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Diagnostic Correlation of Antinuclear Antibodies Detected by ELISA and Indirect Immunofluorescence in Patients Attending a Private Laboratory in Ciudad Bolívar, Venezuela

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Abstract

Background and Objectives: Antinuclear antibodies (ANA) are immunoglobulins directed against nuclear and cytoplasmic components of autologous cells, reflecting the loss of immunological tolerance and having a multifactorial origin. This study relates antinuclear antibodies by ELISA and indirect immunofluorescence (IFA) in patients attended at Ciudad Bolívar, Venezuela 2022. **Patients and Methods**: A descriptive and cross-sectional study was conducted to relate antinuclear antibodies by ELISA and indirect immunofluorescence (IFA) in patients attended at Ciudad Bolívar, Venezuela 2022. The population consisted of 70 patients of both genders. **Results***:* Of the population studied, 29 patients tested positive by indirect immunofluorescence (IFA) (41.43%) and 7 patients by the ELISA method (10.00%). Regarding gender, it was observed that the total population that tested positive was female (IFA: 41.43%. ELISA: 10%). According to Fisher's test (equal to 0.04), this result was significant ($p < 0.05$). Of the 29 patients who tested positive by (IFA), 13 patients (18.58%) were in the age range of 25 - 45 years. Similarly, through the ELISA method, of the 7 patients (10.00%) who tested positive, 4 (5.71%) were in the age range of 25 - 45 years, these results according to Fisher's exact test were not significant. *Conclusions:* After describing the positive and negative results by (IFA) and ELISA methods, it is observed that 58.57% of the patients had negative results in both methods. 31.43% had a positive result by the IFA method and negative by ELISA, and 10.00% were positive by both (IFA) and ELISA. It is noteworthy that patients positive by ELISA had an antibody titer greater than 1:160 in the

IFA test.

Subject Areas

Immunology, Infectious Diseases

Keywords

Antinuclear Antibodies, Indirect Immunofluorescence (IFA), ELISA, Rheumatology, LES

1. Introduction

Antinuclear antibodies (ANA) are immunoglobulins directed against nuclear and cytoplasmic components of autologous cells, which reflect the loss of immunological tolerance, giving rise to autoimmunity. Their production depends on genetic makeup, environment, and hormonal changes, among others [1].

Positive antinuclear antibodies detected by IIF should be reported based on the pattern and dilution titer. Patterns with titers equal to or greater than 1:160 are considered truly positive [2]. The next recommended step is the characterization of specificity (reactivity against extractable nuclear antigens (ENA), dsDNA, Smith, among others). This is useful for the diagnosis and monitoring of patients with autoimmune diseases. Therefore, their detection should be performed in an orderly and reasonable manner using guidelines or strategies focused on the proper use and interpretation of autoantibodies [3] [4].

The IIF antibody technique is the gold standard for detecting antinuclear antibodies, as it demonstrates binding to specific intracellular structures within cells, resulting in a series of staining patterns that are generally classified according to the cellular components recognized and the degree of binding, as reflected in fluorescence intensity or titer. Antinuclear antibody patterns can guide useful confirmatory testing to elucidate a specific clinical diagnosis or prognosis [5] [6]. However, routine use of the antinuclear antibody test (IFA) as a comprehensive screening test is hampered by its labor intensity, subjectivity, and limited diagnostic specificity [7].

The main advantages of the IFA (antinuclear antibody test) are the wide range of detectable antibodies, high sensitivity, and the ability to simultaneously determine reactivity, titer, and immunofluorescence pattern. However, visual IFA has some substantial disadvantages. These assays require expert reading, which is time-consuming and labor-intensive. Furthermore, correct pattern recognition depends on the individual qualifications and experience of the investigator [8].

Over the past two decades, antinuclear antibody testing using the ELISA technique has been introduced with the goal of saving the time and effort required for ANA-IFA and attempting to improve the performance of antinuclear antibody testing. However, previous reports have shown that solid-phase assays still have

lower sensitivity compared to IFA [9].

ELISA is one of the most common and versatile techniques available in clinical laboratories. Commercially available ELISAs for antinuclear antibodies have various configurations of antigens and secondary antibodies. In the case of ELISA, the solid phase, a plate, is coated with a mixture of relevant autoantigens, either purified or recombinant, specific for the autoantibodies that may be present in serum samples. If autoantibodies are present in a patient's serum, they adhere to their corresponding autoantigen and are bound by an enzyme-linked detection antibody, generating a colorimetric reaction that can be measured in a spectrophotometer [10].

The ELISA technique, although widely used, has shown sensitivity and specificity issues, leading to erroneous results in the identification of antinuclear antibodies. This phenomenon not only affects the quality of medical care but can also result in late or incorrect diagnoses, negatively impacting patient health. By correlating ELISA and indirect immunofluorescence (IIF) methods, this study seeks to establish a more reliable diagnostic approach, allowing healthcare professionals to make more informed and accurate decisions. Furthermore, this work will contribute to the development of a local database that can be used for future research, thereby improving the quality of diagnosis in the country and, potentially, in other regions with similar contexts.

2. Materials and Methods

2.1. Patients and Study Design

A descriptive, cross-sectional study was conducted that included patients who visited a private laboratory in Ciudad Bolívar, requesting the determination of antinuclear antibodies using the indirect immunofluorescence (IFI) method between July and September 2022. The inclusion criteria for the selection of participants were: 1) patients with a request for antinuclear antibody analysis; 2) patients of both genders; 3) patients of legal age; 4) patients with a clinical diagnosis of autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, or Sjögren's syndrome; and 5) patients who had not received immunosuppressive treatment in the six months prior to the study. Exclusion criteria were established that included: 1) patients with active infectious diseases, including HIV, hepatitis, and any other infection that could compromise the immune system; 2) patients who had received blood or blood product transfusions in the previous three months; 3) patients with primary or secondary immunodeficiencies; and 4) patients who were unwilling to participate or who decided to withdraw their consent at any point during the study. These criteria were essential to ensure the validity and integrity of the results obtained.

2.2. Ethical Aspects and Informed Consent

The study was conducted in accordance with the ethical principles for medical

research in human subjects of the Declaration of Helsinki [11], with the corresponding signed informed consent of all patients.

2.3. Instruments

Data such as first name, last name, age, diagnostic impression, and laboratory results were collected using a specially designed instrument. It also included clinical and epidemiological data, including the following diagnostic impressions: systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and systemic sclerosis.

2.4. Procedure

The purpose and scope of the study were explained to the patients, and their informed consent was obtained. The tubes were previously identified with the patient's form number and first and last names. Under the supervision of the bioanalyst in charge of the area, the patient serum samples were processed using the AESKULIDES antinuclear antibody assay (IFI) and the ORGENTEC ELISA method.

2.5. Statistical Analysis

The results were presented using frequency distribution tables and contingency tables using absolute and relative values. The databases were created using Microsoft Excel® 2010 and the IBM SPSS Windows version 23 statistical package for analysis. Descriptive statistics and clinical percentages of patients were used for statistical analysis. To compare the variables, Fisher's exact test (bilateral) was applied because these are qualitative variables. The confidence margin will be taken as results greater than 95% or when $p < 0.05$, which were considered as statistically significant results.

3. Results

3.1. Positive Antinuclear Antibodies

It is evident that, of 70 patients, 29 tested positive by the IFA method (41.43%). On the other hand, only 7 patients tested positive by the ELISA method (10.00%) (See Table 1).

Table 1. Positive antinuclear antibodies by the indirect immunofluorescence method and the ELISA method. Private laboratory, Ciudad Bolívar, Bolívar State. July-September 2022.

	Positive		Negative		Total	
	n	%	n	%	n	%
Indirect immunofluorescence	29	41.43	41	58.57	70	100.00
ELISA	7	10	63	90	70	100.00

Source: Researcher data, November 2022.

3.2. Antinuclear Antibodies by IFA by Gender and Age

It was found that, among female participants, 41.43% (n = 29) of the participants had positive results, while 50.00% (n = 35) had negative results. In contrast, all male patients included in the study tested negative, 8.57% (n = 6). Regarding age distribution, the 25 - 45 age group showed a higher prevalence of both positive and negative results. Specifically, 18.58% (n = 13) positive cases and 32.85% (n = 23) negative cases were recorded in this age range. In addition, statistically significant differences ($p < 0.05$) were identified in the relationship between gender and antinuclear antibody positivity (See Table 2).

Table 2. Antinuclear antibodies by the indirect immunofluorescence (IIF) method by gender and age. Private laboratory, Ciudad Bolívar, Bolívar State. July-September 2022.

	Antinuclear antibodies by IFA method					
	Positive		Negative		Total	
	n	%	n	%	n	%
Gender						
Female	29	41.43	35	5000	64	91.43
Male	-	-	6	8.57	6	8.57
Subtotal	29	41.43	41	58.57	70	100.00
Age (years)						
Apr-24	6	8.57	7	10.00	13	18.57
25 - 45	13	18.58	23	32.85	36	51.43
46 - 66	9	12.85	9	12.86	18	25.71
67 - 87	1	1.43	2	2.86	3	4.29
Subtotal	29	41.43	41	58.57	70	100.00

Source: Researcher data, November 2022. Fisher's exact test (IFI/Gender) = 0.03792 df = 1 ($p < 0.05$) Significant. Cramer's V = 0.258. Fisher's exact test (IFI/Age) = 0.7517 df = 3 ($p > 0.05$) Not significant.

3.3. Antinuclear Antibodies by ELISA by Gender and Age

Numbers of positive cases (n = 7) were identified, 10.00% of whom were female, while 81.43% (n = 57) of this same gender had negative results. The six male patients all had negative results (8.57%). Regarding age, the 25 - 45 age group predominated, both in positive results (n = 4) (5.71%) and in negative results (n = 32) (45.71%). No statistically significant differences ($p > 0.05$) were observed between the variables studied (See Table 3).

3.4. Positive and Negative Results for Antinuclear Antibodies Using the ELISA and IFA Methods (Titer and Pattern)

In the present study, 58.57% of patients (n = 41) were observed to have negative results for antinuclear antibodies using both methods. Among patients who showed negative results using the ELISA test but positive results using the IFA test, titers

of 1:160 dilutions were recorded, with the following fluorescence patterns: 27.14% (n = 19) showed a homogeneous pattern, 1.43% (n = 1) showed a fine granular nuclear pattern, and 2.86% (n = 2) showed a coarse granular nuclear pattern. In contrast, patients who tested positive in both methods exhibited a homogeneous pattern, with dilution titers of 1:640 in 4.29% (n = 3) of cases and 1:320 in 5.71% (n = 4) respectively, when calculating Cohen's Kappa coefficient, a low agreement between the methods was obtained (See Table 4).

Table 3. Antinuclear antibodies by ELISA method according to gender and age. Private Laboratory, Ciudad Bolívar, Bolívar State. July-September 2022.

	Antinuclear antibodies by ELISA method					
	Positive		Negative		Total	
	n	%	n	%	n	%
Gender						
Female	7	10.00	57	81.43	64	91.43
Male	-	-	6	8.57	6	8.57
Subtotal	7	10	63	90.00	70	100.00
Age (years)						
Apr-24	2	2.86	11	15.71	13	18.57
25 - 45	4	5.71	32	45.71	36	51.43
46 - 66	1	1.43	17	24.29	18	25.71
67 - 87	-	-	3	4.29	3	4.29
Subtotal	7	10.00	63	90.00	70	100.00

Source: Researcher data, November 2022. Fisher's exact test (ELISA/Gender) = 1 df = 1 ($p > 0.05$) Not significant. Fisher's exact test (ELISA/Age) = 0.7576 df = 3 ($p > 0.05$) Not significant.

Table 4. Description of the positive and negative results of antinuclear antibodies by the ELISA method and indirect immunofluorescence (titer and pattern) in samples from patients treated at the private laboratory, Ciudad Bolívar, Bolívar State. July-September 2022.

ANA ELISA	ANA IFA	Code pattern	Pattern Immunofluorescence	Titer	n	%
Negative	Negative	AC-0	Negativo	≤1:80	41	58.57
Negative	Positive	AC-1	Homogeneous	0.152778	19	27.14
Negative	Positive	AC-4	Fine granular nuclear	0.152778	1	1.43
Negative	Positive	AC-5	Coarse granular nuclear	0.152778	2	2.86
Positive	Positive	AC-1	Homogeneous	0.486111	3	4.29
Positive	Positive	AC-1	Homogeneous	0.263889	4	5.71
Total					70	100

Source: Researcher data, November 2022. Cohen's Kappa coefficient: $\kappa = 0.27$ (95% CI: 0.02 - 0.52).

4. Discussion

The positivity rate for antinuclear antibodies using the IFA method was 41.43% of the population, while the positivity rate for ELISA was 10.00%. This shows that the IFA method has a higher positivity rate than the ELISA method. These results were similar to those of a study conducted by Shovman [12], which compared the IFA, ELISA, and BioPlex 2200 ANA Screen methods. The frequency of positive results was highest (15.5%) with IFA, compared to 5.5% with the ELISA antinuclear antibody kit and 7.5% with the BioPlex 2200 ANA Screen. Similarly, these findings are inconsistent with those obtained by Berkem *et al.* [13], who concluded that the ELISA technique can be used to confirm results obtained using IFA. Currently, the HEp-2 cell line is the preferred substrate for ANA detection and quantification because cell nuclei are large and express a wide variety of antigens associated with systemic autoimmune rheumatic diseases.

These results suggest that the IIF could be the method of choice for initial ANA detection, especially in populations with a high clinical suspicion of autoimmune diseases, as reported by Rodsaward *et al.* [14] in their research paper "Clinical significance of antinuclear antibodies and specific autoantibodies in patients with juvenile and adult systemic lupus erythematosus." Furthermore, it is important to consider that variability in results may be influenced by factors such as laboratory technique, operator experience, and the quality of the reagents used. Therefore, additional studies comparing these methods in diverse populations and clinical conditions are recommended to validate these findings and establish clearer guidelines for the selection of diagnostic methods for detection.

After comparing the results of antinuclear antibodies using the IFA method, the results obtained were classified according to age and sex. It was observed that women between 25 and 45 years of age represented the group with the highest positivity during the conduct of this study. Antinuclear antibodies are more frequent in women of reproductive age [15] [16], sex hormones (especially estrogens) play a significant role in the development of autoimmune diseases and predispose women to a more frequent onset of these diseases. These results differ from the study conducted by Dinse, in this study, a higher prevalence was observed in males, and the age group with the highest positivity was in the population over 50 years of age. However, there are similarities between the results obtained in the present investigation and those obtained by Gomez *et al.* [17], in which they observed antibody positivity in lupus patients of reproductive age. In addition to hormonal and age factors, genetics and environment also play a role in the predisposition to developing ANA.

Antinuclear antibody positivity was also assessed using the ELISA method, and the results were consistent with the research conducted by Edwards [18], suggesting consistency in the scientific evidence on the relationship between antinuclear antibodies and certain risk factors. The data revealed that 10.9% of men (73 individuals) and 12.2% of women (81 individuals) were positive for antinuclear antibodies. Furthermore, the highest prevalence of positivity was observed in the 25 -

45 age groups. Of the seven patients who tested positive for ANA using ELISA, 100% of the patients had suspected systemic rheumatic diseases, primarily SLE. Ten percent had positive results for both ELISA and IIF, which were associated with homogeneous immunofluorescence patterns with titers greater than 1:160 dilutions. Furthermore, 27.14% were positive only by IFA and negative by ELISA, with a homogeneous pattern notable, with titers up to 1:160 [19]. No cases were observed with positive ELISA results but negative results by IFA, nor were positive ELISA results observed in patients with positive patterns other than homogeneous, which could suggest that the antigens present in these patterns are absent or degraded in solid-phase recombinant immunoassays, making the test less sensitive [20] [21].

The results obtained were consistent with the study conducted by Choi [22]-[24], who studied 805 patients with SLE over a 5-year period. At the time of recruitment, antinuclear antibody positivity (≥1:80) was 96.1% by IFA1 (mean titer 1:1280), 98.3% by IFA2 (1:2560), and 96.6% by ELISA. At least one antinuclear antibody assay was positive for 99.6% of patients at enrollment. At year 5, antinuclear antibody positivity by IFA (IFI1 95.2%; IFA2 98.9%) remained high, while there was a decrease in ELISA positivity (91.3%, $p < 0.001$). It can be seen that, in both studies, the ELISA method only yielded a positive result if the patient had a high antibody titer, and the IFA was more sensitive for patients with lower antibody titers [25].

In this study, a sample size of 70 patients who met the inclusion and exclusion criteria was selected to preliminarily understand the relationship between antinuclear antibodies detected by ELISA and indirect immunofluorescence (IFA) in a specific population in Ciudad Bolívar, Venezuela. While a larger sample size could improve the statistical power and generalizability of the findings, the current sample is adequate for a descriptive, cross-sectional analysis. The observed positivity rates (41.43% for IFA and 10.00% for ELISA) suggest a significant difference in the detection of antinuclear antibodies, especially in women, as indicated by the Fisher test p-value of 0.04. However, the small number of patients with positive ELISA results limits the possibility of drawing firm conclusions about the correlation between both methods. Therefore, while a formal power analysis was not performed, the findings provide valuable information, although with the understanding that further studies with larger samples are needed to obtain more definitive conclusions.

This study has certain limitations that offer opportunities for future research. The single-center study with a small sample size opens the door to multicenter studies with larger samples that could validate and expand the current findings. The lack of clinical follow-up also suggests the need for longitudinal research to assess the evolution of outcomes over time.

5. Conclusion

The evaluation of antinuclear antibody positivity in the patients tested reveals sig-

nificant findings that merit attention in the context of research and healthcare. The notable discrepancy between the positivity of the IIF method (41.43%) and the ELISA method (10.00%) suggests that the IIF technique is more sensitive and indicated for detecting the presence of these antibodies, especially in young women aged 25 to 45 years, who constitute the predominant group in the study. Furthermore, the finding that 31.43% of patients had positive IIF and negative ELISA results indicates the possibility that the ELISA method may not capture all positive cases, raising questions about its applicability in the Venezuelan population. The observed correlation between elevated IIF titers and positivity suggests that a more detailed approach to result interpretation may be necessary to improve the diagnosis and management of autoimmune diseases.

6. Recommendations

Establish clear protocols for the determination of antinuclear antibodies, prioritizing IFN-γ over enzyme-linked assays, and foster communication between primary care physicians, rheumatologists, and clinical laboratories to improve test referrals and interpretation. Conduct a cost-benefit analysis for the acquisition of immunofluorescence equipment. Finally, promote studies that validate the efficacy of IFN-γ.

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Conflicts of Interest

The authors declare no conflicts of interest.

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