Original Article

Evidence for immunothrombosis according to some markers of thromboinflammation in women with unexplained recurrent reproductive failures

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ABSTRACT

Background: Unexplained recurrent reproductive failures (URRF) is a term that describes three different conditions of infertility, namely unexplained infertility (UI), unexplained recurrent miscarriage,and unexplained recurrent implantation failures. It is a global reproductive health problem. The aim of the present study was to compare some systemic markers of thromboinflammation in women with URRF to controls. **Materials and Methods:** This was a case–control study involving 70 subjects (35 cases of URRF and 35 healthy control women). The systemic cellular markers of immunothrombosis involving the platelet count, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), neutrophil-to-lymphocyte ratio (NLR), and the platelet-to-lymphocyte ratio were determined using the Sysmex XN-10 Automated Hematology Analyzer, Sysmex Corporations Japan. Data were analyzed using one-way analysis of variance in GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA) and presented as mean±standard deviation. Statistical significance was defined as $p<0.05$. **Results:** The MPV, PDW, and NLR were significantly increased ($p=0.002$, 0.007, and 0.028), respectively, in the subjects with URRF compared to the healthy controls. **Conclusion:** This finding supports evidence for immunothrombosis in patients with URRF.

Key words: Low-grade chronic inflammation, Low-grade intravascular coagulation, Unexplained infertility, Unexplained recurrent implantation failures, Unexplained recurrent miscarriage

Unexplained recurrent reproductive failures (URRF) are a clinical term that broadly defines the inability to conceive or the incapacity to maintain pregnancy to term in a healthy woman during her reproductive age [1-3]. It comprises three different clinical conditions of infertility, namely repeated pre-implantation failures that occur in a natural reproductive cycle (unexplained infertility [UI]) repeated implantation failures that occur in assisted reproductive therapy cycles (unexplained recurrent implantation failures [URIF]) and repeated post-implantation failures that occur in a natural reproductive cycle (unexplained recurrent miscarriages [URM]) [4,5]. It accounts for 80% of all cases of reproductive failures and 50% of all cases of infertility [6,7]. The etiology of URRF is considered a maternal disease involving failure of alloimmune crosstalk between the mother and the conceptus due to an underlying chronic inflammation, which results in a thrombotic state. Immunothrombosis describes the intricate crosstalk between low-grade chronic inflammation and low-grade intravascular coagulation. Research is currently geared toward understanding the role of immunothrombosis in maternal predisposition to microvascular failure and non-specific inflammatory reaction against the deposited sperm cells, the newly formed zygote, the implanting embryo, or the fetal allograft [8,9]. The platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) are efficient systemic cellular markers of low-grade chronic inflammation and low-grade intravascular coagulation (immunothrombosis) [10]. There is currently a paucity of data on the levels of these parameters in Nigerian women with URRF. The present study was therefore conducted to determine the levels of PLT, MPV, PDW, PCT, NLR, and PLR determined from the routine complete blood count in women with different categories of URRF compared to healthy controls.

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MATERIALS AND METHODS

Study Design, Population, and Setting

This was a case–control study involving 70 subjects (35 women with URRF and 35 healthy controls). The 35 cases of URRF comprised 12 women with UI, 13 women with URM, and 10 women with URIF. This study was carried out at the Enugu State University of Science and Technology Teaching Hospital between November 2022 and May 2023.

Subjects Exclusion Criteria

The following criteria were excluded from the study:

1. Women diagnosed with hematological disorders, hormonal disorders, infectious disease, thyroid disorders, autoimmune disorders, and systemic disorders like diabetes mellitus
2. Women with existing or previous ultrasonographic evidence of uterine malformations
3. Women with a history of smoking, contraception, alcohol, or substance abuse
4. Rhesus-negative women with rhesus-positive partners
5. Women with body mass index (BMI) >24.99 kg/m^2 and/or age >40 years.

Subjects Inclusion Criteria

The following criteria were included in the study:

1. A total of 35 apparently healthy women with good obstetrics and gynecology history who had recorded normal live births were chosen as the controls
2. A total of 12 women who failed to achieve a clinical pregnancy after at least 2 years of unprotected sexual intercourse and who has been diagnosed with normal results of fertility with normal partners were chosen as the cases for UI
3. A total of 13 women with a history of >3 repeated miscarriages were chosen as the cases for URM
4. A total of 10 women who failed to achieve a clinical pregnancy after a transfer of at least 3 good quality embryos in at least 3 assisted reproduction therapy cycles were chosen as the cases for URIF.

Sample Size

The sample size for the study was calculated using the Leslie Kish formula [11].

$$n = \frac{Z^{\alpha 2} PQ}{D^2}$$

where

n = Minimum required sample size when the population of the study is >10,000

Z^{α} = The α level of the coefficient interval or the standard normal deviate set at 1.96 corresponding to a 95% confidence interval

P = The proportion in the target population estimated to have URRFs [12].

D = The width of the confidence interval set at 0.05

Q = (1-p); the proportion of non-occurrence.

Substituting

$$n = \frac{1.96 \times 1.96 \times 0.58\ (1 - 0.58)}{0.05 \times 0.05} = 374$$

However, an estimate of 26 women with URRF attended the clinics in the past 1 year. Since this is <10,000, the sample size was adjusted using the formula.

$$nf = 1 + \frac{347}{26} = 24$$

where

nf = Calculated sample size

n = Minimum sample size

N = Population size

Substituting

$$nf = 1 + \frac{347}{26} = 24$$

Considering the anticipated response rate of 90%, the sample size was further adjusted to accommodate attrition using the formula

$Ns = Nf/r$

where

Ns = Adjusted sample size for response rate

Nf = Calculated sample size

Substituting

$$Ns = \frac{24}{0.9} = 27$$

Sample Collection and Preparation

Ten milliliters (10 mL) of venous blood samples were collected from each subject following standard venipuncture techniques. Five milliliters were dispensed into ethylene diamine tetra-acetic bottles for the determination of the PLT, MPV, PDW, PCT, and NLR.

Determination of the Parameters

The hematological parameters involving the PLT, MPV, PDW, PCT, NLR, and the PLR were determined using the Sysmex XN-10 Automated Hematology Analyzer, Sysmex Corporations, Japan. The sample was aspirated by letting the machine sample probe into the blood sample bottle and then pressing the probe button. Approximately 20 mL of blood was aspirated by the machine. The values of the PLT, MPV, PDW, and PCT were displayed in the screen after 30 s as part of the full blood count result. A printout copy of the results was released on the thermal printing paper while the values of the NLR and PLR were calculated manually from the values of the neutrophils, lymphocytes, and PLTs.

Statistical Analysis

Data were analyzed using one-way analysis of variance in GraphPad Prism version 8.0 (San Diego, California, USA) and presented as mean±standard deviation with statistical significance set at $p<0.05$.

Ethical Consideration

Ethical clearance was obtained from the hospital management ethical committee (ESUT/C-MAC/Vol 4/43) and informed consent from the subjects.

RESULTS

The results of the demographic information showed no significant differences for the age and BMI (p=0.212 and 0.366, respectively) between the subjects and controls (Table 1). The MPV, PDW, and NLR were significantly increased (p=0.002, 0.007, and 0.028), respectively, in the women with different categories of URRF (UI, URM, and URIF) compared to the control (Table 1). We also observed a non-significant increase in the PLT, PCT, and PLR (p=0.102, 0.690, and 0.096), respectively, for the women with different categories of URRF compared to the control (Table 1).

DISCUSSION

URRF occur both in the natural and assisted cycles of reproduction. In a natural cycle of reproduction, it is estimated that 60% of conceptions are lost in healthy women from fertilization to birth. On the other hand, it is estimated that the maximum chance of pregnancy per cycle in assisted reproductive therapy is as low as 20% in healthy couples [13]. Although it has been suggested that the coagulation cascade and inflammation pathways are closely linked together, the data on the role of low-grade intravascular coagulation and low-grade chronic inflammation in URRF are still a puzzling issue [14]. Low-grade chronic inflammation and low-grade intravascular coagulation are both pathological states lacking overt inflammation and coagulation but are characterized by continuous and unresolved activation of inflammation and coagulation, respectively [15]. The observed increase in the PLT, PCT as well as the PLR ratio in the women with different categories of URRF and a significant increase in the PDW, MPV and the NLR are suggestive of the occurrence of both low-grade chronic inflammation and low-grade intravascular coagulation in these patients. This could trigger an adverse maternal immune response to the deposited sperm cell, zygote, implanting embryo, and/or the fetal allograft [16]. During alloimmune response, inflammatory mediators particularly proinflammatory cells induce the activation of coagulation proteins, causing a decrease in natural anticoagulation proteins and a decrease in fibrinolytic activity resulting in a prothrombotic state [16]. The outcome of this is a failure of microvascular circulation which may result to failure of implantation, placental abruption, fetal growth restriction, and spontaneous abortion [17]. Some studies have reported that the *in vitro* fertilization program may induce coagulopathy in subjects which may affect the process of implantation during assisted reproductive therapy cycles [17,18]. This has been suggested to be intrinsic or due to the hormone treatment preceding the *in vitro* fertilization program [14]. This may actually account for the increased concentrations of the markers of low-grade chronic inflammation and low-grade intravascular coagulation particularly the PDW, MPV, and NLR recorded for our subjects with URIF. The significant increase in the PDW, MPV, and NLR recorded for subjects with URRF is not consistent with the findings of some studies which reported no significant differences in these parameters in subjects with recurrent pregnancy loss and recurrent implantation failures compared to healthy controls [19,20]. However, our finding is consistent with the findings of another study which recorded a significant increase in these parameters in Yemeni subjects with recurrent pregnancy loss compared to healthy controls [10]. A combined increase in the PDW, MPV, and NLR as recorded in the present study has been identified as an efficient marker of non-specific systemic inflammation and coagulation activation [10,21]. Thus, a combined effect of low-grade chronic inflammation and low-grade intravascular coagulation may be a common underlying mechanism responsible for the different conditions of URRF, namely UI, URM, and URIF.

The small sample size and the fact that the study was conducted in a single center are its limitations, which may have an impact on the validity of the data and the applicability of the findings.

Table 1: Markers of immunothrombosis in women with different categories of unexplained recurrent reproductive failures and healthy controls

Parameter	Control	UI (n=12)	URM (n=13)	URIF (n=10)	p-value
PLT ($\times 10^9$/L)	202.00±30.82	205.00±52.82	203.08±66.22	207.19±36.90	0.102
MPV (FL)	6.32±2.27	16.40±3.79	14.81±2.07	15.35±2.86	0.002*
PDW (FL)	1.91±2.11	16.68±2.63	15.30±4.20	16.06±4.41)	0.007*
PCT (%)	0.22±0.14	0.26±0.11	0.28±0.17	0.3±0.21	0.690
NLR	1.77±0.96	6.89±0.68	8.52±0.84	6.70±0.44	0.028*
PLR	6.09±1.61	6.48±1.52	6.61±1.43	6.67±1.41	0.096
BMI (kg/m^2)	22.01±4.74	21.96±2.8	22.06±3.73	22.02±4.1	0.366
Age(year)	26±7.8	26±5.3	25.88±2.9	25.71±4.6	0.212

UI: Unexplained infertility, URM: Unexplained recurrent miscarriage, URIF: Unexplained recurrent implantation failures, PLT: Platelet count, MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: plateletcrit, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, BMI: Body mass index. *Significant at p<0.05

CONCLUSION

The significant increase in the NLR, MPV, and PLR in the subjects with different categories of URRF in the present study suggests a role for immunothrombosis in patients with URRF; however, further randomized prospective studies are required to support the present findings. The combination of subjects from the three different categories of URRF, namely URM, UI, and URIF could be considered a strength in the present study as there are limited studies that have considered these three cases in a single study in literature. However, the small sample size as well as the use of a single center for the present study could be considered a limitation. Further large-scale studies are needed to support the present findings.

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