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Understanding the ANA prevalence and its common pattern in seroconverted dengue infected patients

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Abstract
Background: A common complication after viral infection is the onset of autoimmune diseases. Dengue viremia may provoke the immune complex formation in patients who are predisposed to develop autoimmune diseases.
Aim: To assess relation between dengue and autoimmune diseases.
Methodology: A prospective study was conducted over clinically symptomatic dengue patients, who tested positive for IgMAb + NS1 Ag, by examining them after six month duration for presence of IgGAb and ANA and their patterns, using Indirect Immuno fluorescence test (IIFT). Frequency, Mean and standard deviation were calculated, followed by Mann-Whitney U test and Chi square test for categorical data.
Results: 765 cases, among 2249 suspected dengue patients, were found positive for IgM and NS1. After six months, 163 patients were assessed further; out of whom, 120 had developed IgG; while 22 were found ANA positive. Nuclear homogeneous pattern (AC-1) was the most common pattern for ANA Ab.
Conclusion: The patients who are dengue seropositive should be periodically screened for ANA and autoimmune disease development.

Keywords: Dengue, IGG antibodies, autoimmune diseases, antinuclear antibody, indirect immunofluorescence test

Introduction
Dengue fever is an intense febrile arbo-viral ailment influencing the tropical and subtropical locales of the world. Dengue is presently endemic in all WHO regions. Dengue perpetrates a significant health, monetary and social burden on the populaces of endemic areas [1]. It is a single-stranded RNA virus, which is transmitted by vectors like *Aedes aegypti* and *Aedes albopictus*. The spectrum of infection with Dengue virus varies and ranges from asymptomatic infection at one end to Dengue Haemorrahgic fever and Dengue Shock Syndrome at the other extreme [2]. After Dengue virus infection, Immunoglobulin M (IgM) antibodies are the primary immunoglobulin isotype to show up within the first week of illness. Immunoglobulin G (IgG) is generally imperceptible at the end of the first week of illness, increasing slowly thereafter and remaining perceivable in serum over 2-3 months, probably even for life [3]. The immune system functions to protect the body by responding to invading microorganisms. Autoimmunity is the failure of an organism in recognizing its own constituent parts as self, thus leading to an immune response against its own cells and tissues. Any disease that results from such an aberrant immune response is termed an autoimmune disease, and is marked by the presence of auto-antibodies [4]. These auto-antibodies may be found in normal individuals, but their expression usually increases after certain inciting events like any infection or tissue damage resulting from trauma or ischemia. There is significant evidence suggesting that different classes of pathogens (bacteria, viruses and parasites) are involved in triggering or propagating self-reactive immune responses [3, 4].
The immune-pathogenesis of dengue incorporates antibody-dependent enhancement, cellular immune response, soluble factors (such as cytokines and complements), and autoantibodies [5], which also play significant roles in the development of other autoimmune diseases. The viremia caused due to dengue can act as a trigger for immune complex formation in patients who are predisposed to develop autoimmune diseases.

Some cases of dengue-associated autoimmune diseases have been reported, such as Systemic Lupus Erythematosus (SLE), Systemic Vasculitis, Acute Disseminated encephalomyelitis (ADEM), Neuromyelitis Optica, Transverse Myelitis and Subacute thyroiditis [3-5].

The Antinuclear antibodies (ANA) assay detects a range of antibodies that react with antigens in the nucleus, nucleolus, cytoplasm and mitotic cellular apparatus [6]. The International Consensus on Antinuclear Antibody Patterns (ICAP) developed a coding method for reporting for ANA testing. These codes allow for uniform, easy and objective access and reference to the web-based consensus patterns available on the ICAP website (www.ANApatterns.org) [7]. Many of these tests exhibit great clinical utility and may play role as diagnostic markers, prognostic indicators and for the monitoring of autoimmune diseases. ANA detection by the Indirect Immunofluorescence assay (IIFA) is a 'gold' standard technique, utilizing HEp-2 cells or its variable cell lines [8].

There has been no study relating autoimmune disorders to diseases like dengue conducted in this region. This study aimed to assess the risk of autoimmune diseases in dengue infected patients by detection of antinuclear antibodies (ANA) and their patterns using Indirect Immuno Fluorescence test (IIFT).

Material and Methods

This was a prospective observational investigation study conducted in Department of Microbiology, in association with Department of Medicine, at a medical college in Central India, between January 2019 and June 2020. The patients with febrile sickness coming to our department, who were clinically suggestive for dengue virus infection and tested positive for Dengue IgM + NS1 antigen or Dengue IgM were included in the study. A voluntary written consent was taken from all patients, and those with any co-morbid condition were excluded from study sample. A minimum sample size of 100 participants was considered to be adequate.

A structured assessment form was utilized to obtain detailed demographic and clinical history from the patients including clinical manifestations and signs. 5ml of blood was collected from each participant and allowed to clot at room temperature (20-25 ºC), followed by centrifugation. The serum that was thus separated was stored in refrigerator at 2-8 ºC for serological tests to be performed over time.

The patient's serum was tested for Dengue IgM + NS1 antigen using NIV DENGUE IgM Capture ELISA Kit and J.MITRA and Co. DENGUE NS1 Ag MICROLISA Kit, respectively as per the kit literature. Semi-quantitative results were obtained by calculating the ratio of the optical density (OD) of each sample to the OD of the calibrator, according to the manufacturer's instructions. Results and data were recorded.

Dengue IgM positive and Dengue IgM+ NS1 positive patients were recalled after 6-9 months and consent was taken to participate in the study. Patient's clinical status and history was re-recorded at this visit. 5 ml of blood was collected from each patient and centrifuged for serum extraction. The serum was then tested for Dengue IgG + Antinuclear Antibody using J. MITRA and Co. DENGUE IgG MICROLISA Kit and EUROIMMUN IIFT Antinuclear Antibody Detection Kit, respectively, as per the kit literature. Results and data were recorded and analyzed.

Data Analysis

Data was entered in Microsoft Excel software and then analyzed using Statistical Package for the Social Sciences (SPSS v 20; SPSS, Chicago, IL, USA). Frequency and percentage were calculated to show the prevalence. Mean, Standard deviation, Median and Inter Quartile range were calculated for the quantitative variables. Univariate analysis was done for gender distribution, age group distribution and symptomatic or asymptomatic infection distribution across different serological status. Mann-Whitney U test and Chi square test were applied for analysis of categorical data. Statistically significance was measured at p value <0.05.

Results

A total of 2249 patients with clinically presumed dengue fever were tested for serological markers of dengue in Department of Microbiology during the study period. 765 cases were found positive for serological tests (Seropositivity 34%). Among these 765 patients, 477 (62.35%) had IgM Ab, 162 (21.17%) had NS1 Ag and remaining 126 (16.47%) had both the markers. Out of these, 603 patients, who had IgM Ab and IgM Ab + NS1 Ag, were approached after 6 months for further testing. Among these, only 163 patients (27%) gave consent for further study. Their serum was tested and 120 patients (74%) were found to have IgG Ab.

The mean age of IgG positive patients was 26.67 years, while the mean age of IgG negative patients was 19.86 years. (Table 1) Average age of the patients was found significantly higher in IgG positive finding. (p value < 0.05). Male preponderance was seen among the IgG positive patients, 60.8% of these were male while 39.2% were female. (Table 2) The male: female ratio was 1.55:1. There was no statistical difference detected for gender distribution among IgG positive and negative patients (p value > 0.05).

Table 1: Age comparison in patients with IgG antibody

IgG	Frequency	Agein years		Mann-Whitney U TEST	P value
		Mean ± SD	Median (IQR)		
Positive	120	26.67 ± 17.5	20(20)	1816.0	0.004**
Negative	43	19.86 ± 15.4	16(8)		

Table 2: Association of gender with IgG antibody

IgG	Gender		Total	Chi-Square	P value
	Female (60)	Male (103)			
Positive	47(39.2%)	73(60.8%)	120	1.086	0.297
Negative	13(30.2%)	30(69.8%)	43		

Amongst 120 IgG positive patients, serum samples of 22 patients (18.33%) were detected as being ANA positive. In ANA-positive patients, the mean age was 23.27 (±18.419) years and the median age was 15.50 years. (Table 3) The age difference among ANA positive and negative patients was not found to be statistically significant (p value > 0.05). Among the 22 ANA positive patients, 11 (50%) were males and 11 (50%) were females. (Table 4) According to our study, gender was not found to be associated with ANA (p value > 0.05).

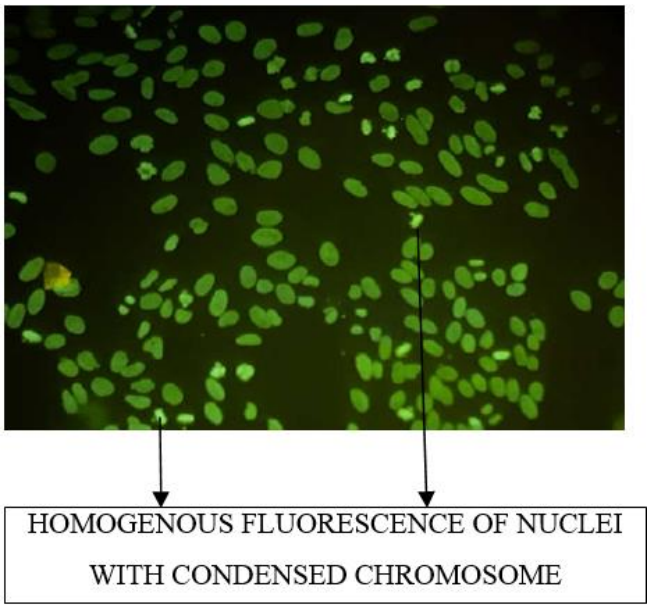
Table 3: Age comparison of ANA positive patients

ANA	Frequency	Age in years		Mann-Whitney U TEST	P value
		Mean ± SD	Median (IQR)		
PRESENT	22	23.27± 18.42	15.50(15)	821.0	0.081
ABSENT	98	27.43± 17.3	20 (22)		

Table 4: Association of gender with ANA detection

ANA	Gender		Total	Chi Square	P value
	Female (47)	Male (73)			
PRESENT	11(50.0)	11(50.0)	22	1.327	0.249
ABSENT	36(36.7)	62(63.3)	98		

The predominant ANA patterns observed in dengue infected patients included Nuclear homogeneous pattern (AC-1) in 18 patients (81.81%), Smooth nuclear envelope pattern (AC-11) in 3 patients (13.63%), followed by Nuclear fine speckled pattern (AC-4) in 1 patient (4.5%).



Discussion

This study was a prospective study conducted in dengue seropositive patients, to assess association between antibodies against Dengue infection and presence of ANA. Our study found seropositivity for dengue serological markers in 34% patients. The seropositivity reported in various studies ranged from 11.9% to 40% [9-13]. This difference could be due to the geographical distribution and the duration of the study period.

This study examined 163 patients who were IgM and NS1 positive and 120 (74.0%) patients among these had IgG antibody. This prevalence was lesser than that found by Luo S *et al*. among dengue symptomatic patients (96.6%) [14].

In our study, dengue symptomatic participants showed significantly higher prevalence of IgG antibodies than asymptomatic individuals after six months of infection. This study finding was in accordance with findings of Ren J *et al*. in which dengue IgG antibodies prevalence was higher in symptomatic patients [15].

Gender distribution was found as an insignificant predictor of seroprevalence of dengue IgG antibodies. This may be because gender distribution was random in our study or may be due to small sample size in our study. In our study, we found that mean age among IgG positive patients was 26.67 years while mean age of IgG negative patients was 19.86 years. Average age of the patients was found significantly higher in IgG positive patients, which was not in accordance with earlier study done by Luo S *et al*. which revealed gender and age as insignificant predictors of seroprevalence of dengue IgG in limited sample size studies [14]. More research ought to be carried out to investigate the connection between age and gender of individuals affected with dengue and formation of dengue IgG antibodies.

In the current study, out of 120 dengue IgG antibody positive patients, 22 patients (18.33%) were found to be ANA positive. The mean age was 23.27 years (±18.4) in ANA positive patients. Among these 22 ANA positive patients, 11 (50%) were males and 11(50%) were females. According to this finding, ANA positivity rate was not affected by gender of the participants in our study. This finding of equal prevalence of ANA positivity in both the genders was not in concordance with the earlier studies done by Watanabe A *et al*. [16]. Their study suggested that there is a female dominance in ANA production due to hormonal or certain other factors in females which play a pivotal role in this process. The hormonal theory remains most compelling, although it lacks a consistent specific path [17]. The higher prevalence of autoimmune diseases in females is not completely understood till date.

The most common ANA patterns observed in our study were nuclear homogenous pattern (AC-1) in 81.81% patients, smooth nuclear envelope pattern (AC-11) in 9.09% patients followed by nuclear fine speckled pattern (AC-4) in 4.5% patients. There are more than 100 autoantigens present in HEp-2 cells. The known target antigens in homogeneous pattern is dsDNA, ssDNA, core histones, and/or nucleosomes, which has clinical association with Systemic Lupus erythematous (SLE), drug induced SLE and juvenile idiopathic arthritis. Smooth Nuclear envelope pattern or Nuclear membrane pattern (AC-11) is associated with target antigens like gp210, lamin A, B and C and lamin B receptor which are clinically associated with primary biliary cholangitis. Nuclear fine speckled pattern (AC-4) is associated with known target antigens like SS-A and SS-B

which are clinically associated Sjogren's syndrome, SLE and neonatal LE [18, 19].

Albeit ANA pattern distributions among healthy individuals vary among studies, a homogenous nuclear pattern is usually the most commonly identified pattern, as was observed in the present study. Similar distribution was observed in the study done by Fritzler MJ *et al*. [20] Studies conducted by Siriwan Wananukul *et al*. in 2005 also reports that the common pattern obtained in healthy individuals is homogeneous pattern (46.7%), followed by speckled (20%) and nucleolar pattern (10%) [21]. The possible explanation of presence of ANA in general population is intrinsic immunological disturbances among humans, as reported by Pisetsky *et al*. [22] Positive ANA finding without any physical signs and symptoms has limited diagnostic utility and ought to be deciphered by a physician in the context of clinical symptoms and results of laboratory tests for specific autoantibodies [23]. ANA production sometimes may happen by the actions of specific genes that promote immune cell activity.

There were certain limitations in our study. Firstly, serum samples were assessed for Dengue IgG and ANA detection, according to manufacturer instructions, only at 1:100 dilution; other dilution titers were not investigated. Secondly, titres of IgG antibody and ANA were not measured.

We recommend that dengue infection control should be given more emphasis among deprived socio-economic populations and young children, by promoting use of personal measures (e.g. use of mosquito repellants, wearing full sleeves shirts, pants, socks, etc.) and environmental management measures (e.g. detection and elimination of mosquito breeding sites). Both types of assays namely, NS1 and IgM detection should be performed together in a single sample for better detection of dengue. The patients who are dengue seropositive, especially children and young adults, should be periodically screened for ANA and Autoimmune disease development.

Though, our study had a small sample size, was uni centric and only one type of viral infection was assessed, we can conclude that there is some association between viral infection and presence of ANA. Further research is recommended to establish a definitive association between the two.

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