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Compare the effectiveness of ELISA and Immunofluorescence tests for the diagnosis of autoantibodies among Antenatal Mothers

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Abstract

Background: The effectiveness of ELISA (enzyme-linked immunosorbent assay) and immunofluorescence tests for the diagnosis of autoantibodies among antenatal mothers is to detect the presence of autoantibodies in pregnant women. Autoantibodies are antibodies that mistakenly target and attack the body's own tissues and organs, leading to autoimmune diseases. **Aim and objective:** compare the effectiveness of ELISA and immunofluorescence tests for the diagnosis of autoantibodies among antenatal mothers. **Material and method:** Both testing methods were applied to 200 samples. Of these, 100 samples were from the test group, and 100 samples were controls (negative and positive controls). Of the 100 test samples. **Observation and Results:**The study revealed that 70% of the cases yielded a positive result when subjected to the ELISA method, whereas 71% of the cases tested positive when subjected to the IFAT method. The ELISA method yielded a positive result in 5% of individuals in the control group, whereas the IFAT method yielded a positive result in 6% of the same group. **Conclusions:** The findings of this study suggest that the use of indirect immunofluorescence assays (IFA) is more effective than enzyme-linked immunosorbent assays (ELISA) for the purpose of ANA testing.

INTRODUCTION

The effectiveness of ELISA (enzyme-linked immunosorbent assay) and immunofluorescence tests for the diagnosis of autoantibodies among antenatal mothers is to detect the presence of autoantibodies in pregnant women. Autoantibodies are antibodies that mistakenly target and attack the body's own tissues and organs, leading to autoimmune diseases. [1] During pregnancy, the mother's immune system changes to tolerate the developing fetus, and this can sometimes trigger the production of autoantibodies. These autoantibodies can potentially cause complications in pregnancy, such as miscarriage, stillbirth, or preterm labor.

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Therefore, detecting the presence of autoantibodies in pregnant women is important to identify any potential risks to the mother and the developing fetus.

ELISA and immunofluorescence tests are two commonly used laboratory techniques for detecting the presence of autoantibodies. ELISA involves the use of specific antigens and antibodies that bind to each other, and the resulting reaction produces a measurable signal. Immunofluorescence, on the other hand,uses fluorescent dyes to detect the presence of autoantibodies in tissue samples. By using these tests to detect autoantibodies in antenatal mothers, healthcare providers can identify any potential risks to the mother and the developing fetus and take appropriate measures to manage the pregnancy. This can include close monitoring of the mother and the fetus, the administration of medications to manage autoimmune diseases, and timely delivery of the baby if necessary.

Anatomic abnormalities account for 10–15% of recurrent pregnancy losses in the second trimester. There may be congenital or acquired causes. A Mullerian duct fusion or resorption defect can result in congenital anomalies (e.g., unicornuate, bicornuate, septate, or double uterus). Acquired anomalies include intrauterine adhesions, uterine fibroids, endometriosis, and cervical incompetence. [2]

The immunological relationship between mother and fetus is bidirectional, determined by antigen presentation by the fetus and recognition and reaction by the maternal immune system. To maintain a pregnancy, an immune response that is balanced is required, and any imbalance can result in early conception loss.

Autoantibodies are responsible for early pregnancy rejection in 30% of women. The culprits are antinuclear antibodies and antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibodies). These women are more likely to miscarry at lower gestational ages. Placental vascular atherosis, intervillus thrombosis, and decidual vasculopathy with fibrinoid necrosis are the most common causes of fetal loss. Pregnancy complications include miscarriages, intrauterine growth retardation, pre-eclamptic toxaemia, stillbirth, preterm labor, and placental abruption. [3]

The mother's immune system is bombarded with a slew of internal and foreign antigens during pregnancy. Abortions become more common when the mother's immune system is weakened. Autoimmune disorders affect women 6 to 10 times more than men, and they are more likely to manifest during the reproductive years. Lupus, scleroderma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, Sjogren's syndrome, or type 1 diabetes affect one out of every nine women. The general population prevalence of SLE is estimated to be 40:100,000, but this condition may affect 1 in 1000 pregnant women. It is estimated that 10% of all lupus patients are diagnosed during pregnancy or shortly after birth. SLE has a prevalence of 3.2 per 100,000 people in India (Medscape), with an annual incidence of 5–100 per 100,000 people; more than 90% of patients are females of reproductive age.

Autoantibodies in antenatal mothers can be detected using both ELISA and immunofluorescence tests. These tests can aid in the diagnosis of autoimmune disorders such as systemic lupus erythematosus (SLE), which can cause pregnancy

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complications. By detecting autoantibodies early in pregnancy, doctors can better monitor the mother and baby and take appropriate measures to protect the fetus.

Materials and Methods

This was a prospective study conducted in the research laboratory, Dept. of Microbiology,Index Medical College, and RC, Indore. Prior to the test, informed consent from patients was obtained. ANA by IF and ELISA was carried out on 200 blood samples. Out of these samples, 100 were taken from patients with clinical symptoms of autoimmune diseases.

TARGET POPULATION

All pregnant women from the age group 21–40 years with a history of previous unfavorable fetal outcomes who visited outpatient OBG departments at Index Medical College Hospital in Indore were included in this study.

SAMPLE

When conducting research, a sample can be defined as a subset of a larger population that will be studied. (S.K. Sharma) The participants in this study were all pregnant women from the age group of 21–40 years with a history of previous unfavorable fetal outcomes who visited outpatient OBG departments in Index Medical College hospitals in Indore with symptoms.

INCLUSIONCRITERIA:

The inclusion criteria for cases were pregnant women with a history of previous unfavorable fetal outcomes (2 or more spontaneous abortions) and a previous history of stillbirths, intrauterine deaths, or neonatal deaths.

EXCLUSIONCRITERIA:

Malignancy, History of Rh-incompatibility, Acute illness, History of sexually transmitted diseases, History of uterine fibroids, endometriosis, PID, Uterine anomalies: septate uterus, bicornuate uterus

Ethical Clearance

In order to conduct the study involving blood samples from pregnant women, the researchers obtained institutional ethical clearance from Index Medical College Hospital in Indore.

Observation and Results:

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Table 1: Age of the Participants

Demographic Variables	**Cases**		**control**	
Age in Years	No.	%	No.	%
21-25	11	11	09	09
26–30	24	24	26	26
31–35	46	46	44	44
36-40	19	19	21	21
Total	100	100	100	100

Table 1: According to the results of this study, 46% of cases were mostly in the 31–35 year age range. In the participants cases, only 11% were between 21 and 25 years old, respectively. In controls, 44% were mostly in the 31–35-year age range. Of the participants, only 9% of the controls were between 21 and 25 years old.

Table 2: Compare the antinuclear antibody positivity among cases and controls.

	Positive		**Negative**	
	f	%	f	%
Cases	71	71	29	29
Control	06	06	94	94

Table 3: The findings indicate that out of a total of 100 cases, 71% of the samples tested positive for antinuclear antibodies, while 29% tested negative. Among the controls, 6% of the samples tested positive for antinuclear antibodies, while the remaining 94% tested negative.

Table 2: Antinuclear antibody positivity based on gravida status

	Variables	**Antinuclear antibody positivity**			
		Cases(f)	**%**	**Controls(f)**	**%**
Gravida Status	2ndGravida	34	34	06	06
	3rdGravida	20	20	00	00
	4thGravida	17	17	00	00
	Total	71	71	06	06

Table 3: The presented data depicts the prevalence of antinuclear antibody positivity in relation to gravida status among both case and control groups. The study found that 34% of cases involving 2nd gravida, 20% of cases involving 3rd gravida, and 17% of cases involving 4th gravida tested positive. In comparison, only 6% of 2nd gravida controls tested positive, while none of the controls from 3rd or 4th gravida tested positive.

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Table 4: Immunofluorescence analysis of antinuclear antibodies in cases and controls

	IFAT Positive		IFAT Negative		p -Value
	f	%	f	%	p-value 0.001
Cases	71	71	29	29	
Control	06	06	94	94	X2 89.2197
Total	77	38.5	123	61.5	Df-1
					Significant

Table 4: The results suggest that among the 100 cases examined, 71% (71 out of 100) were found to be positive for antinuclear antibody through the use of immunofluorescence, while 29% (29 out of 100) were negative for antinuclear antibody. Out of the entire cohort of 100 women who had multiple pregnancies, six of them, constituting 6% of the sample, yielded positive results for antinuclear antibodies through the use of the indirect immunofluorescence assay technique. The remaining 94 women, comprising 94% of the sample, tested negative for the aforementioned antibody. The chi-square value is greater than the table value; hence, it shows The statistical significance of the association between cases and controls was determined through the use of immunofluorescence.

Table 5: Evaluate the HEp-2 cell pattern among IFAT positives.

No	HEp-2 cell pattern	IFAT positives	
		f	%
1	Anti-centromere	18	23.38
2	Homogenous	35	45.45
3	Coarse speckled	10	12.99
4	Fine speckled	08	10.39
5	Others: vimentin, nuclear rim	06	7.79
	Total	77	100

n-77

Table 5: shows the distribution of the HEp 2 cell pattern within the cohort of 77 individuals who tested positive for IFAT, encompassing both the experimental and control groups. Among the 77 positive cases, a homogenous pattern was observed in 45.45% of cases, while an anticentromere pattern was observed in 23.38% of cases. Fine speckled and coarse speckled patterns were observed in 10.39% and 12.99% of cases, respectively. Additionally, vimentin and nuclear rim patterns were observed in 7.79% of patients, respectively.

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Table 6: Evaluate the seropositivity among cases and controls using ELISA.

	Positive		Negative		p -Value
	f	%	f	%	p-value < 0.001
Cases	70	70	30	30	
Control	05	05	95	95	X2 90.1333
Total	75	37.5	125	62.5	Df-1
					Significant

Table 6: shows that out of a total of 100 cases, 70% yielded positive results for antinuclear antibodies (ANA) through the use of an enzyme-linked immunosorbent assay (ELISA), while the remaining 30% of cases were negative for ANA. Out of the entire cohort of 100 subjects under investigation, a fraction of 05% tested positive for antinuclear antibody through the employment of the enzyme-linked immunosorbent assay (ELISA), while the remaining 95% yielded negative results. The chi-square value is greater than the table value; hence, it shows Statistical significance was observed in the association between antinuclear antibody positivity for both the case and control groups.

Table 7: Compare the ELISA and IFAT tests for antinuclear antibody positivity in cases and controls.

	ELISA Positive		IFAT Positive		p- Value
	f	%	f	%	p-value >.754362
Cases	70	70	71	71	
Control	05	05	06	06	X2 -0.0979.
Total	75	37.5	77	38.5	Df-1
					Not significant

Table 7: presents a comparative analysis of antinuclear antibody positivity between cases and controls using two distinct methods, namely ELISA and IFAT. The data is presented in tabular format. In the study, it was found that 70% of the cases tested positive through the ELISA method, while 71% of the cases tested positive through the IFAT method. In the control group, 5% of individuals tested positive using the ELISA method, while 6% tested positive using the IFAT method. In certain instances, IFAT identified an additional positive sample that was not detected by ELISA in the case or control. The chi-square value is less than the table value; hence, no statistical significance was observed in the association between antinuclear antibody positivity for both the case and control groups in ELISA and IFAT tests.

Discusion

The effectiveness of ELISA (enzyme-linked immunosorbent assay) and immunofluorescence tests for the diagnosis of autoantibodies among antenatal mothers is to detect the presence of autoantibodies in pregnant women.

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Autoantibodies are antibodies that mistakenly target and attack the body's own tissues and organs, leading to autoimmune diseases.

The aim of the study is to compare the effectiveness of ELISA and immunofluorescence tests for the diagnosis of autoantibodies among antenatal mothers. The study was conducted at Index Medical College hospitals in Indore. All pregnant women from the age group 21–40 years with a history of previous unfavorable fetal outcomes who visited outpatient OBG departments at Index Medical College Hospital in Indore were included in this study. The samples were 100 pregnant women with a history of previous unfavorable fetal outcomes as cases and 100 healthy pregnant women as controls. The researcher used random sampling for the study.

Our study found that 46% of the cases were mostly in the 31–35 age range. In the participants cases, only 11% were between 21 and 25 years old, respectively. In controls, 44% were mostly in the 31–35-year age range. Of the participants, only 9% of the controls were between 21 and 25 years old.

Our study found that out of a total of 100 cases, 71% of the samples tested positive for antinuclear antibodies, while 29% tested negative. Among the controls, 6% of the samples tested positive for antinuclear antibodies, while the remaining 94% tested negative.

The findings are consistent with Gupta P. et al. (2022). In recent times, there has been an increase in the prevalence of autoimmune disorders, including connective tissue diseases, in India. Antinuclear antibodies are indicative of a systemic autoimmune response and can serve as both a screening mechanism and a means of bolstering the diagnosis of systemic autoimmune disease. [4]

Prevalence of antinuclear antibody positivity in relation to gravida status among both case and control groups The study found that 34% of cases involving 2nd gravida, 20% of cases involving 3rd gravida, and 17% of cases involving 4th gravida tested positive. In comparison, only 6% of 2nd gravida controls tested positive, while none of the controls from 3rd or 4th gravida tested positive.

The results suggest that among the 100 cases examined, 71% (71 out of 100) were found to be positive for antinuclear antibody through the use of immunofluorescence, while 29% (29 out of 100) were negative for antinuclear antibody. Out of the entire cohort of 100 women who had multiple pregnancies, six of them, constituting 6% of the sample, yielded positive results for antinuclear antibodies through the use of the indirect immunofluorescence assay technique. The remaining 94 women, comprising 94% of the sample, tested negative for the aforementioned antibody. The chi-square value is greater than the table value; hence, it shows The statistical significance of the association between cases and controls was determined through the use of immunofluorescence.

A similar study by Liu T. et al. found that over the last ten years, there has been a notable rise in the occurrence of recurrent pregnancy loss (RPL). As a potential factor contributing to RPL, immunological disorders have been under consideration. [5]

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HEp 2 cell pattern within the cohort of 77 individuals who tested positive for IFAT, encompassing both the experimental and control groups. Among the 77 positive cases, a homogenous pattern was observed in 45.45% of cases, while an anticentromere pattern was observed in 23.38% of cases. Fine speckled and coarse speckled patterns were observed in 10.39% and 12.99% of cases, respectively. Additionally, vimentin and nuclear rim patterns were observed in 7.79% of patients, respectively.

Anupriya Asaithambi et al. found antibodies among pregnant females with a history of adverse obstetric events (BOH) in comparison to healthy multigravid women residing in the vicinity of Tirunelveli district. [6]

Categorization of fluorescence intensity within the cohort of 77 individuals who tested positive. The study observed that 31.17% of patients exhibited 3+ positive results, while 45.45% of patients exhibited 2+ positive results. Additionally, 20.77% of patients exhibited one (or more) positive results, and 2.59% of participants exhibited borderline positive results.

According to Tarp B. (2021) et al., the most widely accepted technique for identifying anti-nuclear antibodies is the indirect immunofluorescence method. Hep-2 cell line-coated slides are used for the identification and detection of diverse fluorescence patterns generated by anti-nuclear antibodies. [7]

Out of a total of 100 cases, 70% yielded positive results for antinuclear antibodies (ANA) through the enzyme-linked immunosorbent assay (ELISA), while the remaining 30% of cases were negative for ANA. Out of the entire cohort of 100 subjects under investigation, a fraction of 05% tested positive for antinuclear antibody through the employment of the enzyme-linked immunosorbent assay (ELISA), while the remaining 95% yielded negative results. The chi-square value is greater than the table value; hence, it shows Statistical significance was observed in the association between antinuclear antibody positivity for both the case and control groups.

Kamal AM et al.'s study, which suggested that toxoplasmosis is recognized as a significant risk factor for adverse obstetric outcomes and a leading etiology of congenital infections. The objective of the current investigation was to assess the prevalence of T. gondii seropositivity and its corresponding risk factors among individuals attending the high-risk pregnancy and low-risk antenatal care clinics at Minia Maternity and Paediatric University Hospital in Minia, Egypt. [8]

A comparative analysis of antinuclear antibody positivity between cases and controls using two distinct methods, namely ELISA and IFAT. The data is presented in tabular format. In the study, it was found that 70% of the cases tested positive through the ELISA method, while 71% of the cases tested positive through the IFAT method. In the control group, 5% of individuals tested positive using the ELISA method, while 6% tested positive using the IFAT method.

Therefore, females lacking a history of autoimmune disorders but experiencing pregnancy complications exhibit an increased quantity of autoantibodies. Additional investigation is necessary to determine whether the presence of autoantibodies is a temporary phenomenon that is primarily observed during pregnancy or postpartum or

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if it is indicative of forthcoming immune-related ailments. This research is crucial in order to prevent repeated instances of fetal loss.

Simillar study Ashish Tayde et al. found that individuals diagnosed with autoimmune connective tissue disorders frequently exhibit the presence of antinuclear antibodies (ANA), which are antibodies that target various nuclear antigens. The detection of antibodies is accomplished through the utilization of two distinct testing methodologies, namely the enzyme-linked immunosorbent assay (ELISA) and the indirect immunofluorescence (IF) technique. While ELISA may be a more cost-effective approach, the indirect immunofluorescence assay (IF) is the preferred method for detecting antinuclear antibodies (ANA). The present study involved a comparison of two techniques in terms of their diagnostic performance. Both testing methodologies were implemented on a total of 155 specimens. Out of the total samples, 135 belonged to the experimental group, while the remaining 20 samples were categorized as controls, including both negative and positive controls. Out of the total of 135 test samples, the IF test yielded positive results in 25 cases, which accounts for 18.51% of the total samples. Conversely, negative results were obtained in 110 cases, which represents 81.49% of the total samples. The ELISA assay yielded positive results in 20 cases, accounting for 14.81% of the total sample, while negative results were obtained in 115 cases, representing 85.19% of the sample. The results indicate that 18 samples, accounting for 13.33% of the total, yielded positive outcomes with both methods. Conversely, 108 samples, representing 80.0% of the total, produced negative results with both methods. A total of 7 cases, representing 5.18% of the sample, were identified as yielding negative outcomes through the enzyme-linked immunosorbent assay (ELISA) method. However, subsequent testing utilizing the indirect immunofluorescence (IF) technique revealed these same cases to be positive. A total of two samples, representing 1.48% of the sample population, exhibited positive results when tested via ELISA but conversely yielded negative results when subjected to IF analysis. The study conducted a comparison between the sensitivity and specificity of ELISA and IF, revealing values of 90.0% and 93.9%, respectively. [9]

Our study suggests that the use of indirect immunofluorescence assays (IFA) is more effective than enzyme-linked immunosorbent assays (ELISA) for the purpose of ANA testing. 85

Conclusion

The results of the present investigation indicate that the use of indirect immunofluorescence assay (IFA) is a more efficacious approach compared to enzyme-linked immunosorbent assay (ELISA) in the context of ANA testing.

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