immunological disorder' and signs of organ damage.[7] Present study found that homogenous pattern was associated with renal, musculoskeletal, hematological, neuro-psychiatric and hepatic systems and speckled pattern had association with renal manifestations.

ANA positivity is a risk factor for the development of uveitis, especially in patients with oligoarticular arthritis.[20] In the presentstudy 27%of oligoarticularJIA patients had ANA positivity. Nordalet al reported ANA positivity in 27.4% among total JIA patients in their Cohort.[25] In previous studies, the incidence of uveitis was reported to be between 10- 20%,[26] but in the present study 15.2% patients had uveitis.These findings were consistent with the studydone in Turkey, where 10 -20% of JIA patients had uveitis.[27]

Pachman et al found ANA positivity in 70-80% of their JDM patients, majority of whom had coarse speckled pattern.[28] In the present study ANA positivity was 40% among JDM patients and both homogenous and speckled patterns were found among them in equal frequency.[28]

Chung et al in their study found that majority (95%) of Scleroderma patients had ANA positivity and patterns were homogeneous and centromeric.[29] The present study found ANA positivity in 31.5% scleroderma cases and majority had coarse speckled patterns followed by homogenous pattern. Among the polyarteritisnodosa cases 16.5% were ANA positive with homogenous staining pattern. No similar study was available to compare our result.

**Conclusion**

ANA positivity was highest amongMCTD followed by SLE cases and majority had homogeneous pattern. A good number of other PRD cases (OligoJIA, JDM, PAN, SS) were also ANA positive. Staining patterns showed strong association with clinical manifestations in SLE and JIA cases.

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and their parents.The authors declare that they have no competing interest.

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