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Original article

The Frequency of Immunofluorescence Antinuclear Antibody and Patterns in Autoimmune Diseases in Benghazi City

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Abstract
Autoimmune diseases, characterized by a loss of immune self-tolerance, represent a significant and growing global health burden. The detection of Antinuclear Antibodies (ANA) serves as a crucial serological hallmark for diagnosing systemic autoimmune rheumatic diseases. However, epidemiological data on ANA prevalence and patterns remain limited in many regions, including Libya. This study aimed to determine the seroprevalence, patterns, and titers of ANA in a population from Benghazi, Libya, and to investigate their association with demographic characteristics. A retrospective cross-sectional study was conducted on 775 serum samples referred for ANA testing at Al Saleem Laboratory between January and May 2024. ANA detection and pattern analysis were performed using indirect immunofluorescence (IIF) on HEp-2 cells. The study population had a mean age of 40.93 ± 15.885 years and was predominantly female (81.5%). The overall ANA positivity rate was 30% (233/775). A significant female predominance was observed among positive cases (85.8%), yielding a female-to-male ratio of 6:1. The highest ANA positivity was found in individuals over 40 years (54.1%). The homogeneous pattern was most frequent (45.5%), followed by speckled (27.0%), nucleolar (12.9%), cytoplasmic (6.9%), peripheral (4.7%), and centromere (3.0%). Low titers (1:160) were most common (42.5%), with no statistically significant difference in titer distribution by gender ($p=0.279$). This study establishes a high prevalence of ANA positivity in the Benghazi population, with distinct demographic and serological patterns. The findings align with global trends of female predominance and middle-age onset, while providing foundational data for the Libyan population. These results underscore the importance of ANA testing and appropriate interpretation in clinical practice and highlight the need for expanded autoimmune disease surveillance in the region.
Keywords. Antinuclear Antibodies (ANA), Autoimmune Diseases, Prevalence, Immunofluorescence Patterns, Libya, Benghazi.

Introduction

The human immune system serves as the body's primary defense mechanism against pathogenic organisms [1]. This complex network of cells and proteins protects against infections while maintaining tolerance to self-antigens. However, when this immunological tolerance fails, the immune system may mistakenly attack the body's own tissues, resulting in autoimmune diseases [1,2]. Autoimmune diseases represent a diverse group of disorders characterized by abnormal immune responses against self-antigens [3]. These conditions affect approximately 3-5% of the global population and demonstrate increasing incidence in recent years [4]. The pathogenesis involves complex interactions between genetic predisposition, environmental factors, and immune dysregulation [5]. These conditions are broadly classified into systemic and organ-specific categories based on their target autoantigens and clinical manifestations [6]. Systemic autoimmune diseases, such as Systemic Lupus Erythematosus (SLE), involve multiple organ systems and demonstrate widespread tissue damage [7]. SLE exemplifies this category with its varied clinical presentations ranging from mild cutaneous involvement to severe renal and neurological complications [7].

Other significant systemic autoimmune conditions include rheumatoid arthritis, which primarily affects joints but can involve other systems [8], and systemic sclerosis, characterized by cutaneous and internal organ fibrosis [9]. Sjögren's syndrome represents another systemic disorder that mainly targets exocrine glands but can present with systemic features [10]. The organ-specific autoimmune diseases demonstrate more localized immune responses. Multiple sclerosis involves autoimmune-mediated damage to the central nervous system myelin [11], while autoimmune hepatitis features an immune attack on hepatocytes [12]. Primary biliary cholangitis represents another organ-specific condition targeting small bile ducts [13].

Antinuclear antibody (ANA) detection serves as a fundamental diagnostic tool for systemic autoimmune rheumatic diseases [14]. These autoantibodies target various nuclear components, including DNA, histones, and nucleoproteins [15]. The detection through indirect immunofluorescence on HEp-2 cells provides crucial diagnostic information, with different staining patterns indicating specific autoimmune conditions [16].

The International Consensus on ANA Patterns (ICAP) has standardized pattern interpretation, enhancing diagnostic accuracy across laboratories [17]. However, ANA testing requires careful clinical correlation as positive results can occur in healthy individuals, particularly with advancing age, and in various non-autoimmune conditions [18].

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This study aims to investigate the prevalence and patterns of antinuclear antibodies in the Benghazi population and examine their correlation with demographic characteristics and clinical autoimmune disease presentations. The findings will contribute to understanding the local epidemiology of autoimmune disorders and optimizing diagnostic approaches in our population.

Methods

Study Design and Population

A retrospective cross-sectional study was conducted. The target population consisted of individuals of all ages and both sexes from Benghazi, Libya, who were referred for antinuclear antibody (ANA) testing.

Sample Size and Sampling

A minimum sample size of 318 was calculated to achieve a 95% confidence level with a 5% margin of error. The final study sample included 775 sera, which exceeded this minimum requirement, thereby enhancing the power of the analysis.

Data Collection

Data were extracted from the records of Al Saleem Laboratory in Benghazi, Libya. The dataset comprised 775 consecutive ANA test results from patients referred for clinical suspicion of autoimmune disease (AID) or for follow-up of a known condition. The data collection period spanned from January 1st to May 30th, 2024. All ANA testing was performed using the indirect immunofluorescence (IFA) technique with ORGENTEC Diagnostic GmbH kits on HEp-2 cell substrates.

Data Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 28). Descriptive statistics were presented using frequency tables and graphical representations. Inferential analyses were conducted using the Chi-square test to assess associations between categorical variables. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant for all tests.

Results

Demographic Characteristics of the Study Population

A total of 775 serum samples were included in this study, comprising 632 females (81.5%) and 143 males (18.5%). The age distribution of participants ranged from 1 to 91 years, with a mean age of 40.93 ± 15.885 years (mean ± SD). The detailed demographic characteristics are presented in (Figure 1).

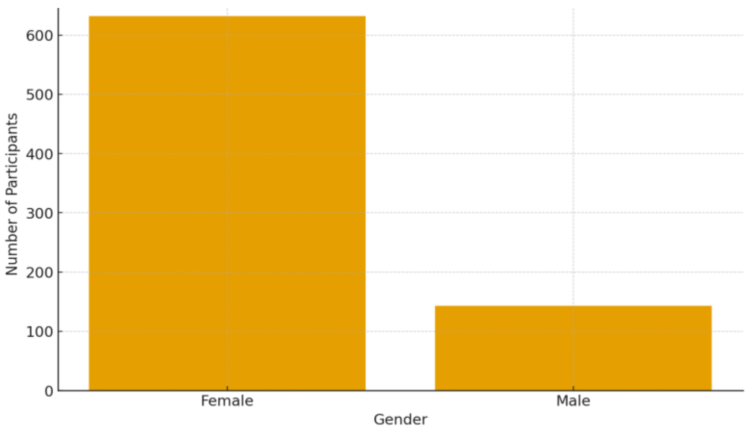

Figure 1. Gender distribution of the study population

Prevalence of Antinuclear Antibody Positivity

Among the 775 tested sera, 233 samples (30.0%) demonstrated positive ANA results, while 542 samples (70.0%) were negative. The distribution of ANA positivity showed significant gender-based variation (x^2 = 4.072, p = 0.044). Female participants accounted for 85.8% (n=200) of positive cases, compared to 14.2% (n=33) in males, resulting in a female-to-male ratio of approximately 6:1 (Table 2).

Table 2. ANA Positivity by Gender

Gender	ANA Positive (n)	ANA Negative (n)	Total (n)	ANA Positive (%)
Female	200	432	632	31.6
Male	33	110	143	23.1
Total	233	542	775	30.0
x^2 = 4.072, p = 0.044				

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Age Distribution Among ANA-Positive Cases

The ANA-positive cohort (n=233) was stratified into three age groups: 1-20 years (13.3%, n=31), 21-40 years (32.6%, n=76), and >40 years (54.1%, n=126). The highest prevalence of ANA positivity was observed in the oldest age group (>40 years), as illustrated in (Figure 3).

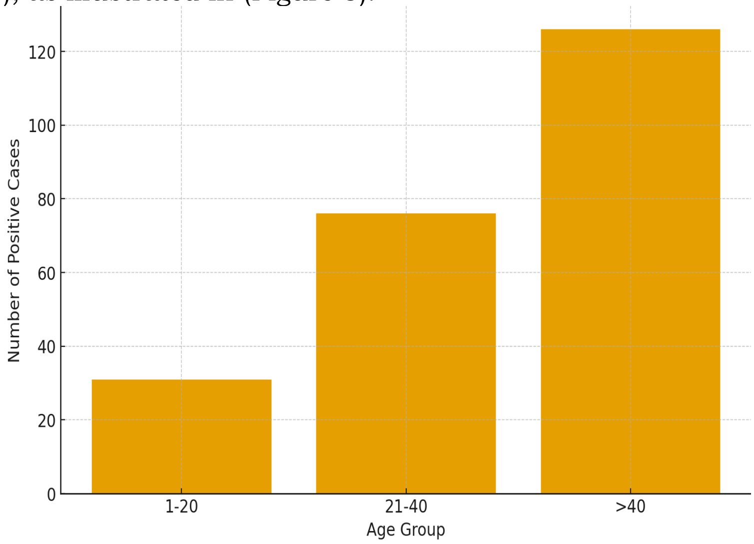

Figure 3. Age distribution among ANA-positive cases

Distribution of ANA Immunofluorescence Patterns

Six distinct ANA patterns were identified among positive samples. The homogeneous pattern was most prevalent (45.5%, n=106), followed by speckled (27.0%, n=63), nucleolar (12.9%, n=30), cytoplasmic (6.9%, n=16), peripheral (4.7%, n=11), and centromere patterns (3.0%, n=7). The overall distribution is detailed in (Figure 4).

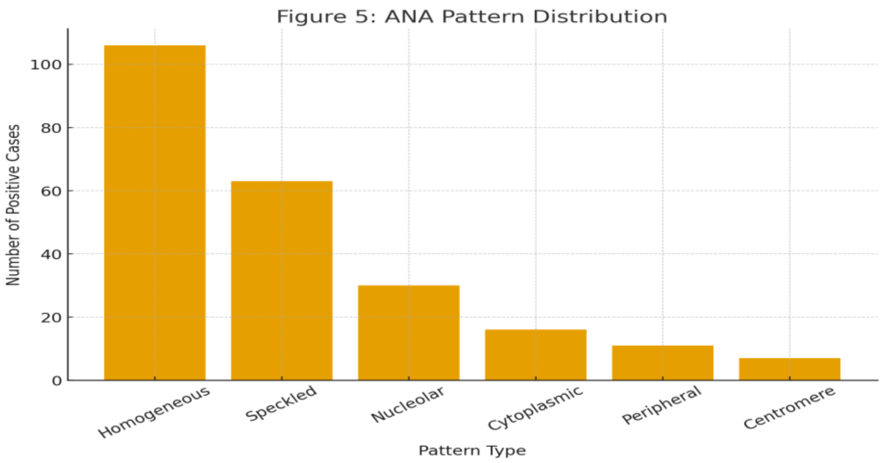

Figure 4. ANA pattern distribution

Gender-specific analysis revealed that females predominated across all ANA patterns (Figure 5). The homogeneous pattern comprised 88 females and 18 males, while the speckled pattern included 59 females and 4 males. Notably, all cases demonstrating the peripheral pattern (n=11) were female.

Age-stratified analysis of ANA patterns demonstrated that the homogeneous pattern-maintained dominance across all age groups: 38.7% (1-20 years), 44.7% (21-40 years), and 47.6% (>40 years). The speckled pattern showed higher prevalence in younger participants (35.5% in 1-20 years) compared to older groups (27.0% in >40 years).

ANA Titer Distribution

ANA titers were categorized into three groups: borderline (20.2%, n=47), low titer (42.5%, n=99), and high titer (37.3%, n=87). Gender-based analysis revealed no statistically significant difference in titer distribution between females and males (x^2 = 2.553, p = 0.279), as shown in Table 2.

Age-stratified titer analysis demonstrated variable distribution patterns, though without statistical significance (x^2 = 4.913, p = 0.296). The 1-20 years group showed the highest prevalence of high titers (45.2%), while the >40 years group demonstrated predominance of low titers (48.4%).

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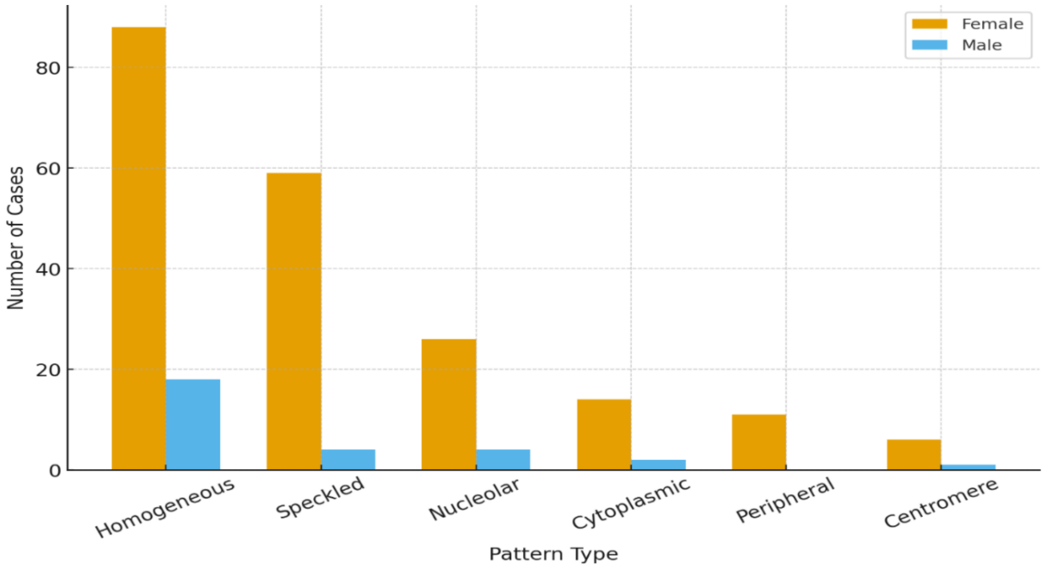

Figure 5. ANA pattern distribution by gender

Table 2. ANA Titer Categories and Distribution by Gender

ANA Titer Category	Female (n)	Male (n)	Total (n)	Percentage (%)
Borderline	39	8	47	20.2
Low Titer	84	15	99	42.5
High Titer	77	10	87	37.3
Total	200	33	233	100.0
$x^2 = 2.553$, $p = 0.279$				

Discussion

The findings of this study contribute to the growing body of evidence documenting the increasing global prevalence of autoimmune disorders and their serological markers [4,5]. Our investigation of 775 patients in Benghazi revealed a 30% prevalence of ANA positivity, providing important regional data that aligns with the recognized pattern of autoimmune disease epidemiology while highlighting population-specific characteristics [4,6]. The observed female predominance in ANA positivity (85.8%) with a 6:1 female-to-male ratio reflects well-established gender disparities in autoimmune conditions [4]. This pattern is consistent with the fundamental immunological differences between sexes, where hormonal influences, genetic factors, and variations in immune response regulation contribute to increased female susceptibility to autoimmune dysregulation [2,3]. The complex interplay between estrogen signaling, X-chromosome inactivation, and immune cell function creates a biological milieu more permissive to loss of self-tolerance in females [2,3].

The age distribution pattern in our cohort, with the highest ANA positivity in individuals over 40 years (54.1%), corresponds with the typical onset patterns of many autoimmune diseases [4,7]. This distribution may reflect the cumulative effects of environmental exposures, age-related immunological changes, and the progressive nature of autoimmune pathogenesis [3,5]. The maturation of the immune system and its gradual decline with age, coupled with lifelong antigen exposure, createconditions favorable for autoimmune manifestations in middle and later life [2,3].

The pattern distribution observed in our study, with homogeneous (45.5%) and speckled (27.0%) patterns predominating, demonstrates consistency with established ANA profiling in autoimmune populations [14,16]. These patterns correspond to specific autoantibody profiles associated with conditions such as SLE and Sjögren's syndrome, where antibodies target nuclear components, including DNA and extractable nuclear antigens [7,10,14]. The International Consensus on ANA Patterns (ICAP) has been instrumental in standardizing the interpretation of these patterns, enhancing their diagnostic utility across different populations and laboratory settings [16,17]. The titer distribution in our study population, with low titers (1:160) comprising 42.5% of positive cases, reflects the spectrum of autoimmune serology seen in clinical practice [6,14]. While higher titers generally show stronger association with active autoimmune disease, the presence of low-positive titers in our population underscores the importance of clinical correlation in ANA interpretation [6,18]. The absence of significant gender-based differences in titer distribution suggests that while females demonstrate higher prevalence of autoimmunity, the quantitative antibody response may follow similar patterns across genders when autoimmunity is present [4,6].

Our findings must be interpreted considering several methodological considerations. The use of indirect immunofluorescence on HEp-2 cells aligns with current recommendations for ANA screening, providing the advantage of pattern recognition while maintaining high sensitivity for autoimmune rheumatic diseases [15,16]. However, the limitations of single-center data and retrospective design highlight the need for broader, prospective studies incorporating clinical diagnosis and follow-up data.

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The 30% ANA positivity rate in our population warrants consideration in the context of global variations in autoimmune prevalence. Differences in genetic background, environmental exposures, and healthcare access may contribute to regional variations in autoimmune serology [4,5]. Future studies investigating specific environmental triggers and genetic markers in our population would provide valuable insights into these observed prevalence patterns.

Conclusion

Our findings establish important baseline data for ANA prevalence and patterns in the Benghazi population. The results highlight the significant burden of autoimmune serology in our region and underscore the importance of appropriate ANA testing and interpretation in clinical practice. The demographic and pattern distributions align with established autoimmune disease epidemiology while providing population-specific insights that can guide diagnostic approaches and resource allocation in our healthcare system.

Conflict of interest. Nil

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