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Current Approach on Recurrent Pregnancy Loss; Clinical and immunological Case-Control Study.

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Abstract:

Background: Recurrent pregnancy loss (RPL) is the most common and challenging condition in the field of reproductive medicine. Only around 50% of RPL cases have defined causes or risk factors, and still there are few diagnostic and treatment strategies available. Therefore, it's essential to establish other evidence-based diagnostic checkpoints aiming at enhancing management and improving pregnancy outcome in such cases.

Methods: A case-control study included 60 pregnant women from the antenatal care clinic at Zagazig University Hospitals during the period April 2020 through April 2024. The study cases were divided into four equal groups: Group 1 normal cases, Group 2 RPL cases, Group 3 RPL cases under treatment, and Group 4 non-recurrent cases. All cases were subjected to thorough history, clinical examination and laboratory investigations that included important immune markers as anti-phospholipid and anti-thyroid antibodies beside routine lab. work. The collected data was analyzed using the appropriate statistical methods.

Results: Educated, rural, and nonsmoking women made up most of the recruited cases. Their age ranges between 20 and 38 years. All cases were matched in terms of demographics, clinical and medical conditions, as well as risk factors for RPL, with no statistically significant differences. In this case-control study, the most common clinical entity with an immunologic basis was anti phospholipid syndrome (5 cases, 33%, in the RPL group and 3 cases ,20.0%, in the RPL group that were under treatment) .This was significantly higher (P = 0.04) than in the control group and Group 4 (0.0%). Conclusion: after the exclusion of all risk factors for RPL, 50% of cases have no causes. APS was the most common cause (33.3%) of RPL .

Keywords: Antiphospholipid Antibodies; Recurrent pregnancy loss: Immunological reaction:

1- Introduction

Miscarriage occurs in up to 15–20% of apparently normal couples and becomes recurrent in 2–3% of these couples ***(Sharma B, et al., 2020).*** The risk of fetal loss rises steeply after the age of 35 years, from 9.5% at 20–24 years to 76% at 45 years and older. In clinical practice, the chances of a successful pregnancy in women ≥40 years are poor when risks of miscarriage, pre-eclampsia, ectopic pregnancy, and stillbirth increase ***(Jeve and Davies, 2014).***

The maternal immune system is intimately involved in the establishment, maintenance, development, and, up to the termination of normal pregnancy. Therefore, it is logical and highly plausible to regulate the immune system at all the phases of the pregnancy ***(Kobayashi et al., 2024).*** Disturbances in the immune mechanisms could be a significant cause of recurrent pregnancy loss (RPL).

Antiphospholipid syndrome (APS) links Antiphospholipid antibodies (aPLs) to venous and arterial thrombosis and embryo-fetal morbidity. It is associated with premature birth, intrauterine growth restriction, early pre-eclampsia, HELLP syndrome, subchorionic hematoma and placental abruption in 5–15% of women who have experienced numerous miscarriages ***(Kayoko Kaneko et al., 2024)***. Women diagnosed with 'obstetric' APS exclusively experience problems related to APLs and do not have a history of thrombosis. APLs harm the

placenta after several miscarriages. Trophoblasts, endothelial cells, and blood cells undergo inflammatory and prothrombotic alterations ***(Ramhorst et al., 2016).***

Patients must meet Sydney criteria for APS. While some researchers advocate for eliminating anticardiolipin antibodies (aCLs) from the lab test, others maintain that all Sydney criteria categories are essential to prevent a false-negative APS diagnosis. Recently, numerous authors have advocated flexibility and/or clinical and laboratory category expansion. Several upcoming investigations should clarify this subject soon ***(Nadkarni & Smith, 2016).***

The new Sydney criteria for antiphospholipid antibody syndrome are finalized. One or more clinical criteria objectively established episodes of arterial, venous, or small artery thrombosis in any tissue organ are considered vascular thrombosis. Unknown fetal deaths after the 10^{th} week of pregnancy (shown by ultrasound or direct examination); unknown early births of a healthy baby before the 34th week due to eclampsia, pre-eclampsia, or placental insufficiency; and unknown triplets before the 10^{th} week of pregnancy.

B. The laboratory criteria require two or more visits separated by 12 weeks.

1. Guidelines from the International Society on Thrombosis and Hemostasis guide lupus anticoagulant detection ***(Kobayashi, et al., 2024).***
2. Standardized ELISA looks for IgG and/or IgM anticardiolipin antibodies in blood or plasma at medium or high levels (>40 GPL or MPL, or >99^{th} centile).
3. An ELISA test found high levels of IgG/IgM antibody to β2-glycoprotein I in serum or plasma (>99^{th} centile); do what the test says and see what happens.

Recommended pregnant APS treatment regimens

A) Women with APS who have had early (pre-embryonic or embryonic) miscarriages in the past and have never had thrombosis should get LDA plus preventive unfractionated heparin (5000–7500 IU SC/12 h) or LMWH (enoxaparin, tinzaparin, bemiparin, or dalteparin, at normal SC/24 h doses) (Grade 1B). Use warfarin or LMWH for thromboprophylaxis for 6 weeks after delivery. **(2015) Garrido-Gimenez & Alijotas-Reig**

A group of women with APS who have never had a thrombosis, a single or repeated loss of a fetus, or an early delivery due to severe pre-eclampsia or placental insufficiency were given either LDA plus prophylactic or intermediate-unfractionated heparin (7500–10000 IU SC every 12 hours, or every 8–12 hours adjusted to keep the mid-interval aPTT 1.5 times the control mean) or LMWH (low or intermediate prophylactic doses, enoxaparin). Use warfarin or LMWH for thromboprophylaxis for 6 weeks after delivery.

(C) Women with APS and prior thrombosis: When you take LDA, you should also take therapeutic unfractionated heparin (enoxaparin 1 mg/kg SC, dalteparin 100 U/kg SC/12 h, or enoxaparin 1.5 mg/kg/day SC) or LMWH (therapeutic dose, enoxaparin 200 U/kg/day SC). We recommend warfarin or LMWH (therapeutic dose) thromboprophylaxis for life after delivery.

2- Objectives

It is essential to establish other evidence-based diagnostic checkpoints to enhance management and improve pregnancy outcomes in cases of RPL with no defined cause especially immunologic factors, looking for immunoglobulin's IgG, IgM and IgA against different antigens e.g., Thyroid, Lupus, G.lycoprotein and Rubella etc….

3- Methods

Study Design: A case-control study.

Study Setting: Obstetrics and Gynecology Department, at Zagazig University Hospitals

Study Duration: From April 2021 to April 2024**.**

Sample Size: 60 females were recruited from the antenatal care outpatient clinic.

Participants (Inclusion and Exclusion criteria): Recruited cases were pregnant women attending regular follow-up examinations in the first trimester (between 8 and 13 weeks), as documented by sure dates and early ultrasound scanning (USS).

We excluded pregnancies with bad medical or obstetric history other than recurrent or intermittent miscarriage. We also excluded pregnancies with clinically significant uterine malformations, uterine fibroids, gross endocrinopathy as well as those with known genetic disorders ***(Bender Atik et al., 2018 & Karlsen K et al., 2020).***

Study Groups: Cases in this study were divided into 4 equal groups (each with 15 cases).

Group A: Cases with normal pregnancy (control).

Group B: Cases of recurrent pregnancy loss (RPL) that are not under treatment. Recurrent pregnancy loss (RPL) is defined by the American Society for Reproductive Medicine (ASRM) and The European Society of Human Reproduction and Embryology (ESHRE) as having two or more pregnancy losses in a row before 22 weeks of pregnancy. ***(Bender Atik, et al., 2018 & Karlsen K, et al., 2020).***

Group C: Cases with RPL that are currently under treatment, e.g., cases with overt or subclinical autoimmune hypothyroidism and cases with APS; however, there is ongoing debate about how to manage them.

***Group D*:** Cases with non-recurrent pregnancy loss.

Clinical and diagnostic measurements:

Clinical data were collected from patients' medical records.

Thorough history:

•Demographic and personal history: age, education, occupation, residence, and special habits.

•Medical history: for comorbidity and drug therapy.

Laboratory investigations:

-Complete blood count, liver and kidney function tests.

-Thyroid functions: The thyroid function was classified as either normal or abnormal. The abnormalities include both hyper and overt as well as subclinical hypothyroidism. Overt hypothyroidism is characterized by low free thyroxine and excessive thyroid-stimulating hormone (TSH), while subclinical hypothyroidism is characterized by elevated TSH levels and normal free thyroxine, is. There is currently disagreement regarding the definition of an increased TSH level; recent guidelines recommend considering an upper limit of 4.0 mIU/L as diagnostic ***(Al Dong AC, et al., 2019).***

-The coagulation profile: Venous blood was collected using 0.109 M tri-sodium citrate and subjected to centrifugation twice at 2500 g for 15 minutes at room temperature to yield plasma with minimal residual platelets. Plasma was subsequently frozen and stored in small aliquots at −20 °C until analysis. Samples anticoagulated with EDTA. The sample was promptly stored at −40 °C. Prothrombin time PT and (aPTT) using standard techniques and activated thromboplastin time were measured. ***(Nassour-Mokhtari et al ,2020).***

-The anti-phospholipid antibodies (IgG, IgM, IgA): Clinical tests for antiphospholipid antibodies are not standard, and the level of evidence does not support routine screening. The only exceptions are anticardiolipin, lupus anticoagulant, anti-ß2-glycoproteinI and ant phosphatidylserine. We prepared anti phospholipid antibodies using the methods described by ***Tripodi et al., 2023. these are:***

- **The solid-phase enzyme-linked immune-specific assay (ELISA)** is used to measure anticardiolipin antibodies IgG, IgA, and IgM idiotypes ***(Tabatabaei MS, and Ahmed, 2022).***
- **The lupus anticoagulant** (with phospholipid confirmation if positive) is determined by the dilute Russell's viper venom time (dRVVT) assay **(*Pengo et al., 2017).***
- **Anti-ß2-glycoproteinI (*Di Simone N et al., 2005).***

4-Results

Table 1: Demographic data and its relationship in the studied groups

	Group A T=15 No (%)	**Group B T=15 No (%)**	**Group C T=15 No (%)**	**Group D T=15 No (%)**	***P***
Age (y)					
Mean ± SD	29.7±9.9	30.7±7.7	31.1±8.1	30.5±9.1	0.79
Range	(20-37)	(21-38)	(20-39)	(20-38)	
Education					
Illiterate	1(6.7)	2(13.3)	1(6.7)	3(20.0)	
Primary	3(20.0)	2(13.3)	3(20.0)	1(6.7)	0.81
Secondary / High	4(26.7)	3(20.0)	5(33.3)	4(26.7)	
University and above	7(46.7)	8(53.3)	6(40.0)	7(46.7)	
Occupation					
Working	8(53.3)	6(40.0)	7(46.7)	9(60.0)	0.11
Not working	7(46.7)	9(60.0)	8(53.3)	6(40.0)	
Residence					
Urban	5(33.3)	7(46.7)	6(40.0)	6(40.0)	0.37
Rural	10(66.7)	8(53.3)	9(60.0)	9(60.0)	
Smoking					
No	11(37.3)	12(80.0)	13(86.7)	12(80.0)	
Passive (husband)	4(26.7)	2(13.3)	2(13.3)	3(20.0)	0.81
Active	0(0.0)	1(6.7)	0(0.0)	0(0.0)	

The demographic and risk factors included comparative data for the four groups of patients (group A, normal pregnancy; group B, recurrent pregnancy loss; group C, recurrent pregnancy loss with medical treatment; and group D, non-recurrent pregnancy loss). Data obtained showed that age ranged approximately between 20 and 38 years old among the four groups with no significant difference ($P = 0.79$). Hence, age appears to have no effect on pregnancy in the studied cases.

There was not a statistically significant ($P > 0.05$) difference among the four studied groups as regards all the demographic characteristics of the recruited women, including age (in years), level of education, residence, occupation, and smoking. ***[Table 1]***

Table 2: Medical, and detailed obstetric history and its relationship among the studied groups

	Group A T=15 No (%)	**Group B T=15 No (%)**	**Group C T=15 No (%)**	**Group D T=15 No (%)**	***P***
a) The medical history					
Comorbidities					
Yes	1(6.7)	5(33.3)	4(26.7)	2(13.3)	**0.03***
No	14(13.3)	10(66.7)	11(37.3)	13(86.7)	
Type of comorbidities	**No =1**	**No=5**	**No=4**	**No=2**	
Diabetes	0(0.0)	1(20.0)	1(25.0)	0(0.0)	
Hypercholesterolemia	1(100.0)	1(20.0)	1(25.0)	1(50.0)	
Hypertension	0(0.0)	1(20.0)	0(0.0)	0(0.0)	
Others	0(0.0)	2(40.0)	2(50.0)	0(0.0)	
b) Detailed Obstetric history					
Number of pregnancies (gravidity)	4(26.7)	4(26.7)	5 (33.3)	4(26.7)	0.81
Number of term pregnancy	3(20.0)	1(25.0)	2(50.0)	2(50.0)	0.04*
Number of abortions	0(0.0)	2(50.0)	2(50.0)	1(25.0)	0.04*
Number of living children	3(20.0)	1(25.0)	2(50.0)	2(50.0)	0.04*

*****P <0.05 there was a statistically significant difference

Regarding the medical and detailed obstetric history, there was a statistically significant difference (P < 0.05) between the studied groups as regards comorbidities, number of pregnancies, number of term pregnancies, number of lost pregnancies, and number of living children**.** ***[Table 2]***

Table 3: Prothrombin time, and activated partial thromboplastin and their relationship among the studied groups

	Group A T=15 No (%)	**Group B T=15 No (%)**	**Group C T=15 No (%)**	**Group D T=15 No (%)**	***P***
Prothrombin time (PT)					
Normal	15(100.0)	10(66.7)	12(80.0)	15(100.0)	0.03*
Abnormal	0(0.0)	5 (33.3)	3(20.0)	0(0.0)	
Activated partial thromboplastin					
Normal	15(100.0)	10(66.7)	12(80.0)	15(100.0)	0.03*
Abnormal	0(0.0)	5 (33.3)	4(26.7)	0(0.0)	

*****P <0.05 there was a statistically significant difference

When compared to other groups, 5 (33.3%) of the women with RPL showed a significant difference in the prothrombin time and activated partial thromboplastin test (P = 0.03). **[Table 3]**

Table 4: Frequency distribution of IgG &IgM Antibodies for RPL and their relationship among the studied groups

	Group A T=15 No (%)	**Group B T=15 No (%)**	**Group C T=15 No (%)**	**Group D T=15 No (%)**	***P***
RPL IgG antibodies #					
• Positive	0(0.0)	2(13.3)	0(0.0)	0(0.0)	0.03*
• Borderline	1(7.7)	3(20.0)	1(7.7)	0(0.0)	
• Negative	14(93.3)	10(63.7)	14(93.3	15(100.0)	
RPL IgM antibodies #					
• Positive	0(0.0)	1(7.7)	0(0.0)	0(0.0)	0.04*
• Borderline	0(0.0)	4(26.7)	3(20.0)	0(0.0)	
• Negative	15(100.0)	10(63.6)	12(80.0)	15(100.0)	
Auto thyroid IgM antibodies					
• Positive	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0.91
• Borderline	0(0.0)	1(7.7)	0(0.0)	0(0.0)	
• Negative	15(100.0)	14(93.3)	15(100.0)	15(100.0)	
Auto thyroid IgG antibodies					
• Positive	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0.98
• Borderline	0(0.0)	0(0.0)	1(7.7)	1(7.7)	
• Negative	15(100.0)	15(100.0)	14(93.3)	14(93.3)	

*****P <0.05 there was a statistically significant difference.

For Torch infection (Toxoplasmosis -HSV-CMV-Rubella)

Regarding rubella antibodies, both IgG and IgM showed statistically significant differences among the studied groups (p < 0.05) to significantly higher among groups B and C. While HSV IgG and IgM antibodies showed no statistically significant difference among the studied groups (P> 0.05).

Table 5: Antiphospholipid Antibodies and their relationship among the studied groups

	Group A T=15 No (%)	Group B T=15 No (%)	Group C T=15 No (%)	Group D T=15 No (%)	*P*
Antiphospholipid					
Normal	15(100.0)	10(66.7)	12(80.0)	15(100.0)	0.03*
Abnormal	0(0.0)	5 (33.3)	3(20.0)	0(0.0)	
P-2-glycoprotein-I					
Positive	15(100.0)	11(73.3)	12(80.0)	15(100.0)	0.04*
Negative	0(0.0)	4(26.7)	3(20.0)	0(0.0)	
Anticardiolipin antibodies					
Positive	15(100.0)	13(86.7)	14(93.3)	15(100.0)	0.11
Negative	0(0.0)	2(2(13.3)	1(7.7)	0(0.0)	
Lupus anticoagulants					
Positive	15(100.0)	11(66.7)	13(86.7)	15(100.0)	0.08
Negative	0(0.0)	5(33.3)	2(13.3)	0(0.0)	

*****P <0.05 there was a statistically significant difference

In this case-control study, antiphospholipid antibodies were found in 5 women in GB (the RPL group) (33.3%) and 3 women in GC (the RPL group that was given medicine) (20.0%). This is significantly (P = 0.04) higher than in groups A and D (0.0%). **[Table 5]**

Table 6: The thyroid function tests among the studied groups

	Group A T=15 No (%)	Group B T=15 No (%)	Group C T=15 No (%)	Group D T=15 No (%)	*P*
Thyroid function test					
Normal	15(100.0)	9(60.0)	13(86.7)	15(100.0)	0.04*
Abnormal#	0(0.0)	6(40.0)	2(13.3)	0(0.0)	

*****P <0.05 there was a statistically significant difference.

Abnormal, including overt hypothyroidism and subclinical hypothyroidism.

There is a statistically significant difference among the studied groups regarding the thyroid function being significantly abnormal among RPL group 6 (40.0%) and PRL group with medication 2 (13.3%) compared to the normal and non-RPL groups. **[Table 6]**

5.Discussion

5.1 Regarding the coagulation profile; This case-control study compared to other groups, 5 (33.3%) of the women with RPL showed a significant difference in the prothrombin time and activated partial thromboplastin test (P = 0.03).in agreement with ***(Kobayashi, T et al.,2024)***

5.2 Regarding the anti-phospholipid antibodies in this case-control study, 4 women in group B (the RPL group) (26.7%) and 3 women in group C (the RPL group that received medicine) (20.0%) had antiphospholipid antibodies. The P-value of 0.04 indicates a significantly higher prevalence of antiphospholipid antibodies compared to groups A and D (0.0%). Recurrent pregnancy losses are associated with the antiphospholipid syndrome. According to other studies ***(Bick RL and Frenkel EP. 1999; Bick RL, 2000),*** 5% to 20% of patients with recurrent pregnancy loss have positive antiphospholipid antibodies (aPLs). Several research groups have used different lab tests to find these antibodies. The results range from 8% to 42%. The tests are anticardiolipin antibody (aCL), lupus anticoagulant (LA), and anti-B2glycoprotein I.

The presence of antiphospholipid antibodies (aPLs) contributes to the diagnosis of autoimmune pregnancy loss. The aPLs were completely unique for each patient. The APLs could react directly with phospholipids, the protein cofactors attached to plastic (such as ELISA plates), or only when the cofactors bind to phospholipids ***(Kayoko Kaneko et al.,2024)***. Monospecific APLs react with one antigen, while cross-reactive APLs react with multiple antigens. These APLs have different effects on the trophoblast. For example, they stop

extravillous cytotrophoblasts from entering the decidua and villous cytotrophoblasts from differentiating. They also kill syncytiotrophoblasts and start inflammatory pathways on their surface (***Bick RL, 2000).***

5.3 ***Regarding the thyroid function: The*** study found a big difference in thyroid function between the normal and non-RPL groups and between the RPL group 6 (40.0%) and the PRL group with medication 2 (13.3%). in agreement with many Numerous observational studies ***(Abalovich, M. et al., 2002; Benhadi, N. et al., 2009; Wang, S. et al., 2012; Taylor, P.N. et al., 2014)*** have reported this association.

The mechanism underlying the association between RPL and thyroid autoimmunity remains unclear. Lazzarin et al. (2012) propose that thyroid autoimmunity may serve as an indicator of subtle thyroid dysfunction. Nonetheless, the observed absence of a correlation between subclinical hypothyroidism and recurrent pregnancy loss appears to contradict this notion. Researchers have proposed a broader immunological mechanism that includes NK cells, but the findings of ***Mariee et al. (2012) contradict this claim. The findings of Mariee et al. (2012) and Lazzarin, N. et al. (2012) contradict this claim.***

The relationship between thyroid autoimmunity and the likelihood of subsequent miscarriages remains ambiguous. The link between thyroid autoimmunity and a higher risk of miscarriage in women without a history of recurrent pregnancy loss (RPL) suggests that this link may also exist for women who have experienced RPL, despite the lack of definitive proof. The studies conducted on this topic in women with recurrent pregnancy loss have exhibited insufficient statistical power. ***(De Leo, S., and Pearce, E.N. 2018)***

6. Conclusion

It has become clear that the maternal immune system is intimately involved in the establishment, maintenance, development, and termination of normal pregnancy. In this context, it is logical and highly plausible that the immune system regulates all phases of pregnancy and possibly reproduction.

7- List of abbreviations

Anticardiolipin antibody (aCL)

Enzyme-linked immune-specific assay (ELISA)

Lupus anticoagulant (LA)

Antiphospholipid antibodies (aPLs).

Prothrombin time PT and (aPTT)

Recurrent pregnancy loss (RPL)

Russell's viper venom time (dRVVT)

Thyroid-stimulating hormone (TSH)

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