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Assessment of Antinuclear Antibody (ANA) Levels in Patients from Al-Bayda, Libya

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تقييم مستويات الأجسام المضادة للنواة (ANA) لدى المرضى في مدينة البيضاء، ليبيا

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Abstract: This cross-sectional study aimed to determine the prevalence and distribution of antinuclear antibody (ANA) levels among patients referred for laboratory testing at a specialized medical laboratory in Al-Bayda, Libya, during 2024-2025. A retrospective analysis of 244 patient records was conducted, examining age, sex, test year, and ANA results (categorized as normal or elevated). The findings revealed that the overwhelming majority of tested individuals (92.2%) exhibited ANA levels within normal reference ranges, while only a small subset (7.8%) showed elevated levels. The study population demonstrated a mean age of 42.3 years with a notable female predominance (71.7%). Among the 19 positive cases, females showed higher positivity rates (9.1%) compared to males (4.3%), and individuals aged ≥40 years constituted 63% of positive results. However, statistical analysis indicated no significant associations between ANA positivity and sex ($\chi^2 = 2.15$, $p = 0.14$) or age ($t = 1.32$, $p = 0.19$). The observed ANA positivity rate (7.8%) was notably lower than rates reported from other Libyan cities (9-11%) and international benchmarks. These findings highlight the need for expanded multicenter studies across Libya to better understand regional variations in autoimmune disease prevalence and improve diagnostic capabilities.

Keywords:
Antinuclear Antibody (ANA) ،Autoimmune Diseases ،Prevalence، Libya ،Indirect Immunofluorescence Assay (IFA) ،Demographic Factors، Seroprevalence.

الملخص:

هدفت هذه الدراسة المقطعية إلى تحديد مدى انتشار وتوزيع مستويات الأجسام المضادة للنواة (ANA) بين المرضى المُحالين للفحص المختبري في مختبر طبي متخصص في البيضاء، ليبيا، خلال عامي 2024–2025. تم إجراء تحليل بأثر رجعي لسجلات 244 مريضاً، وتم فحص العمر والجنس وسنة الفحص ونتائج ANA (والتي تم تصنيفها إلى طبيعية أو مرتفعة). كشفت النتائج أن الغالبية العظمى من الأفراد الذين تم فحصهم (92.2%) أظهروا مستويات ANA ضمن النطاقات المرجعية الطبيعية، بينما أظهرت مجموعة صغيرة فقط (7.8%) مستويات مرتفعة. أظهر مجتمع الدراسة متوسط عمر 42.3 سنة مع هيمنة ملحوظة للإناث (71.7%). من بين الحالات الإيجابية البالغ عددها 19 حالة، أظهرت الإناث معدلات إيجابية أعلى (9.1%) مقارنة بالذكور (4.3%)، وشكل الأفراد الذين تبلغ أعمارهم 40 سنة فما فوق 63% من النتائج الإيجابية. ومع ذلك، أشار التحليل الإحصائي إلى عدم وجود علاقات ذات دلالة إحصائية بين إيجابية ANA والجنس ($\chi^2 = 2.15$, $p = 0.14$) أو العمر ($t = 1.32$, $p = 0.19$). كان معدل إيجابية ANA الملاحظ (7.8%) أقل بشكل ملحوظ من المعدلات المبلغ عنها من مدن ليبية أخرى (11–9%) والمعايير الدولية. تسلط هذه النتائج الضوء على الحاجة إلى دراسات موسعة متعددة المراكز عبر ليبيا لفهم أفضل للتباينات الإقليمية في انتشار أمراض المناعة الذاتية وتحسين القدرات التشخيصية.

الكلمات المفتاحية: الأجسام المضادة للنواة (ANA)، أمراض المناعة الذاتية، الانتشار، ليبيا، مقايسة المناعة غير المباشرة (IFA)، العوامل الديموغرافية، الانتشار المصلي.

1. Introduction

Antinuclear antibodies (ANA) are key immunological markers that commonly appear in autoimmune disorders such as systemic lupus erythematosus and other connective tissue diseases. The presence of ANA is often used as a preliminary diagnostic indicator in clinical settings. Detecting abnormal ANA levels can offer insight into a patient's immune activity and potential underlying conditions (Tan *et al*., 1997). Given the limited local data in Libya, this study seeks to contribute to the understanding of ANA prevalence among a general patient population in Al-Bayda. Antinuclear antibodies serve as important diagnostic markers in the early identification of autoimmune diseases. Their detection is especially relevant in diagnosing lupus and mixed connective tissue disorders (Agmon-Levin et al., 2014). Studies conducted in various regions have demonstrated a higher prevalence of ANA in females compared to males, and an increasing rate among individuals over 40 years of age (Rider *et al*., 2008). A large-scale U.S. study (NHANES) reported ANA positivity in around 13.8% of the general population (Satoh *et al*., 2012), highlighting the necessity of localized studies to capture region-specific trends. However, no comprehensive data currently exists for Libyan populations, which underlines the value of the present study. The primary objective of this study was to examine the prevalence of normal and elevated ANA levels among patients attending a medical diagnostic laboratory in Al-Bayda. It also aimed to evaluate demographic variables (age and sex) and their relationship with ANA results.

2. Materials and Methods

A descriptive cross-sectional study was conducted in Al-Bayda city at a specialized Al-Bayda laboratory during the years 2024 and 2025. The data involved retrospective

review of laboratory records from patients tested for ANA levels. A total of 244 patient records were included. Information regarding age, sex, test year, ANA result values, and diagnostic classifications (normal or high) was retrieved and analyzed.

2.1 Sample Collection and ANA Test Procedure

2.1.1 Sample Collection

2.1.1.1 Specimen Type: Peripheral venous blood.

2.1.1.2 Collection Tube: Blood is drawn using a sterile disposable syringe or a vacutainer system into a **plain red-top tube** (without anticoagulant).

2.1.1.3 Volume Required: Generally, 3 to 5 mL of whole blood is collected.

2.1.1.4 Handling: The collected blood is left to clot at room temperature, followed by centrifugation to separate the serum.

2.1.1.5 Storage Conditions: Serum should ideally be tested immediately or stored at 2–8°C for up to 48 hours. For longer preservation, the serum must be frozen at -20°C (Bizzaro *et al*., 2020).

2.2 Test Principle

The ANA test identifies autoantibodies directed against nuclear components within human cells. It serves primarily as a screening tool for autoimmune disorders, especially systemic lupus erythematosus (SLE) and other connective tissue diseases (Mahler & Fritzler, 2016).

2.3 Methodology

- The test is commonly performed using the Indirect Immunofluorescence Assay (IFA) on HEp-2 cells, which is recognized as the gold standard technique (Agmon-Levin *et al.*, 2014).
- Some laboratories may also utilize automated immunoassays or ELISA (Enzyme-Linked Immunosorbent Assay) methods as alternatives (Fritzler *et al*., 2012).

2.4 Instrumentation

For this study, the ANA testing was conducted using the EUROIMMUN Analyzer I (manufactured by EUROIMMUN AG, Germany), a semi-automated instrument designed to facilitate indirect immunofluorescence testing (EUROIMMUN AG, 2021).

2.5 Testing Procedure

- Patient serum is diluted, typically at a ratio of 1:100, with phosphate-buffered saline (PBS).
- The diluted serum is applied onto microscope slides coated with fixed HEp-2 cells.
- Slides are incubated at room temperature for about 30 minutes.
- After incubation, slides are washed to eliminate unbound antibodies.
- A fluorescein-conjugated anti-human IgG antibody is then added and incubated.

- Following a second wash, the slides are examined under a fluorescence microscope.

The presence, pattern, and intensity of nuclear fluorescence staining are recorded, with common patterns including homogeneous, speckled, nucleolar, and centromere (Fritzler, 2013).

2.6 Interpretation of Results

2.6.1 Positive Result: The detection of nuclear fluorescence indicates ANA positivity. The specific staining pattern can provide clues regarding certain autoimmune diseases.

2.6.2 Negative Result: The absence of fluorescence suggests no ANA detectable at the tested dilution.

Results are generally reported semi-quantitatively with titers such as 1:100 or 1:320 (Mahler & Fritzler, 2016).

2.7 Statistical analysis

Statistical Analysis of ANA Prevalence and Demographic Associations

3. Results

3.1. Demographic Characteristics and ANA Prevalence

The analysis of 244 patient records revealed a predominantly female population (71.7%) with a mean age of 42.3 ± 13.7 years (range: 18-83 years). The overall prevalence of elevated ANA levels was 7.8% (19/244), while 92.2% (225/244) of patients showed normal ANA levels.

3.2 Gender Distribution and ANA Positivity Rates

Further analysis of ANA positivity by gender revealed that 9.1% (16/175) of female patients showed elevated ANA levels compared to 4.3% (3/69) of male patients. Although females demonstrated a 2.1-fold higher prevalence rate, this difference did not reach statistical significance ($\chi^2 = 2.15$, $p = 0.14$).

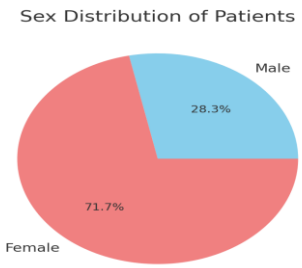

Figure 1: Relationship Between Sex Distribution and ANA Test Positivity

3.3 Annual Distribution of ANA Testing

The distribution of patients by test year showed a higher proportion of tests conducted in 2024 compared to 2025, reflecting potential variations in clinical referrals or testing availability during the study period.

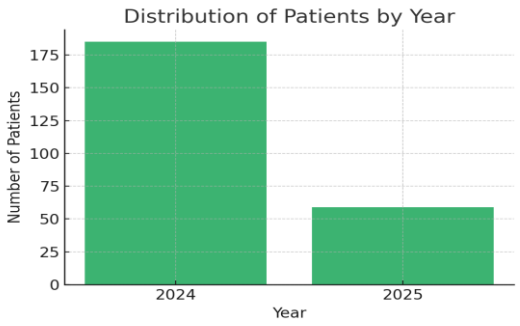

Figure 2: Annual Distribution of ANA Testing

3.4 Demographic and Clinical Characteristics of Patients with Elevated ANA Levels

Age distribution analysis showed that patients aged ≥40 years accounted for 63% (12/19) of positive cases. However, comparative analysis using independent t-test revealed no statistically significant difference in mean age between ANA-positive and ANA-negative groups ($t = 1.32$, $p = 0.19$).

Table. 1 Demographic and Clinical Characteristics of Patients with Elevated ANA Levels

Gender	Age	Result
F	34	3.14
F	**42**	3.03
F	52	4.18
F	83	1.77
F	54	1.88
M	19	1.69
F	31	1.6
F	58	5.22
F	60	3.45
F	30	2.45
F	44	4.89
F	37	4.1
F	38	1.4
F	56	3.6
M	38	7.19
F	65	3.16
F	36	1.83
F	40	2.15
F	**42**	7.25

4. Discussion

In this study, the frequency of ANA positivity among patients tested in Al-Bayda was relatively low (7.8%). Although females and individuals over 40 years showed higher proportions of positive results, statistical testing confirmed that these differences were not significant. This suggests that while demographic trends may point toward certain risk groups, the evidence from this dataset alone is not strong enough to confirm such associations.

The seroprevalence of ANA has been investigated in various regional and international contexts, providing a framework for comparing the findings from Al-Bayda. Within Libya, studies from major urban centers have reported consistently higher rates. For instance, research in Benghazi identified an ANA positivity rate of 9-11% among patients with suspected autoimmune diseases, confirming a higher prevalence in females (Elfituri *et al*., 2021). Similarly, a study in Tripoli focusing on rheumatologic disorders found a comparable positivity rate of 9-11%, reinforcing the role of ANA as a key diagnostic marker in the Libyan population (Abushufa *et al*., 2022).

Beyond Libya, studies from neighboring North African countries report even higher seroprevalence. In Tunisia, a prevalence of approximately 12% was documented among patients tested for autoimmune conditions (Ben Ayed et al., 2018), while an Egyptian cohort reported rates ranging from 10% to 15% (El-Sherbiny *et al*., 2019). Globally, large-scale epidemiological studies provide a broader benchmark. The U.S. NHANES study reported an ANA prevalence of 13.8% in the general population, highlighting a significant baseline level of autoimmunity that is considerably higher than what is observed in the current study (Satoh *et al.*, 2012). Collectively, this body of literature establishes a clear gradient of ANA prevalence, against which the lower rate found in Al-Bayda can be contrasted, suggesting potential regional variations within Libya itself.

The observed 7.8%prevalence of ANA positivity establishes an important regional baseline for autoimmune serology in eastern Libya. While numerical trends toward higher prevalence in females and older individuals align with global patterns, the lack of statistical significance likely reflects the limited sample size of positive cases. These findings emphasize the necessity for larger multicenter studies to achieve sufficient statistical power for robust demographic analysis and to establish comprehensive national reference data for autoimmune markers in Libya.

4.1 Conclusion

Most patients in this study had ANA levels within the reference range, with only a small proportion testing positive. Although women and older patients appeared to have higher positivity, these findings were not statistically significant. Compared with regional and international reports, the observed prevalence in Al-Bayda was somewhat lower.

To improve understanding of autoimmune conditions in Libya, future research should involve larger, multi-center populations and apply additional diagnostic markers such

as anti-dsDNA and ENA profiles. Broader surveillance and improved laboratory capacity will be crucial for strengthening early detection and patient care.

4.2 Recommendations

1. Promote early screening of ANA in patients with clinical indicators of autoimmune conditions.
2. Encourage health institutions to educate physicians about autoimmune diagnostics.
3. Integrate confirmatory testing, such as anti-dsDNA and ENA profiles, for individuals with positive ANA findings.
4. Expand regional studies to include other parts of Libya for more representative data.

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