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COMPARATIVE ANALYSIS OF VARIOUS DIAGNOSTIC MODALITIES (ANA BY ELISA AND IFA) FOR AUTOIMMUNE DISORDERS IN A TERTIARY CARE CENTRE IN WESTERN RAJASTHAN

Immunology

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ABSTRACT

Background : Anti-nuclear antibodies (ANAs) are specific antibodies directed against a variety of nuclear antigens which constitute the basis for diagnosis and treatment of rheumatic diseases like Systemic Lupus Erythematosus (SLE), Juvenile Idiopathic Arthritis, Systemic sclerosis, Poly Arteritis Nodosa etc. ANA test is 95-100% sensitive for the diagnosis of SLE and is commonly diagnosed by indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). **Methods:** A 3-month prospective study was conducted in Immunology Lab, Dr S.N. Medical College, Jodhpur wherein all samples with history and suspicion of autoimmune disorders were included in study. A total of 762 samples suspected of autoimmune disorder were processed using ELISA kit (CALBIOTECH) according to the kit .Amongst these,75 positive ELISA and 25 random negative samples were further subjected to IFA testing using IFA kit (Immuno Concepts) and the results were then comparedand evaluated. **Results:** Out of 75 positive ELISA samples, 18 (24%) were males and 57 (76%) females and in IFA out of 79 positive samples 16 (20.25%) were males and 63 (79.74%) females. The majority of positive cases by both ELISA and IFA were found in the (31-40) age group, followed by (21-30). The most frequently detected pattern by IFA was speckled (39.00%), followed by speckled + SSA/RO and homogenous + speckled (both 10.00%). Other patterns included homogenous (8.00%), SSA/RO (9.00%) and centromere (3.00%). Negative results were seen in (21.00%) of cases. **Conclusion:** In this study, both ELISA and indirect immunofluorescence assay (IFA) demonstrated effectiveness in screening for antinuclear antibodies (ANA) associated with autoimmune disorders. While IFA remains indispensable for comprehensive ANA analysis, ELISA serves as a valuable complementary tool, especially in large-scale population screenings.

KEYWORDS

Autoimmune diseases, ANA, ELISA, IFA

INTRODUCTION

Autoimmune disorders are conditions in which the immune system is unable to differentiate between healthy tissue and potentially harmful antigens which can be explained through the concept of molecular mimicry. Autoantibodies can induce damage to the body by binding to self-tissues, activating the complement cascade and inducing lysis. Due to the chronic nature of most autoimmune diseases, autoantibodies appear long before clinical symptoms; in fact, the risk of developing an autoimmune disease rises from about 10% if one autoantibody is present to around 60-80% if three autoantibodies are present for a particular autoimmune disease[1]. Nearly 4% of the worldwide population is affected by more than 80 different types of ADs, which encompass both systemic and tissue-specific ailments including, systemic lupus erythematosus, insulin-dependent diabetes mellitus, autoimmune hepatitis, rheumatoid arthritis, thyroiditis, Crohn's disease, psoriasis, multiple sclerosis, etc.; with a higher prevalence rate in women in comparison to men.

Anti-nuclear antibodies (ANAs) are specific antibodies directed against a variety of nuclear antigens those have been detected in the serum of patients with many rheumatic and non-rheumatic diseases. These antibodies are involved not only in the disease pathogenesis, but they also constitute the basis for diagnosis and treatment of childhood rheumatic diseases like Systemic Lupus Erythematosus (SLE), Juvenile Idiopathic Arthritis (JIA), Systemic sclerosis (SSc), Poly Arteritis Nodosa (PAN) etc. The ANA test is 95-100% sensitive for the diagnosis of SLE. The many known subtypes of ANA can be grouped in 2 main categories, namely, autoantibodies against DNA and histones, and autoantibodies against extractable nuclear antigens (ENAs). The first group includes antibodies against dsDNA (double-stranded DNA) and against histones. Antibodies against dsDNA in high titters are considered to confirm SLE diagnosis. Antibodies against histones indicate drug-induced SLE.

The second group includes antibodies against Smith antigen (SM), which are specific for SLE, anti–SS-A/Ro, and anti–SS-B/La (these 2 types are specific for Sjögren syndrome, subacute cutaneous SLE, and neonatal lupus syndrome), anticentromere (considered specific for limited cutaneous SS), Jo-1 (considered specific for PM), anti–U3-RNP, and Scl-70 (these final 2 are considered specific for SS).

Despite that there are many tests available for detection of ANAs, the ones most commonly used in daily practice are the indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA).

Aims & Objectives

1. To evaluate and compare the sensitivity, specificity and diagnostic accuracy of ELISA and IFA methods for detecting antinuclear antibodies (ANA) in patients with suspected autoimmune disorder.
2. To determine the concordance between ELISA and IFA detection.

MATERIALS & METHODS

A 3-month duration prospective study was conducted in Microbiology Department, Immunology lab, Dr S.N Medical College Jodhpur in which all the samples received in Microbiology lab from patients suspected of SLE or other autoimmune disorder or considered at risk were processed using ELISA and IFA methods for detecting antinuclear antibodies (ANA). The patients with established diagnosis of autoimmune disorder or under treatment were excluded from study.

A total of 762 samples suspected of autoimmune disorder were received during our study period and were processed using ELISA kit (CALBIOTECH) according to the kit protocols and cutoff determined as <0.9 (Negative),0.9-1.1 (Borderline positive) and >1.1 Detectable ANA IgG by ELISA(Positive).

75 positive samples obtained from ELISA and 25 randomly selected negative samples were then subjected to IFA testing in which IFA slide was prepared using IFA kit (Immuno Concepts) with 1:80 sample dilution and observed under a fluorescent microscope using a blue filter (wavelength 450–550 nm) in a dark room and various patterns like homogenous, speckled, SSA/Ro

The co-relation between the two diagnostic modalities was then established.

RESULTS:

Out of 762 blood samples tested for antinuclear antibodies (ANA) using the ELISA method, 75 samples (9.8%) yielded positive results.

To further evaluate the accuracy and concordance between methods, indirect immunofluorescence assay (IFA) was subsequently performed on all 75 ELISA-positive samples, along with 25 randomly selected ELISA-negative samples, totalling to 100 samples. From 75 ELISA positive samples, 18 (24%) were males and 57 (76%) were females, IFA results revealed 79 samples to be ANA-positive and 21 to be ANA-negative, indicating a notable number of additional positives not initially detected by ELISA. out of 79 positive IFA samples 16 (20.25%) are males and 63 (79.74%) are females.

The most frequently detected pattern by IFA was speckled (39.00%), followed by speckled + SSA/RO and homogenous + speckled (both 10.00) **(Table 1 & Figure 1)**

Table 1 : Distribution of samples with various Fluorescent pattern detected

S.No.	Detected pattern	Pattern type	Percentage
1.	Speckled	39	39.00%
2.	SSA/RO	9	9.00%
3.	Speckled+ SSA/RO	10	10.00%
4.	Homogenous	8	8.00%
5.	Homogenous+ speckled	10	10.00%
6.	Centromere	3	3.00%
7.	Negative	21	21.00%

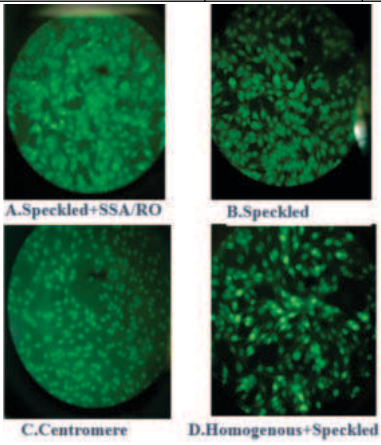

Figure 1 : Fluorescent patterns detected

The majority of positive cases by both ELISA and IFA were found in the (31-40) age group, followed by (21-30). Fewer cases were seen in the younger (1-20) and older (>60) age group. **(Table 2)**

Table 2 : Comparative Analysis Of ELISA And IFA Positivity Among Different Age Groups Of Diagnosed Patients

S.no.	Age group	ELISA	IFA
1.	1-10.	2	1
2.	11-20.	8	7
3.	21-30	15	15
4.	31-40	27	29
5.	41-50	12	13
6.	51-60	8	9
7.	>60	3	5
8.	Total	75	79

DISCUSSION :

In our study, ELISA testing of 762 samples revealed 75 positive (9.8%), 12 borderline (1.6%), and 675 negative (88.6%) results. These findings align with previous reports, including Li et al. (2011)[2].

A notable gender disparity was observed, with ANA positivity significantly higher among females (76%, 57 out of 75) than males (24%, 18 out of 75). This trend was similarly reflected in IFA results, with 79.74% of IFA-positive samples being female. Such a female predominance is well-documented in autoimmune literature, as highlighted by Ashish Tyade et al. (2018)[3] and Ranganathan et al. (2020)[4], and is likely attributed to a combination of hormonal, genetic, and immunological factors that contribute to heightened autoimmune susceptibility in women.

Age-wise distribution indicated that ANA positivity was most frequent in individuals aged 31–40 years, with 27 ELISA-positive and 29 IFA-positive cases, followed by the 21–30 and 41–50 age groups. This pattern is consistent with Jain et al. (2021) who also reported higher ANA prevalence in young to middle-aged adults. Lower detection rates were observed in both paediatric (1–10 years) and older (>60 years) populations, reinforcing the concept that autoimmune diseases predominantly manifest in early to mid-adulthood.

In a subset of 100 samples tested with both ELISA and IFA, ELISA detected 75 positives and 25 negatives, whereas IFA identified 79 positives and 21 negatives, reaffirming the slightly higher sensitivity of IFA. This observation aligns with findings by Choi et al. (2022)[5], Khalifah et al. (2022)[6], and Karumanchi et al. (2018)[7], who also reported the superior diagnostic accuracy of IFA over ELISA. Nevertheless, the practicality of ELISA in initial screening remains invaluable, especially for processing large sample volumes efficiently.
In this study, both ELISA and indirect immunofluorescence assay (IFA) demonstrated effectiveness in screening for antinuclear antibodies (ANA) associated with autoimmune disorders. However, IFA on HEp-2 cells continues to be recognized as the gold standard for ANA detection due to its superior sensitivity, broader autoantibody detection range, and its ability to reveal distinct nuclear and cytoplasmic staining patterns that aid in disease-specific interpretation.

Although ELISA exhibited slightly lower sensitivity—failing to detect four positive cases identified by IFA—it offers notable practical benefits, such as automation compatibility, cost-effectiveness, and ease of result interpretation. These features make ELISA particularly suitable for high-throughput and routine diagnostic laboratories. Thus, while IFA remains indispensable for comprehensive ANA analysis, ELISA serves as a valuable complementary tool, especially in large-scale population screenings.

CONCLUSION:

The present study underscores the complementary roles of ELISA and IFA in the screening and confirmation of antinuclear antibodies (ANA), which are pivotal in the diagnosis of autoimmune disorders. While ELISA proved valuable for high-throughput preliminary screening due to its simplicity, cost-effectiveness, and automation, IFA on HEp-2 cells reaffirmed its position as the gold standard, offering higher sensitivity and the ability to identify specific nuclear and cytoplasmic staining patterns**.**

Limitations Of The Study: Due to financial constraints, the sample size selected for IFA screening was limited.

Relevance Of Study: The study contributes significantly to autoimmune disease diagnostics by bridging methodological, demographic, and clinical aspects, and offers a strong foundation for improving laboratory protocols, informing public health policies, and guiding future research focused on early detection and management of autoimmune disorders.

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Conflict Of Interest: None declared

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