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Research Article

Autoantibody profile in first trimester recurrent pregnancy loss - a tertiary care hospital study in North East India

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ABSTRACT

Background: Recurrent pregnancy loss (RPL), defined as the loss of two or more pregnancies before 20 weeks, is often linked to genetic, anatomical, endocrine, or autoimmune causes, though the reason remains unknown in about 50% of cases. This study aimed to assess the burden of autoimmune disease in RPL patients attending antenatal OPD and to profile associated autoimmune antibodies. A strong association was found between RPL and the presence of antiphospholipid (APA), antinuclear (ANA), and anti-thyroid peroxidase (Anti-TPO) antibodies.

Methodology: 121 pregnant women in first trimester with a history of RPL were screened for Anti phospholipid, Anti Cardiolipin, Anti Thyroid Peroxidase and Anti Beta 2 Globulin 1 antibodies. The levels of Complement C3 and C4 were also evaluated.

Results: 65(53.7%) cases were positive for either of the autoantibodies. Anticardiolipin IgG, Anticardiolipin IgM, Anti Phospholipid IgM, Anti Phospholipid IgG, Beta-2 glycoprotein IgM and Beta-2 glycoprotein IgG were found to be positive in 41(33.8%),3(2.4%), 10(8.2%), 4(3.3%),6(5%) and 1(0.8%) respectively. Anti TPO was positive in a significant number of participants 25(21%) despite having normal TSH levels. Complement C3 and C4 were however within normal ranges in all participants. Significant finding of the current study is that with one unit change in Anticardiolipin IgG, the level of C3 was increased by more than two times. Additionally mutual positive association was observed among the antibodies.

Conclusion: Current study throws light on the prevalence of autoantibodies in recurrent pregnancy loss and opens avenues for further research to facilitate early management of RPL and better pregnancy outcomes.

Keywords: Recurrent Pregnancy Loss, Autoantibody, Complement, TSH, Cardiolipin, Antinuclear Antibody

INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as the loss of two or more pregnancies before 20 weeks of gestation. Epidemiologic studies suggest around 1-5% of couples have recurrent pregnancy loss.[1] Although no reliable published data have estimated the probability of finding an aetiology for RPL in a population with two versus three or more miscarriages, available literature strongly recommend evaluating such patients after just two losses in patients with no prior live births. [2]Common aetiologies include genetic, anatomic anomalies, endocrine and autoimmune. However, even after evaluation for these causes, approximately half of all cases remain unexplained[3].

Current diagnostic procedures identify etiological factors, such as translocations, immunologic factors, endocrine disorders and uterine abnormalities in 50% of these couples. However, despite the available diagnostic platforms, in 50% of cases causes of RPL still

remain in the unexplained category[2]. In this context, an inadequate immunological interaction between mother and the embryo could be attributed as the main factor related to adequate placental development, embryo survival, and maintenance of early pregnancy[6].
The immune status of a mother has long been thought to be related to the spontaneous pregnancy loss[1]. Higher pregnancy losses have been reported in patients with the presence of autoantibodies. A strong association has been observed between the presence of antiphospholipid antibodies (APA), antinuclear antibodies (ANA) and Antithyroid peroxidase antibodies (Anti TPO) recurrent pregnancy loss. APA include a group of autoantibodies like Anti Cardiolipin antibodies (ACA), Lupus Anticoagulant 4(LA) and anti-beta 2 glycoprotein while Anti-Nuclear antibodies comprise a group of antibodies with different specificities against antigens of the cell nucleus [5].
Commonest clinical manifestation in APA is recurrent miscarriage. The incidence of APA has been reported to be 41.26% and ANA 22.7%[7]. The mechanisms by which APS results in RPL are incompletely understood.
Antiphospholipid antibodies (APA) are heterogeneous immunoglobulins of G, M or A classes with specificity directed towards anionic phospholipids. The APA interfere with the natural anti coagulation pathway and thereby contribute to arterial or venous thrombosis. Apart from this hypothesis, another mechanism suggested is that they would appear as a secondary response to the phospholipid self-antigens[8]. APA are also known to interfere with the physiological changes that take place during pregnancy[4].
A definite relationship has also been proposed between the gestational week of abortion and prevalence of APA. Incidence of APA in women with one or more 1st trimester miscarriage varies between 14% and 42% [9].
Another autoantibody implicated in the RPL is the antithyroid peroxidase antibodies. Anti–thyroid peroxidase antibody (TPO-Ab), which is the known cause of autoimmune thyroid disease, enhances proinflammatory cytokine responses. Anti-TPO positive euthyroid females had a higher prevalence of infertility, anaemia as well as preterm delivery[10]. A statistically significant relationship between anti-TPO antibodies and recurrent miscarriage has also been detected in a study conducted by Ali Ghalib et al [11].
The role and clinical significance of complement is unclear in patients with unexplained recurrent miscarriage, though low levels of complement 3 (C3) and/or complement 4 (C4) are reported to be associated with the antiphospholipid syndrome [12].
The high baseline rate of spontaneous isolated and recurrent pregnancy losses in the general population, the lack of consistent definition for RPL, combine to frustrate aims at diagnostic and therapeutic recommendations. RPL is a complex challenge as it poses lot of challenges on the treating physician along with the patients due to the lack of a universally accepted definition. Even after thoroughly evaluating recurrent pregnancy loss aetiology and risk factors, almost half of the cases cause remain unexplained[13].
No data exists on the association of autoantibodies with recurrent pregnancy loss in in north-east region of India. Despite the intense interest in this potential aetiology for RPL, there is no consensus on appropriate diagnostic workup or therapy.
As such this study was aimed to evaluate the burden of autoimmune disease related recurrent pregnancy losses and the profile of autoantibodies in RPL cases during their first trimester.

MATERIALS AND METHODS

The study was carried out over a period of two years from March 2023 to February 2025 in the Department of Microbiology in association with Department of Obstetrics and Gynaecology. All pregnant women with history of two or more spontaneous and consecutive pregnancy loss less than 12 weeks of gestation attending the antenatal OPD were included in the study. Patients having known history of malignancy, HIV, receiving immunosuppressive therapy were excluded from the study.
A special proforma was designed for the present study and accordingly the obstetric history of each woman was recorded. A total of 121 cases fulfilling the inclusion criteria was included in the study. Blood was collected from the subjects and serum was separated. Serum samples were stored at -200C and thawed when the tests were conducted.
This prospective study obtained ethical clearance from the Institutional Ethical Committee vide certificate no MC/190/2007/Pt.II/March -2023/6 dated 10.04.2023.
Autoantibodies to Anticardiolipin (ACA) IgG and IgM (HIGHTOP, China), Antiphospholipid IgG

AND IgM (DiAMetra. SRL, Italy), Anti Beta 2-glycoprotein-1 (Generic assay), Anti Thyroid peroxidase (TPO) (Biogenix) was carried out by ELISA using the commercial kits as per manufacturer's instructions. Autoantibodies such as anti-nuclear antibodies (ANA) were qualitatively and quantitatively tested by indirect immunofluorescence (IIFA) using HEp-2 cells (Emergo, Europe) and a cut off for positivity was as per the manufacturer's instructions. Complement assays (C3 & C4) were done by quantitative method (Genrui, Netherlands) by nephelometry.

RESULTS

In a total of 121 pregnant women with a history of RPL, 65(53.7%) of them were positive for either of the autoantibodies being tested. Anticardiolipin IgG and Anticardiolipin IgM were found to be positive in 41(33.8%) and 3(2.4%) respectively, while Anti Phospholipid IgM and Anti Phospholipid IgG were found to be positive in 10(8.2%) and 4(3.3%) of the participants. Beta-2 glycoprotein IgM was found to be positive in 6(5%), while Beta-2 glycoprotein IgG was positive in only 1(0.8%) participant (Fig1).

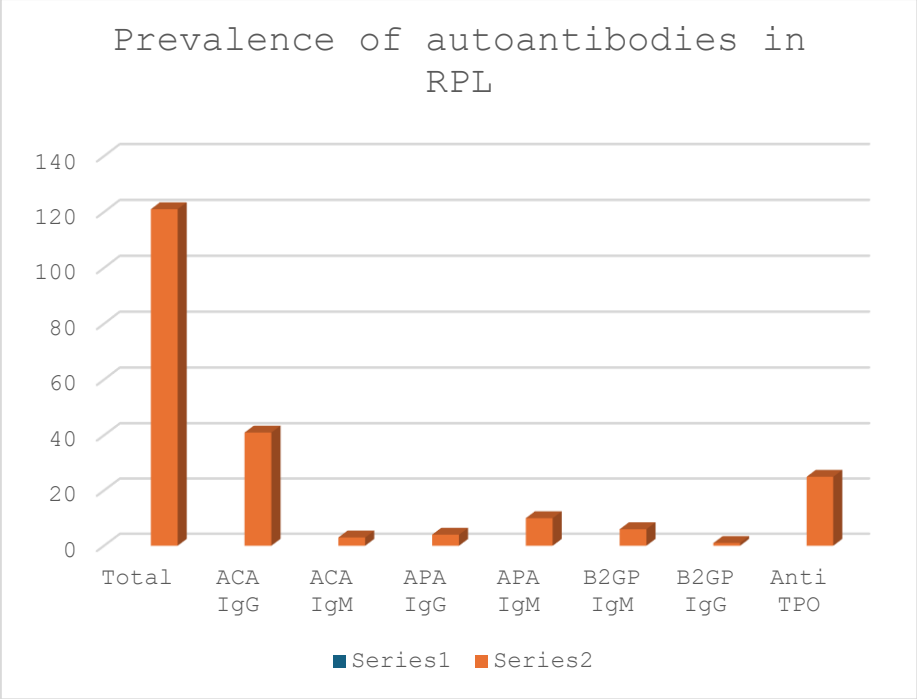

Fig 1: Prevalence of different types of autoantibodies in RPL cases

A significant percentage of the study group [25(21%)] showed high levels of Anti-Thyroid peroxidase antibodies levels (anti TPO) depicted in Fig2, however only 3 of them showed high TSH levels.

Complement C3 and C4 levels in all participants were however within -normal ranges. Only 3 participants (2.47%) showed positive anti-Nuclear Antibodies through IFA. Regression analysis show significant increase of the level of C3 in positive cases of Anti-Cardiolipin IgG ($p<0.0001$, 95% of CI 1.001 to 3.377).

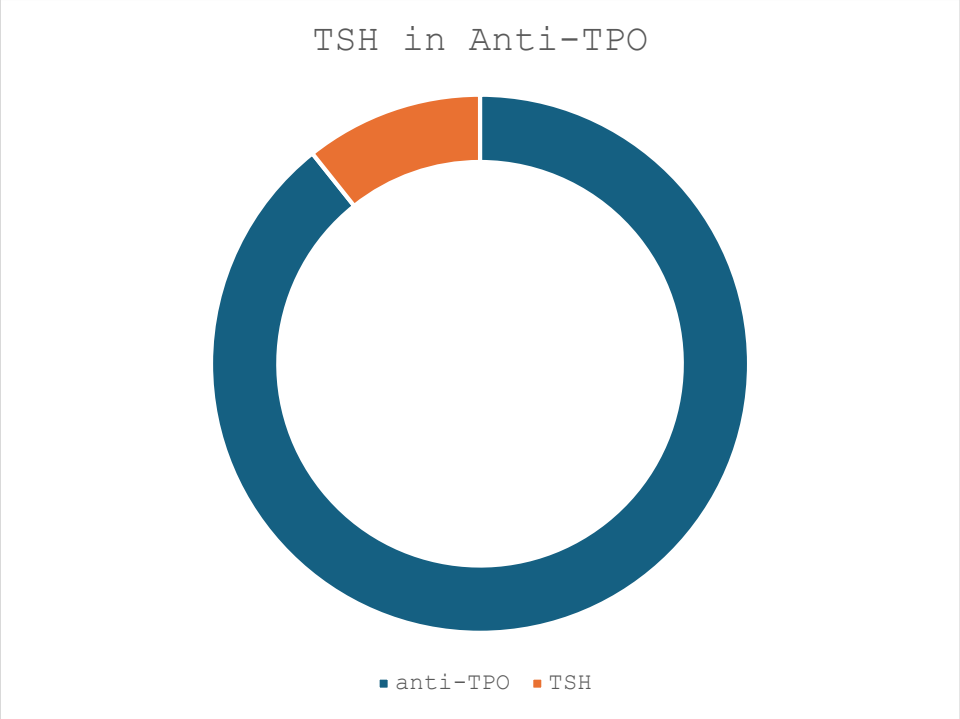

Fig 2: High TSH in Positive Anti-TPO cases

DISCUSSION

In this study, we estimated the prevalence of autoantibodies in pregnant women with the history of recurrent pregnancy loss. Very few studies have been done in the recent past in India regarding autoimmunity in recurrent pregnancy loss, and none so in the Northeastern region of India, where the prevalence of autoimmune diseases have been found to have a slightly higher prevalence than the rest of India[14]. Therefore, the current study would open a wider scope for further research for better patient care and successful pregnancy outcomes.

We found the total prevalence of autoantibodies to be 53.7% in a study group of 121, while Shankarkumar et al found a prevalence of 34%.[15] The positivity of Anticardiolipin IgG was 33.8%, which was similar to the study done by Khan et al, however the positivity of ACA IgM was quite low[1]. Both APA IgG and IgM were low compared to the studies done by Shankarkumar et al and Khan et al [1,15]. The prevalence of anti-TPO was 21% in comparison to the study done by Godines-Enriquez et al where it was 14.8%, despite all patients being in an euthyroid state[16]. Though previous cohort and meta-analytic studies have found no significant improvement with levothyroxine therapy in euthyroid patients in presence of anti TPO Abs, however the 2017 American Thyroid Association guidelines suggests that women with a prior history of loss may be considered for low dose LT4 treatment (25–50 µg) given its potential benefits in comparison with its minimal risk [17-19]. Previous studies correlating complement C3 and C4 levels with successful pregnancy outcomes in RPL cases found higher levels of both C3 and C4 in the study population than the control group, indicating a positive correlation [20,21]. However, due to lack of a control group in our study, a definite correlation couldn't be established between the complement levels and prediction of successful live births.

CONCLUSION

The current study underscored the significant knowledge gap in management of RPL cases. To mitigate the physical and emotional constraints faced by this group of patients, a robust screening of all possible risk factors including autoantibodies, should be done early, for better management and successful pregnancy outcomes. Screening of thyroid antibodies, even with normal TSH levels, is recommended. A low dose levothyroxine might be considered in these patients, due to its potential benefit over its minimal risk.

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REFERENCES

1. Gausiya AR Shaikh Khan, Shashikant P Vaidya, Subhash A Angadi and Geeta V Koppikar. Study of autoantibodies in recurrent foetal loss in tertiary care hospital. International Journal of Appl ied Research 2018; 4(12): 71-74
2. Rai R, Regan L. Recurrent miscarriage. Lancet. 2006; 368:601-611.
3. Holly B. Ford, MD, Danny J. Schust, MD. Recurrent Pregnancy Loss: Etiology, Diagnosis, and Therapy. Rev Obstet. Gynecol. 2009;2(2):76-83.
4. Pattison NS, Birdsall MA. Recent advances in Obstetrics & Gynaecology. Editor John Bonner. Publisher: Churchill Livingstone, 1994: 18: 23-50.
5. Bradwell AR, Stokes RP, Johnson GD. Historical Chapter II Atlas of Hep-2 patterns and laboratory techniques. 1995: 12 Kwak YHJ, Gilman-Sachs
6. Kwak-Kim, J.; Bao, S.; Lee, S.K.; Kim, J.W.; Gilman-Sachs, A. Immunological modes of pregnancy loss: Inflammation, immune effectors, and stress. Am. J. Reprod. Immunol. 2014, 72, 129–140.
7. Kwak YHJ, Gilman-Sachs A, Kenneth D. Reproductive outcome in women with recurrent spontaneous abortions of all immune and autoimmune causes: preconception versus post conception treatment. Am. J Obstet. Gynecol. 1992; 166(6):1787-1798
8. Scrobohaci ML, Freyssinet JM. Anticorps antiphospholipides--syndrome antiphospholipides. Antiphospholipid antibodies--antiphospholipid syndrome. Cause or consequence of thrombosis. Pathol. Biol (Paris). 1991 Sep;39(7):709-15.
9. Brown HL. Anti-phospholipid antibodies and recurrent pregnancy loss. Clinical Obstetrics & Gynecology. 1991; 34(17):17-26
10. Monika Meena, Seema Chopra, Vanita Jain, Neelam Aggarwal. The Effect of Anti-Thyroid Peroxidase Antibodies on Pregnancy Outcomes in Euthyroid Women. Journal of Clinical and Diagnostic Research. 2016 Sep, Vol-10(9): QC04-QC07
11. H.H. Ali Ghalib, C. Najmaddin Fattah, A.K. Mohammed. Association between antithyroid peroxidase antibody and recurrent miscarriage. Eur Rev Med Pharmacol Sci 2023; 27 (7): 3003-3008 DOI: 10.26355/eurrev_202304_31933
12. Sugiura-Ogasawara M, Nozawa K, Nakanishi T, Hattori Y, Ozaki Y. Complement as a predictor of further miscarriage in couples with recurrent miscarriages. Hum Reprod. 2006 Oct;21(10):2711-4. doi: 10.1093/humrep/del229. Epub 2006 Jun 21. PMID: 16790609.
13. Turesheva A, Aimagambetova G, Ukybassova T, Marat A, Kanabekova P, Kaldygulova L, Amanzholkyzy A, Ryzhkova S, Nogay A, Khamidullina Z, Ilmaliyeva A, Almawi WY, Atageldiyeva K. Recurrent Pregnancy Loss Etiology, Risk Factors, Diagnosis, and Management. Fresh Look into a Full Box. J Clin Med. 2023 Jun 15;12(12):4074. doi: 10.3390/jcm12124074. PMID: 37373766; PMCID: PMC10298962.
14 Sharma D., & Sangma D. (2024). Lupus Nephritis in Northeastern India: Navigating the complex landscape of clinical challenges and emerging healthcare opportunities. Medical Research Archives, 12(2). doi:10.18103/mra. v12i2.5123
15 Shankarkumar U, Pradhan VD, Patwardhan MM, Shankarkumar A, Ghosh K. Autoantibody profile and other immunological parameters in recurrent spontaneous abortion patients. Niger Med J 2011; 52:163-6.
16 Godines-Enriquez, M.S.;Miranda-Velásquez, S.;Enríquez-Pérez, M.M.; Arce-Sánchez,L.; Martínez-Cruz, N.; Flores-Robles,C.M.; Aguayo-González, P.;Morales-Hernández, F.V.;Villarreal-Barranca, A.; Suárez-Rico,B.V.; et al. Prevalence of Thyroid Autoimmunity in Women with Recurrent Pregnancy Loss. Medicina 2021, 57, 96. https://doi.org/10.3390/medicina57020096
17 Leduc-Robert, G.; Iews, M.; Abdelkareem, A.O.; Williams, C.; Bloomenthal, D.; Abdelhafez, F.; Bedaiwy, M.A. Prevalence of thyroid autoimmunity and effect of levothyroxine treatment in a cohort of 1064 patients with recurrent pregnancy loss. Reprod.Biomed. Online 2020, 40, 582–592.
18 Dong, A.C.; Morgan, J.; Kane, M.; Stagnaro-Green, A.; Stephenson, M.D. Subclinical hypothyroidism and thyroid autoimmunity in recurrent pregnancy

loss: A systematic review and meta-analysis. Fertil. Steril. 2020, 113, 587–600. e1.

19 Alexander, E.K.; Pearce, E.N.; Brent, G.A.; Brown, R.S.; Chen, H.; Dosiou, C.; Grobman, W.A.; Laurberg, P.; Lazarus, J.H.; Mandel,S.J.; et al. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid 2017, 27, 315–389.

20 Zhengxin Zhou, Huixia Xie, Miao Liu, Ruopeng Li, Wenjing Jiang, Yan Zheng, Qun Lv. Expression and Correlation of Complement C3 and C4 in Serum and Maternal-Fetal Interface in Patients with Unexplained Recurrent Spontaneous Abortion: A Prospective Cohort Study. Clin. Exp. Obstet. Gynecol. 2023, 50(11), 252. https://doi.org/10.31083/j.ceog5011252

21 Mayumi Sugiura-Ogasawara, Kyoko Nozawa, Tamao Nakanishi, Yukio Hattori, Yasuhiko Ozaki, Complement as a predictor of further miscarriage in couples with recurrent miscarriages, Human Reproduction, Volume 21, Issue 10, 1 October 2006, Pages 2711–2714, https://doi.org/10.1093/humrep/del229