



ORIGINAL ARTICLE

## Estimation of lupus anticoagulant in women with recurrent miscarriages.

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**ABSTRACT... Objective:** To determine the frequency of lupus anticoagulant (LA) in women presenting with recurrent miscarriages and to measure the LA in women with recurrent miscarriage in our setup. **Study Design:** Cross-sectional Descriptive study. **Setting:** Department of Pathology, Islamic International Medical College (IIMC), Rawalpindi. **Period:** Oct 2020 to Oct 2021. **Methods:** Total sample of 162 females was recruited for this study with three consecutive miscarriages before 24 weeks of gestation. All the patients were screened for Lupus anticoagulant (LA) by screening (LA1) reagent. Samples with positive (LA1) were then repeated after 6 weeks. **Results:** A total of 10 (6.1%) patients tested positive for LA on the first screening test. There were 152 (93.8%) patients with negative results at this stage. When the test was repeated after six weeks, only four out of the 10 tested positive. Thus, a total of four (2.4%) out of 162 patients were categorized as seropositive for LA. There were 158 (97.5%) negative results at this stage. **Conclusion:** In conclusion, the detection of Lupus Anticoagulant (LA) poses ongoing challenges, primarily due to the absence of specific tests. Our study underscores the urgent necessity for a truly specific diagnostic tool. Despite the costly automated testing methods and the employment of LA-sensitive APTT, our findings reveal a scarcity of confirmed and repeated LAC positivity, even within high-risk patient cohorts.

**Key words:** Coagulation Abnormalities, LA Seropositive, Lupus Anticoagulant, Pregnancy Outcomes, Recurrent Miscarriages.

### INTRODUCTION

During pregnancy, the body undergoes significant hemodynamic alterations, leading to a hypercoagulable state characterized by venous stasis from the gravid uterus and alterations in coagulation dynamics. This increased propensity for blood clotting poses a potential risk factor for recurrent miscarriages.<sup>1,2</sup> According to the definitions provided by reputable bodies like The Royal College of Obstetricians and Gynecologists (RCOG) and the European Society of Human Reproduction and Embryology (ESHRE), recurrent miscarriage is clinically identified as “three or more consecutive losses before 24 weeks of gestation.” Understanding the intersection between pregnancy-related hypercoagulability and recurrent miscarriages is vital for effective clinical management and risk assessment.<sup>3</sup> Healthcare providers need to be vigilant in recognizing these interconnected

factors to provide appropriate interventions and support for women experiencing recurrent pregnancy loss. This knowledge underscores the importance of multidisciplinary collaboration between obstetricians, hematologists, and reproductive specialists to optimize care and improve outcomes for affected individuals.<sup>4</sup>

Recurrent miscarriage is often attributed to various contributing factors, with antiphospholipid syndrome (APS) emerging as a significant cause, acknowledged by experts in the field. APS, an acquired thrombophilic condition, poses considerable risks of morbidity and mortality in affected individuals. Within APS, antiphospholipid antibodies such as Lupus anticoagulant (LA), Anticardiolipin (aCL), and anti- $\beta$ 2 glycoprotein I antibodies (B2GP-I) play a central role. These antibodies bind to negatively charged phospholipids, potentially leading to

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fetal loss and thrombotic events.<sup>5</sup> Research suggests a strong association between the presence of these antibodies and an elevated risk of both miscarriage and thrombosis, highlighting their clinical significance. Understanding the role of antiphospholipid antibodies in recurrent miscarriages is essential for accurate diagnosis and appropriate management strategies. Multidisciplinary collaboration among healthcare professionals is crucial to effectively address the complexities of APS and optimize patient care and outcomes.<sup>6</sup>

The International Society on Thrombosis and Haemostasis (ISTH) recently updated its recommendations for Lupus anticoagulant (LA) diagnosis, advocating for the use of two tests: the LA sensitive partial thromboplastin time (PTT-LA) and the dilute Russell viper venom time (DRVVT).<sup>7</sup> LA, an in vitro phenomenon, involves antiphospholipid antibodies that impede clot formation, prolonging clotting time. Accurate assessment of LA is crucial for diagnosing and managing patients with antiphospholipid syndrome (APS). The ISTH underscores the importance of employing these tests for precise evaluation, as outlined in recent guidelines. Effective diagnosis and management strategies rely on the precise identification of LA, emphasizing its significance in APS management protocols. Healthcare providers must adhere to recommended testing protocols to ensure optimal patient care and outcomes in APS cases.<sup>8,9</sup>

Despite ongoing treatment, patients with antiphospholipid syndrome (APS) still face significant morbidity and mortality risks. Therefore, it's crucial to carefully evaluate prognostic indicators and implement appropriate therapeutic interventions to mitigate adverse outcomes. This emphasizes the importance of proactive management strategies to improve the prognosis and quality of life for individuals with APS.<sup>10,11</sup>

The estimation of lupus anticoagulant in women with recurrent miscarriages serves as a crucial diagnostic tool. Given the potential association between lupus anticoagulant and recurrent

miscarriages, assessing its presence aids in identifying underlying thrombotic disorders. This evaluation informs clinical decision-making, guiding appropriate interventions to mitigate risks and improve pregnancy outcomes. By understanding the role of lupus anticoagulant in recurrent miscarriages, healthcare providers can offer tailored management strategies, including anticoagulant therapy, to reduce the likelihood of future pregnancy loss. Such comprehensive evaluation underscores the importance of multidisciplinary approaches in addressing the complex interplay between immunological factors and reproductive health. Addressing this gap in knowledge is vital for optimizing clinical management strategies and improving pregnancy outcomes for affected individuals.

## METHODS

This study was conducted in the Department of Pathology, Islamic International Medical College (IIMC), Rawalpindi from October 2020 to October 2021. The Institutional Review Committee has granted ethical permission (Application Number: Riphah/IRC/20/240 Dated: October, 19<sup>th</sup> 2020), and informed consent has been obtained from all people involved.

A total of 162 Pregnant females with gestational age < 24weeks were included for data collection by convenient sampling. Informed consent was taken from all participants and their anonymity was maintained. Data collected from the women with history of three consecutive miscarriages attending OPD or admitted in Gynae/obs wards for investigation & treatment of recurrent miscarriages.

A detailed history and physical examination was conducted and were recorded on Performa. Patients with >24 weeks of gestation, SLE, Preeclampsia, Cervical and uterine abnormalities, Blood group incompatibilities, Uncontrolled DM/Gestational diabetes, Syphilis and other STDs were excluded from the study. About 1.8cc venous blood samples were obtained in trisodium citrate vial (blue cap) under sterile conditions. Lupus anticoagulant was estimated using three dimensional, Coagulation Analyzer

SYSMEX CS-1600. About 1.8cc of venous blood was transferred to a sodium citrate vial (blue cap) for lupus anticoagulant. Subsequently, the blood samples were promptly transported to the laboratory, where Platelet poor plasma was prepared using a process of double centrifugation lasting 10 minutes at a speed of 4000rpm, in order to prevent the inclusion of platelets. (2) The blood samples from the patient were kept at room temperature. The plasma was separated and stored at a temperature between 2-8°C. Within 4 hours of collecting the blood, the plasma was separated to ensure the accuracy and precision of the results. The procedures were performed in accordance with the manufacturers' instruction manual.

The data analysis was conducted using the Statistical Programme SPSS version 21.0. Basic descriptive statistics, including frequencies and percentages, were calculated for each categorical category, such as age, duration of marriage, and number of live births. Mean and standard deviation (SD) were used to express the data values for continuous variables. The independent T-test, also known as the two-sample T-test, was employed to compare the means.

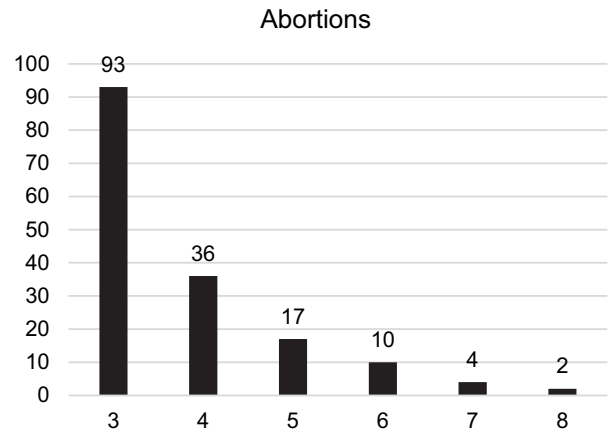
**RESULTS**

A total sample of 162 females was recruited for this study. There were minimum of three abortions (as per the study inclusion criteria) and a maximum of eight abortions. A total of with 57.4 % (n=93) females had a history of three abortions, 22.2% (n=36) had four abortions, 10.5% (n=17) had five abortions, 6.2 % (n=10) had six abortions, 2.5 % (n=4) had seven abortions, while only 1.2 % (n=2) had eight abortions (Figure-1). The overall mean number of abortions was 3.78 + 1.14.

**Lupus Anticoagulant Antibodies**

A total of 10 (6.1 %) patients tested positive for Lupus Anticoagulant Antibodies (LA) on the first screening test. There were 152 (93.8%) patients with negative results at this stage. When the test was repeated after six weeks, only four out of the 10 tested positive. Thus, a total of four (2.4 %) out of 162 patients were categorized as seropositive for LA. There were 158 (97.5 %) negative results

at this stage shown in Table-I.



**Figure-1. Frequency of abortions in all participants (n = 162)**

Screening Test(LA1)		Confirmatory Test(LA2)	
Total	Positive	Total	Positive
162	10 (6.1%)	10	04(2.4%)

**Table-I. Frequency of positive tests in LA1 and LA2 groups**

In the LA1 group, out of a total of 162 tests conducted, 152 (93.8%) were negative. In contrast, within the LA2 group, among a total of 10 tests administered, 6 (60%) yielded negative results upon confirmatory testing shown in Table-II.

Screening Test(LA1)		Confirmatory Test(LA2)	
Total	Negative	Total	Negative
162	152 (93.8%)	10	6 (60%)

**Table-II. Frequency of negative tests in LA1 and LA2 groups**

**Lupus Anticoagulant Positivity and Age**

A two-stage analysis was conducted to evaluate any relationship between LA positivity and age (Table-III). As shown, the mean age of LA positive patients was significantly lesser than that of LA negative patients at both the screening test stage (p < 0.001) as well as the second test stage (p = 0.007).

	Mean Age		P-Value
	LA Positive	LA Negative	
Screening Test	27.50+1.64	30.43+0.34	<0.001
Confirmatory Test	26.25+1.49	30.35+0.34	0.007

**Table-III. Descriptive statistics of age (years). Statistical significance was kept as p < 0.05**

## DISCUSSION

The present study has been carried out to determine the association between Lupus anticoagulant (LA) and recurrent miscarriages. Our results are similar to the study conducted by Christine A et al., in 2020 in Canada conducted a study where 2257 high-risk patients were tested for Lupus anticoagulant (LA) over a period of 6 years and documented only 5 (0.02 %) LA positive patients with early recurrent miscarriage. Because a confirmed, repeated Lupus anticoagulant was exceedingly uncommon even in a high-risk scenario, this study does not support two assays recommended by International Society on Thrombosis and Haemostasis as sufficient to detect and describe patients with LA.<sup>12</sup>

In contrast to the results reported by Gebhart J et al. in Austria in 2019, our findings indicate that women with persistent Lupus anticoagulant (LA) exhibit a significantly elevated risk of experiencing severe, potentially life-threatening pregnancy complications and unfavorable pregnancy outcomes. Likewise, research conducted in Lahore, Pakistan, in 2018 by Nazia Farooq and colleagues revealed a strong association between a high prevalence of lupus anticoagulant antibodies and recurrent miscarriages.<sup>14</sup> The study by Alaa El-Deen M. Ismail et al., in 2013, in Egypt Lupus anticoagulants was positive in forty two cases (29.8 %) of abortion group.<sup>15</sup>

In a study conducted by Sheela et al. in 2017 in India, it was noted that out of the 33 patients, seven of them (21.21%) exhibited positive antiphospholipid antibody titers. The positivity was confirmed by repeating the tests after 12 weeks. Additionally, five patients (15.15%) tested positive for ACA antibody. Four patients, accounting for 12.12% of the total, tested positive for both LA and B2GP1.<sup>3</sup>

In present study 97.5 % cases were negative for Lupus anticoagulant, which is consistent with another retrospective study from 2016 to 2018 by Pramod Kumar et al., in 2020, India<sup>16</sup> In 2015, Mahsa Besharat et al., in Iran, conducted a study indicated that these tests should be carried out.<sup>17</sup> As mentioned above in present study majority

of patients show negative results. Heterogeneity of lupus anticoagulant leads to the inability to create a single gold standard diagnostic assay; so different assays should be conducted to get accurate results.

## CONCLUSION

Due to the lack of available diagnostic assays, the clinical laboratory faces a difficult task when attempting to diagnose Lupus anticoagulant (LA). If we want to standardise diagnostic techniques and help doctors manage the uncommon but potentially fatal APS disorders, we need a test that can diagnose Lupus anticoagulant with absolute certainty.

## FUTURE RECOMMENDATIONS

We recommend that these antibodies must be tested in all the patients with recurrent miscarriages, and they should be offered appropriate treatment. Standardized methods to test these antibodies should be affordable, more widely available and used.

## LIMITATIONS

Our study's limitations include a potentially limited sample size, variability in laboratory techniques, retrospective design, confounding variables, and limited external validity, which may impact generalizability and statistical power.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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




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No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
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2	Ayesha Nayyar	Supervision of project.	
3	Fakhra Noureen	Validation visualization, Writing - review & editing.	
4	Ama tul Naval	Validation correction and review.	
5	Humaira Abid	Data collection and review.	
6	Muhammad Awais Niaz	Data analysis.	