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Original Article

Evaluation of Antinuclear Antibodies in Liver Diseases

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ABSTRACT

Background: Liver diseases comprise a heterogeneous group of disorders with varying etiologies, including autoimmune, metabolic, viral, and alcohol-related conditions. Antinuclear antibodies (ANA) are well-established markers of autoimmune hepatitis but may also be detected in other chronic liver diseases. Their clinical significance in different liver disorders remains an area of ongoing investigation.

Aim: To evaluate the prevalence of antinuclear antibodies in patients with liver diseases and to assess their association with clinical characteristics, biochemical parameters, disease etiology, and severity.

Materials and Methods: This hospital-based, observational, cross-sectional study was conducted in the Department of Biochemistry in collaboration with the Departments of General Medicine and Gastroenterology at MGM Medical College and Hospital, Kamothe, Navi Mumbai, over a period of 1.5 years. A total of 50 adult patients with clinically, biochemically, and/or radiologically confirmed liver diseases were enrolled using consecutive sampling. Demographic details, clinical features, laboratory investigations, liver function tests, viral markers, imaging findings, and ANA status were recorded. Serum ANA testing was performed using the indirect immunofluorescence assay (IIFA) on HEp-2 cells. Statistical analysis was carried out using IBM SPSS Statistics version 26.0, with a p-value <0.05 considered statistically significant.

Results: The majority of patients were males (64%), with the highest proportion belonging to the 41–50-year age group (26%). Non-alcoholic fatty liver disease was the most common diagnosis (28%), followed by alcoholic liver disease (22%) and viral hepatitis (18%). ANA positivity was detected in 28% (14/50) of patients and was significantly higher among patients with autoimmune hepatitis (100%; $p<0.001$). The homogeneous fluorescence pattern (35.7%) and an ANA titre of 1:160 (35.7%) were the most frequent findings. ANA-positive patients had significantly higher serum AST, ALT, and bilirubin levels and significantly lower serum albumin compared with ANA-negative patients ($p<0.05$). Female gender, jaundice, elevated INR, and hypoalbuminemia were also significantly associated with ANA positivity.

Conclusion: ANA positivity was observed in more than one-fourth of patients with liver diseases and was strongly associated with autoimmune hepatitis and greater biochemical evidence of hepatic injury. Indirect immunofluorescence-based ANA testing serves as a valuable adjunct in the evaluation of liver diseases and may facilitate early diagnosis of autoimmune liver disorders, assessment of disease severity, and appropriate clinical management.

Keywords: *Antinuclear antibodies, Autoimmune hepatitis, Liver diseases, Indirect immunofluorescence assay, Non-alcoholic fatty liver disease, Autoantibodies.*

INTRODUCTION

Liver diseases constitute a major global health burden and encompass a wide spectrum of disorders ranging from metabolic, infectious, alcohol-related, autoimmune, and cholestatic conditions to end-stage liver cirrhosis and hepatocellular carcinoma. According to the Global Burden of Disease Study, liver diseases account for more than two million deaths annually worldwide, representing nearly 4% of all global deaths. Chronic liver diseases are associated with significant morbidity, mortality, and healthcare expenditure, particularly in developing countries where viral hepatitis and alcohol-related liver disease remain highly prevalent. In recent years, the incidence of non-alcoholic fatty liver disease (NAFLD) has increased substantially due to the rising prevalence of obesity, diabetes mellitus, and metabolic syndrome, making it the most common chronic liver disease globally. (1,2)

The liver plays a crucial role in immune regulation and tolerance because of its continuous exposure to gut-derived antigens. Disruption of immune tolerance may lead to autoimmune-mediated liver injury characterized by the production of various autoantibodies and chronic hepatic inflammation. Among these autoantibodies, antinuclear antibodies (ANA) are one of the most frequently detected serological markers in autoimmune liver diseases. ANA are directed against various nuclear antigens and are commonly associated with autoimmune hepatitis (AIH), although they may also be detected in other liver disorders including primary biliary cholangitis, NAFLD, chronic viral hepatitis, alcoholic liver disease, and liver cirrhosis. (3,4)

Autoimmune hepatitis is a chronic inflammatory liver disease characterized by elevated serum aminotransferases, hypergammaglobulinemia, circulating autoantibodies, and characteristic histopathological findings. ANA, together with smooth muscle antibody (SMA) and liver kidney microsomal antibody type-1 (anti-LKM1), forms an important component of the diagnostic criteria established by the International Autoimmune Hepatitis Group (IAIHG). Early identification of ANA-positive patients facilitates prompt initiation of immunosuppressive therapy, thereby reducing disease progression and improving long-term outcomes. (5,6)

Although ANA positivity is traditionally considered a hallmark of autoimmune hepatitis, several studies have demonstrated the presence of ANA in non-autoimmune liver diseases. Patients with NAFLD, alcoholic liver disease, chronic hepatitis B and C infection, and liver cirrhosis may exhibit ANA positivity despite the absence of classical autoimmune hepatitis. The reported prevalence varies considerably, ranging from 10% to 40%, depending on patient population, disease severity, laboratory techniques, and ANA titre cut-off values. The clinical significance of ANA positivity in these conditions remains controversial, with some studies suggesting an association with advanced fibrosis and disease severity, while others report no significant prognostic value. (7,8)

Indirect immunofluorescence assay (IIFA) using HEp-2 cells remains the gold standard method for ANA detection because of its high sensitivity and ability to identify different fluorescence patterns. Various ANA fluorescence patterns, including homogeneous, speckled, nucleolar, centromere, and cytoplasmic patterns, may provide additional diagnostic clues regarding underlying autoimmune disorders. Furthermore, higher ANA titres have been associated with increased likelihood of clinically significant autoimmune disease. (9)

The evaluation of ANA in patients with liver disease is therefore clinically relevant, not only for diagnosing autoimmune hepatitis but also for identifying patients who may require further immunological work-up or closer clinical follow-up. Several investigators have reported correlations between ANA positivity and elevated liver enzymes, hyperbilirubinemia, hypoalbuminemia, advanced fibrosis, and poor liver function. However, available data remain inconsistent, particularly in heterogeneous populations including multiple etiologies of liver disease. (10,11)

In India, where the spectrum of liver disease includes viral hepatitis, alcohol-related liver disease, NAFLD, and autoimmune liver diseases, limited studies have comprehensively evaluated ANA positivity across different liver disorders. Understanding the prevalence and clinical significance of ANA in Indian patients may contribute to improved diagnostic strategies and patient management.

Therefore, the present study was undertaken to evaluate the prevalence of antinuclear antibodies among patients with various liver diseases attending MGM Medical College and Hospital, Kamothe, Navi Mumbai, and to determine the association of ANA positivity with demographic characteristics, clinical presentation, biochemical parameters, disease etiology, and severity.

MATERIALS AND METHODS

Study Design and Setting

This was a hospital-based, observational, cross-sectional study conducted in the Department of Biochemistry in collaboration with the Department of General Medicine and Department of Gastroenterology at **MGM Medical College and Hospital, Kamothe, Navi Mumbai**, over a period of **1.5 years**. The study was designed to evaluate the prevalence

and clinical significance of **antinuclear antibodies (ANA)** in patients with various liver diseases and to determine their association with clinical, biochemical, and etiological characteristics.

Study Population

The study included **50 consecutive patients** diagnosed with liver disease who fulfilled the eligibility criteria and attended the inpatient or outpatient departments during the study period.

Sample Size

A total of **50 patients** with confirmed liver diseases were enrolled in the study using consecutive sampling.

Inclusion Criteria

- Patients aged **18 years and above**.
- Patients with clinically, biochemically, and/or radiologically confirmed liver disease.
- Patients diagnosed with:
    - Autoimmune hepatitis (AIH)
    - Alcoholic liver disease (ALD)
    - Non-alcoholic fatty liver disease (NAFLD)
    - Viral hepatitis (HBV/HCV)
    - Chronic liver disease (CLD)
    - Liver cirrhosis
- Patients willing to participate and providing written informed consent.

Exclusion Criteria

- Patients younger than 18 years.
- Pregnant women.
- Patients already receiving immunosuppressive therapy before ANA evaluation.
- Patients with previously diagnosed systemic autoimmune diseases such as systemic lupus erythematosus, systemic sclerosis, rheumatoid arthritis, or Sjögren syndrome.
- Patients with malignancy involving the liver.
- Patients who refused consent or had incomplete clinical or laboratory data.

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Data Collection

After obtaining informed consent, detailed demographic and clinical information was recorded using a structured case record form.

The following variables were collected:

- Age
- Gender
- Body mass index (BMI)
- Alcohol consumption
- History of diabetes mellitus
- Hypertension
- Drug history
- Family history of liver disease
- Duration of symptoms
- Clinical presentation (jaundice, abdominal pain, fatigue, abdominal distension, pruritus, edema, gastrointestinal bleeding)
- Physical examination findings
- Final diagnosis of liver disease

Laboratory Investigations

All patients underwent routine laboratory investigations including:

Hematological Investigations

- Complete blood count (CBC)
- Hemoglobin
- Total leukocyte count
- Platelet count
- Erythrocyte sedimentation rate (ESR)

Liver Function Tests

- Total bilirubin
- Direct bilirubin
- Indirect bilirubin
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Alkaline phosphatase (ALP)
- Gamma-glutamyl transferase (GGT)
- Total protein
- Serum albumin
- Serum globulin
- Albumin/globulin ratio

Coagulation Profile

- Prothrombin time (PT)
- International Normalized Ratio (INR)

Renal Function Tests

- Blood urea
- Serum creatinine

Viral Markers

- Hepatitis B surface antigen (HBsAg)
- Anti-HCV antibody

Imaging Studies

- Ultrasonography (USG) abdomen
- FibroScan or liver stiffness assessment (where clinically indicated)
- CT scan or MRI abdomen (when required)

Antinuclear Antibody (ANA) Testing

Approximately 3–5 mL of venous blood was collected from each participant under aseptic precautions. Blood samples were allowed to clot and serum was separated by centrifugation.

Serum ANA testing was performed using the Indirect Immunofluorescence Assay (IIFA) on HEp-2 cells, which is considered the gold standard for ANA detection. Commercially available ANA HEp-2 kits were used according to the manufacturer's instructions.

The procedure included:

- Dilution of patient serum.
- Incubation on HEp-2 cell substrate slides.
- Washing to remove unbound antibodies.
- Addition of fluorescein-labeled anti-human IgG conjugate.
- Final washing and mounting.
- Examination under a fluorescence microscope.

ANA results were reported as:

- Positive
- Negative

Positive samples were further categorized according to:

- Fluorescence pattern
    - Homogeneous
    - Speckled
    - Nucleolar
    - Centromere
    - Cytoplasmic
    - Mixed pattern
- ANA titre (1:80, 1:160, 1:320, ≥1:640)

Diagnosis of Liver Diseases
The diagnosis of liver diseases was established using a combination of:

- Clinical history
- Physical examination
- Liver function tests
- Viral serology
- Autoimmune markers
- Radiological imaging
- Liver biopsy findings (where clinically indicated)
- Standard national and international diagnostic guidelines.

Outcome Measures
Primary Outcome

- Prevalence of ANA positivity among patients with liver diseases.

Secondary Outcomes

- Distribution of ANA patterns and titres.
- Association of ANA positivity with different liver diseases.
- Correlation between ANA positivity and liver function tests.
- Association between ANA positivity and severity of liver disease.
- Relationship of ANA positivity with demographic and clinical characteristics.

Statistical Analysis
Data were entered into Microsoft Excel 2021 and analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

Continuous variables were expressed as mean ± standard deviation (SD) for normally distributed data or median with interquartile range (IQR) for skewed data.

Categorical variables were presented as frequencies and percentages.
Comparisons between ANA-positive and ANA-negative groups were performed using:

- Independent Student's *t*-test for normally distributed continuous variables.
- Mann–Whitney U test for non-normally distributed variables.
- Chi-square test or Fisher's exact test for categorical variables.

Association between ANA positivity and liver function parameters was evaluated using Pearson's or Spearman's correlation coefficient, depending on data distribution. Multivariate logistic regression analysis was performed to identify independent predictors of ANA positivity where appropriate. A p-value <0.05 was considered statistically significant.

RESULTS AND OBSERVATIONS
A total of **50 patients** with clinically and/or radiologically confirmed liver diseases were included in the study. The demographic profile, clinical presentation, laboratory findings, etiological spectrum, and antinuclear antibody (ANA) status were analysed.

Table 1. Demographic Characteristics of the Study Population (n = 50)

Age Group (Years)	Number (n)	Percentage (%)
18–30	8	16.0
31–40	11	22.0
41–50	13	26.0
51–60	10	20.0
>60	8	16.0
Total	**50**	**100.0**

Gender Distribution

Gender	Number (n)	Percentage (%)
Male	32	64.0
Female	18	36.0
Total	**50**	**100.0**

Observation: Most patients belonged to the **41–50 years** age group (26%), with a predominance of males (64%).

Table 2. Etiological Distribution of Liver Diseases

Diagnosis	Number (n)	Percentage (%)
Non-alcoholic fatty liver disease (NAFLD)	14	28.0
Alcoholic liver disease (ALD)	11	22.0
Viral hepatitis (HBV/HCV)	9	18.0
Liver cirrhosis	8	16.0
Autoimmune hepatitis (AIH)	5	10.0
Other chronic liver diseases	3	6.0
Total	**50**	**100.0**

Observation: NAFLD was the most common liver disease (28%), followed by alcoholic liver disease (22%).

Table 3. Clinical Presentation

Clinical Feature	Number (n)	Percentage (%)
Fatigue	38	76.0
Jaundice	31	62.0
Abdominal pain	27	54.0
Abdominal distension	20	40.0
Loss of appetite	29	58.0
Pruritus	12	24.0
Pedal edema	10	20.0
Gastrointestinal bleeding	6	12.0

Observation: Fatigue (76%) and jaundice (62%) were the most common presenting complaints.

Table 4. Liver Function Test Parameters

Parameter	Mean ± SD
Total Bilirubin (mg/dL)	3.42 ± 2.51
AST (IU/L)	118.4 ± 70.6
ALT (IU/L)	102.8 ± 63.5
ALP (IU/L)	214.6 ± 96.8
GGT (IU/L)	178.5 ± 104.7
Albumin (g/dL)	3.32 ± 0.74
INR	1.42 ± 0.39

Observation: Elevated transaminases and cholestatic enzymes were observed in the majority of patients.

Table 5. ANA Positivity Among Study Participants

ANA Status	Number (n)	Percentage (%)
Positive	14	28.0
Negative	36	72.0
Total	**50**	**100.0**

Observation: ANA positivity was detected in **28%** of patients with liver diseases.

Table 6. ANA Positivity According to Liver Disease

Liver Disease	Total Cases	ANA Positive	ANA Negative	p-value
Autoimmune hepatitis	5	5	0	<0.001
NAFLD	14	3	11	
Alcoholic liver disease	11	2	9	
Viral hepatitis	9	2	7	
Liver cirrhosis	8	1	7	
Other CLD	3	1	2	

Observation: ANA positivity was significantly higher in patients with autoimmune hepatitis (**$p < 0.001$**).

Table 7. ANA Fluorescence Patterns (n = 14)

Pattern	Number (n)	Percentage (%)
Homogeneous	5	35.7
Speckled	4	28.6
Nucleolar	2	14.3
Cytoplasmic	2	14.3
Mixed	1	7.1

Observation: Homogeneous fluorescence was the most common ANA pattern observed.

Table 8. ANA Titre Distribution (n = 14)

ANA Titre	Number (n)	Percentage (%)
1:80	4	28.6
1:160	5	35.7
1:320	3	21.4
≥1:640	2	14.3

Observation: Most ANA-positive patients demonstrated a titre of **1:160**.

Table 9. Comparison of Liver Function Tests According to ANA Status

Parameter	ANA Positive (n=14) Mean ± SD	ANA Negative (n=36) Mean ± SD	p-value
AST (IU/L)	152.3 ± 64.8	105.2 ± 59.7	0.018
ALT (IU/L)	132.8 ± 57.5	90.4 ± 52.8	0.011
Bilirubin (mg/dL)	4.48 ± 2.61	2.98 ± 2.12	0.034
Albumin (g/dL)	3.02 ± 0.69	3.44 ± 0.71	0.047

Observation: ANA-positive patients had significantly higher AST, ALT, and bilirubin levels, while serum albumin was significantly lower.

Table 10. Association of ANA Positivity with Selected Clinical Variables

Variable	ANA Positive (n=14)	ANA Negative (n=36)	p-value
Female gender	8	10	0.041
Jaundice	12	19	0.039
Cirrhosis	4	4	0.083
Elevated INR (>1.5)	6	5	0.047
Hypoalbuminemia (<3.5 g/dL)	10	14	0.032

Observation: ANA positivity showed significant associations with female gender, jaundice, elevated INR, and hypoalbuminemia.

DISCUSSION

The present hospital-based observational study evaluated the prevalence and clinical significance of antinuclear antibodies (ANA) in patients with various liver diseases. Among the 50 enrolled patients, ANA positivity was observed in 28% of cases. Autoimmune hepatitis demonstrated the highest prevalence of ANA positivity, while lower frequencies were observed in NAFLD, alcoholic liver disease, viral hepatitis, liver cirrhosis, and other chronic liver diseases. Furthermore, ANA-positive patients exhibited significantly higher serum AST, ALT, bilirubin levels, lower serum albumin concentrations, and greater prevalence of jaundice and coagulation abnormalities, suggesting an association between ANA positivity and more severe hepatic dysfunction.

The majority of patients belonged to the 41–50-year age group, with males constituting 64% of the study population. Similar demographic findings have been reported by Czaja, who observed that although autoimmune liver diseases frequently affect women, alcohol-related and metabolic liver diseases predominantly occur in middle-aged men because of higher exposure to alcohol consumption and metabolic risk factors. (5)

NAFLD represented the most common liver disease in the present study (28%), followed by alcoholic liver disease (22%) and viral hepatitis (18%). This distribution reflects the changing epidemiology of liver diseases worldwide, where metabolic dysfunction-associated fatty liver disease has become increasingly prevalent due to obesity and diabetes mellitus. Younossi et al. similarly reported NAFLD as the leading cause of chronic liver disease globally. (2)

ANA positivity was identified in 28% of patients, which is consistent with previous reports demonstrating ANA positivity in approximately 20–35% of patients with chronic liver diseases. Liberal et al. reported that ANA may occur not only in autoimmune hepatitis but also in chronic viral hepatitis, NAFLD, and alcoholic liver disease, although generally at lower titres than those observed in autoimmune hepatitis. (6)

The strongest association was observed in autoimmune hepatitis, where all five patients were ANA positive (100%). This finding is consistent with international diagnostic guidelines, which recognize ANA as one of the principal serological markers for autoimmune hepatitis. Hennes et al. demonstrated that ANA remains an essential component of the simplified diagnostic scoring system for autoimmune hepatitis, particularly when present at titres of ≥1:80. (12)

In patients with NAFLD, ANA positivity was identified in approximately one-fifth of cases. Previous studies have reported ANA prevalence ranging from 13% to 30% among patients with NAFLD. Ravi et al. suggested that although

ANA positivity may be relatively common in NAFLD, it does not necessarily indicate autoimmune hepatitis but may reflect immune activation associated with chronic hepatic inflammation. (8)

Similarly, low frequencies of ANA positivity were observed among patients with alcoholic liver disease, viral hepatitis, and liver cirrhosis. Autoantibody production in chronic viral infections has been attributed to persistent immune stimulation and molecular mimicry, whereas chronic alcohol-induced liver injury may promote immune dysregulation and autoantibody formation. These observations have been supported by previous studies investigating chronic hepatitis C and alcoholic liver disease. (7,10)

The homogeneous fluorescence pattern was the most frequently observed ANA pattern (35.7%), followed by the speckled pattern. Similar findings have been reported by Agmon-Levin et al., who described homogeneous and speckled patterns as the predominant fluorescence patterns in autoimmune liver diseases. These patterns are commonly associated with antibodies directed against DNA and histone antigens and frequently occur in autoimmune hepatitis. (9)

The majority of ANA-positive patients demonstrated titres of 1:160, whereas higher titres (≥1:640) were less common. Current international recommendations suggest that higher ANA titres have greater diagnostic significance for autoimmune diseases, whereas low titres may occasionally occur in healthy individuals or chronic inflammatory conditions. Therefore, ANA titre interpretation should always be correlated with clinical findings and other laboratory investigations. (3)

An important finding of the present study was the significant association between ANA positivity and elevated serum AST, ALT, bilirubin, reduced serum albumin, and prolonged INR. These findings indicate that ANA-positive patients had relatively greater hepatocellular injury and impaired hepatic synthetic function. Similar observations have been reported by Muratori et al., who demonstrated correlations between autoantibody positivity and disease activity in autoimmune hepatitis. (11)

Female gender showed a statistically significant association with ANA positivity. Autoimmune diseases are well known to occur more frequently in women because of hormonal influences, genetic susceptibility, and immunological differences. Previous epidemiological studies have consistently demonstrated female predominance among ANA-positive autoimmune disorders. (4)

The present study has several strengths. ANA testing was performed using indirect immunofluorescence on HEp-2 cells, which remains the internationally accepted gold standard. The study also evaluated different ANA fluorescence patterns, titre distribution, and their association with clinical and biochemical parameters across multiple liver disease etiologies.

However, certain limitations should be acknowledged. The sample size was relatively small and the study was conducted at a single tertiary care center, which may limit generalizability. Liver biopsy was not available for all patients, and additional autoimmune markers such as anti-smooth muscle antibody, anti-LKM1, anti-SLA, and antimitochondrial antibodies were not routinely evaluated. Longitudinal follow-up was also not performed to determine the prognostic significance of ANA positivity.

Overall, the findings suggest that ANA positivity is relatively common among patients with liver diseases and is particularly frequent in autoimmune hepatitis. ANA-positive patients exhibited significantly greater biochemical evidence of hepatic injury and impaired liver function, emphasizing the value of ANA testing in the comprehensive evaluation of chronic liver diseases.

CONCLUSION

The present study demonstrated that antinuclear antibodies (ANA) were present in a substantial proportion (28%) of patients with liver diseases, with the highest prevalence observed in autoimmune hepatitis. ANA positivity was significantly associated with elevated liver enzymes, hyperbilirubinemia, hypoalbuminemia, female gender, jaundice, and impaired coagulation parameters, indicating more severe hepatic dysfunction. The homogeneous pattern and an ANA titre of 1:160 were the most commonly observed findings. These results highlight the clinical utility of ANA testing, particularly by indirect immunofluorescence, as an important adjunct in the evaluation of liver diseases. Early identification of ANA-positive patients may facilitate timely diagnosis of autoimmune hepatitis, improve disease characterization, and support appropriate clinical management. Larger multicenter studies with extended follow-up are recommended to further establish the prognostic significance of ANA in different liver disorders.

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