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Soluble and Cellular Inflammatory Predictive Markers Associated with Recurrent Pregnancy Loss Among Kazakhstani Women: a Pilot Study

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Abstract

Background: Recurrent pregnancy loss (RPL) is a common complication of pregnancy globally, characterized by multiple miscarriages but with poorly explained etiologies. Insofar as a state of low-grade inflammation (LGI) accompanies RPL, this study explores the link between RPL and markers of LGI among Kazakhstani women.

Methods: The retrospective study was conducted on 112 Kazakh women, comprising 64 with a confirmed diagnosis of RPL and 48 women with two or more uncomplicated pregnancies serving as controls. Statistical analysis was performed on SPSS 29 software.

Results: All tested blood analytes, including CRP, glucose, cholesterol, LDL-cholesterol, Hemoglobin, and RBC counts, were negatively associated with RPL. The only exception was neutrophil values having a positive association with RPL despite a lack of significant correlation between groups.

Conclusion: The study shows a marginal association between the LGI biomarkers considered and the overall risk factors of RPL in Kazakh women, which is in apparent contradiction with earlier studies. The absence of parallel studies in Central Asian countries hampers the analysis of study trends in related communities. Future case-control studies with more sample sizes are needed to explore the RPL biomarkers in depth.

Keywords: recurrent pregnancy loss, low grade inflammation, recurrent miscarriages, RPL markers, Kazakhstan.

Introduction

Miscarriage, defined as the spontaneous loss of a pregnancy before it reaches the viability stage, is a common complication of pregnancy, with 23 million miscarriages occurring every year globally, which translates to 44 pregnancies lost every minute [1]. Repetitive miscarriages, also designated as recurrent pregnancy loss (RPL), vary in frequency according to

the definition of RPL adopted by different international societies. While the American Society for Reproductive Medicine (ASRM) and the European Society of Human Reproduction and Embryology (ESHRE) characterize RPL as two or more miscarriages [2, 3], the Royal College of Obstetricians and Gynaecologists (RCOG) requires three or more failed pregnancies [4]. This translated to RPL prevalence of 2–4% among child-bearing women depending on the definition adopted [5]. Broadly speaking, RPL is classified into primary, secondary, and tertiary categories. The causes of RPL are multifactorial and include genetic (2–5%), anatomic (10–15%), endocrine causes (17–20%), autoimmune (20%), infections (0.5–5%), and environmental [6] factors. Despite identifying these and related RPL risk factors, 50–70% of all cases remain idiopathic [7], thus necessitating the search for additional causative factors of RPL.

Several well-controlled studies have reported on genetic risk factors for RPL but with varied outcomes. We recently reported on the association of the human forkhead Box P3 *(FOXP3)* gene and Human Leukocyte Antigen (HLA) class II genes with idiopathic RPL [8, 9]. It was also shown that a regulated inflammatory response is needed for a successful pregnancy [10]. While chronic low-grade inflammation (LGI) remains largely asymptomatic with a protracted progression, it plays a major role in various chronic disorders. These include cardiovascular and metabolic diseases, neurodegenerative conditions, cancer [11], and various pregnancy complications, including pregnancy loss [12].

It was suggested that LGI is a main contributor to the etiology of RPL [13, 14], highlighted by the suggested association of neutrophil activation, marked by elevated neutrophil counts and neutrophil-to-lymphocyte ratio (NLR), in women with a history of multiple miscarriages [14]. However, the precise cutoff values for these and related parameters remain uncertain necessitating further exploration. While no significant difference in CRP levels allegedly exists between women with RPL and the control group, serum CRP values were higher in RPL patients when serum CRP levels were evaluated in the context of *CRP* gene polymorphisms, as observed in women carrying the rs2794520 T allele [15]. This prompted the speculation that variation in the CRP may influence the risk of repeated miscarriages without affecting CRP levels *per se*. Other inflammation markers, such as neutrophil and lymphocyte counts and NLR, were higher in women with RPL in early pregnancy [10]. Nevertheless, there was no difference between the two observed groups in platelet, and WBC counts and hemoglobin (Hgb) levels [15].

A Turkish study established that unexplained infertility is positively correlated with TGs, LDL-cholesterol, total cholesterol, and CRP, in addition to baseline LGI biomarkers [16]. This research investigates the predictive potential of CBC parameters, CRP, and cholesterol levels as potential contributors to RPL. While studies on the association of LGI biomarkers with altered risk of RPL were reported for Asian, European, and USA subjects, no comparable investigations were done on Kazakhstani women. The present study aims to examine the association between RPL and the presence of LGI in the analysis of CBC parameters, CRP, cholesterol levels, etc. Due to the limited number of studies on this topic, this work will help enhance understanding of the RPL from the perspective of inflammation among women from Kazakhstan.

Material and methods

Study Design and Study Settings

This was a retrospective case-control study involving 112 Kazakhstani women and was conducted from September 2022 to January 2024. These consisted of 64 women with confirmed RPL diagnosis (Cases) and 48 multiparous women serving as Control. RPL definition per the ESHRE guidelines was used [3], with the inclusion criteria considered: women older than 18 with two or more pregnancy losses of unknown etiology with the same partner. The exclusion criteria for the case group included one or more spontaneous miscarriages, older age (> 40 years) at first pregnancy, and/or presence of female genital anatomical abnormalities. Women were also excluded if they reported prior or current autoimmune disorders, liver dysfunction, or managed ovarian hyperstimulation or artificial insemination (ART). Inclusion criteria for the control group were two or more successful pregnancies and deliveries, negative somatic diseases, or the receipt of infertility drug treatment throughout pregnancy.

Study Instrument

A questionnaire in Kazakh and Russian languages was provided depending on the participants' preferences and included sociodemographic and clinical data such as biochemical tests, CBC workups, as well as smoking and drinking habits, and was completed during patients' routine check-ups with a gynecologist. In addition to the questionnaire, available medical data of patients were also taken after an informed consent form was taken from each eligible woman for the inclusion criteria.

Study Variables

Independent variables included sociodemographic characteristics (age, ethnicity, education), and gynecological data such as menarche, menstrual dysfunction, surgeries, infections and illnesses, vaginal swab, and PAP smears. Pregnancy history, including the number of gestations and parity, and clinical and habitual data (smoking and alcohol consumption) were independent variables. Body mass index (BMI) on the height (m) and weight (kg) of study participants was calculated as kg/m2. Chronic illnesses (hypertension, diabetes, thyroid function, and venous thrombosis), and laboratory data (blood Hemoglobin levels, RBC, and WBC, neutrophils, lymphocytes, monocytes, platelet counts), and other analytes including erythrocyte sedimentation rate (ESR), CRP, HDL-cholesterol (HDL-c) and LDL-cholesterol LDL-c), and other vitamins C, D, and B12. The number of spontaneous miscarriages has been considered a dependent variable.

Statistical Analysis

Statistical analysis was done using SPSS v. 29 (IBM, Armonk, NY). The analysis included the calculation of mean and standard deviation to give a comparative description of each normally distributed variable between case and control groups. For the continuous variables student t-test and categorical variables, a chi-square test was performed. Estimation of the student t-test was performed for the parametric values, while for the non-parametric values Mann-Whitney "U" test was used.

Results

Table 1 displays sociodemographic characteristics and clinical profiles of RPL cases and control women. Age ($p = 0.579$), BMI ($p = 0.496$), and ethnic origin ($p = 0.648$) were

not statistically significantly different between the two groups. Most of the study subjects were Kazakh (> 95%), and the remainder were Russians and other ethnicities. The number of live births (p <0.001) were significantly lower, and the number of miscarriages (p< 0.001) were significantly higher in the case group compared to the control group.

Table 1 Sociodemographic characteristics and clinical profiles of RPL cases and control women

Variables	RPL cases (n = 64)	Control (n = 48)	P[3]
Age (years)[1]	33.3 ± 5.9	32.7 ± 5.5	0.579
BMI (kg/m^2)[1]	24.2 ± 4.2	23.7 ± 2.6	0.496
Ethnic origin[1]: Kazakh	50 (94.3)	42 (95.5)	0.648
Russian	2 (3.8)	2 (4.5)	
Others	1 (1.9)	0 (0.0)	
Oral contraceptive[2]: Barrier	2 (3.1)	3 (6.3)	0.507
Hormonal	4 (6.3)	5 (10.4)	
Irregular menses[2]	0 (0.0)	1 (2.1)	1.000
Aspirin intake[2]	6 (9.4)	2 (4.3)	0.463
Progesterone treatment[2]	14 (21.9)	3 (17.6)	1.000
LMW heparin[2]	6 (9.4)	0 (0.0)	0.334
Vitamin B supplements[2]	3 (4.7)	7 (14.6)	0.096
Abnormal Pap smear[2]	7 (10.9)	2 (4.2)	0.296
Smoking[2]	0 (0.0)	4 (8.3)	0.031
Alcohol[2]	0 (0.0)	3 (6.3)	0.076
Pregnancy outcome[1]: Gestation	4.44 ± 1.76	4.00 ± 1.50	0.169
Live births	0.37 ± 0.55	3.52 ± 1.13	<0.001
Still births	0.22 ± 0.45	0.00 ± 0.00	0.001
Miscarriages	3.83 ± 1.54	0.00 ± 0.00	<0.001
Ectopic	0.11 ± 0.44	0.29 ± 0.87	0.159
Artificial (IVF)	0.33 ± 0.92	0.27 ± 0.68	0.692

1. Mean ± SD
2. Number (percent total)
3. Student t-test (continuous variables), chi-square test (categorical variables)

Table 2 The biochemical and haematological characteristics of the study participants

Laboratory variables	RPL cases (n = 64)	Control (n = 48)	P[1]
Glucose[2]	3.9 ± 0.34	4.4 ± 0.50	<0.001
ESR[3]	10.11 (2.00 – 40.00)	4.00 (1.00 – 34.00)	0.068
CRP[3]	1.47 (0.10 – 4.60)	1.90 (0.88 – 2.54)	0.006
Albumin[2]	55.17 ± 8.82	43.35 ± 4.23	<0.001
TSH[3]	2.10 (0.87 – 3.54)	1.44 (0.27 – 3.99)	<0.001
TG (mmol/L)[3]	1.81 (1.00 – 3.80)	1.01 (0.50 – 3.28)	<0.001
Cholesterol (mmol/L)[3]	3.20 (1.80 – 9.90)	4.20 (2.30 – 7.30)	<0.001
HDL-cholesterol (mmol/L)[3]	1.42 (0.80 – 2.20)	1.35 (0.84 – 2.40)	0.062
LDL-cholesterol[3]	1.19 (1.00 – 3.20)	1.83 (1.10 – 3.82)	<0.001
Hemoglobin[3]	11.14 (8.6 – 13.7)	12.17 (8.5 – 15.5)	<0.001
RBC count[2]	3.09 ± 0.70	4.42 ± 0.57	<0.001
WBC count[2]	7.4 ± 3.38	6.42 ± 1.92	0.074
Neutrophils[2]	65.16 ± 11.99	63.41 ± 7.09	0.384
Platelet count[2]	257.54 ± 97.57	275.87 ± 78.72	0.299
Lymphocytes[2]	35.70 ± 11.23	29.86 ± 7.35	0.003

1. Student t-test for parametric, Mann-Whitney U-test for non-parametric
2. Mean ± SD
3. Median (range)

The biochemical and haematological characteristics of the study participants are represented in Table 2. HDL-c, WBC count, neutrophils, platelet count, and lymphocytes were not significantly different between Case and Control groups. RPL cases had significantly lower glucose (3.9 ± 0.34 vs. 4.4 ± 0.50 mmol/L) and cholesterol (3.20 (1.80 – 9.90) vs. 4.20 (2.30–7.30) mmol/L) levels compared with control women. TSH (2.10 (0.87 – 3.54) IU/ml) and TG (1.81 (1.00 – 3.80) mmol/L) values, were also higher in RPL cases compared to healthy participants. Similarly, LDL-c, Hemoglobin and RBC counts were significantly different between the two groups (p<0.001), with the case group (1.19 (1.00 – 3.20) mmol/L, 11.14 (8.6 – 13.7) g/dL, 3.09 ± 0.70 respectively) being lower compared to control women (1.83 (1.10 – 3.82) mmol/L, 12.17 (8.5 – 15.5) g/dL, 4.42 ± 0.57). CRP levels were not markedly altered (1.47 (0.10 – 4.60)) in some cases, while ESR values were greatly increased (10.11 (2.00 – 40.00)). Furthermore, albumin was significantly elevated in RPL cases (p<0.001) than in controls.

Table 3 illustrates haematological and biochemical indices for the RPL cases and control group. There is a significantly higher TG / Glucose and TG / HDL and HDL / LDL ratios in RPL cases than in control group (p < 0.001). In contrast, CRP / Albumin (p adj = 0.002), NLR (p adj = 0.004), and Platelet / Lymphocytes (PLR) (p adj = 0.001) ratios were significantly lower in RPL cases than in control women (Table 3).

Table 3 Haematological and biochemical indices for the RPL cases and control group

Index	RPL Cases	Controls	Z score	P	P_{adj}
TG / Glucose	0.46 (0.23 – 0.92)	0.24 (0.11 – 0.70)	2.992	<0.001	<0.001
CRP / Albumin	0.025 (0.00 – 0.01)	0.043 (0.02 – 0.06)	-2.411	0.016	0.002
TG / HDL	1.28 (0.56 – 2.55)	0.98 (0.33 – 3.55)	1.958	0.011	<0.001
HDL / LDL	1.17 (0.47 – 1.80)	0.67 (0.24 – 2.18)	2.411	<0.001	<0.001
Neutrophil / Lymphocytes	1.88 (0.66 – 5.00)	2.12 (0.97 – 5.54)	-2.237	0.025	0.004
Platelet / Lymphocytes	7.43 (2.62 – 20.09)	8.94 (3.13 – 27.86)	-2.761	0.006	0.001

Discussion

Many women worldwide experience repeated miscarriages, making it a significant global health concern [1], with half of its etiology remains poorly defined [6]. Recent consensus has been on linking RPL with a state of LGI [17]. This study furthers the understanding of the causes and risk factors of RPL in Kazakhstan, as no similar research has been done on women of specific geographical regions of Central Asia.

Results of this study demonstrate that glucose and cholesterol levels greatly vary (p-value < 0.001) between the two groups, prompting the speculation of altered metabolism in the etiology of RPL [18]. This was reminiscent of a prospective study on US women, which established hypercholesterolemia as a risk marker of RPL [19]. Despite RPL's association with inflammation being confirmed by many publications, according to Verit [16] and Yang [20], our results show unexpectedly decreased cholesterol levels in patients with RPL. The data obtained present a weak association of cholesterol with LGI and,

accordingly, RPL, suggesting that it cannot be a reliable source of biomarkers in diagnosing RPL.

The findings of altered HDL-c and LDL-c values in RPL appear contradictory to previous studies [16, 20], as we reported a higher median of HDL-c and decreased LDL-c in RPL patients. These diverges might be linked to diets, smoking, or medication intake that alters HDL-c and LDL-c values [21, 22]. Noteworthy, the RPL subjects' blood parameters specifically lowered Hemoglobin levels and RBC counts and increased TSH and TG values despite those parameters falling in the considered normal reference range [22–25]. Our study showed for the first time a significant range between groups for TSH in disagreement with TSH values established for Palestinian women, which are critical for normal embryonic and fetal development and overall healthy thyroid function, with negligible variations in RPL patients and control group [26].

The lack of differences in CRP values disagreed with studies done in the Netherlands [27] and Sweden [28], in which RPL women established significantly higher median CRP levels compared to healthy women. Despite previous studies evidencing elevated levels of CRP during normal pregnancy, and growing substantially in RPL cases [28] this study distinguishes by showing a weak association between CRP levels and RPL. However, consensus with this study was found in a study done in Turkey [15], claiming that there is no difference in CRP levels between groups. This study demonstrated opposite connotations probably due to the investigated study subjects' ethnical differences, because of the range in dietary patterns [29] and environmental exposures [30]. Also, as suggested by Guvey et al., (2021) the problem will be linked to some specific CRP genes that can cause RPL without increasing CRP values itself, because of this, there is a need to do a DNA test on CRP genes for further subsequent researches.

In a study done in China by Jiang et al., (2021) positive correlation was found between increased neutrophils, NLR, and RPL diagnosis, which was in line with our study [14]. In our study despite having negligible results (p=0.384) between the two investigated groups neutrophil values' mean was higher in RPL cases. However, NLR failed to show the same results being increased in the control group. This prompts neutrophils' importance in the diagnosis of RPL.

Study strengths and limitations. This retrospective study was the first to examine to association of RPL and LGI based on specific blood parameters among Kazakh women by contributing valuable knowledge to the healthcare of Central Asia which is considered an under-represented population. This study expands, even more, the growing body of evidence in this explored link between RPL and LGI because it focuses on a new population and gives a chance to build up even more applicable associations for more clinical interventions to emerge. This study's findings will help to better understand the pathogenesis of RPL and other related disorders. Also will aid in emerging new clinical therapies as insightful information on cholesterol, CRP, neutrophil values, and other blood parameters was shown.

This study was limited by a lack of sample size and scarcity of the information available in Central Asia to make comparisons for a comprehensive understanding of the RPL. Furthermore, the retrospective nature of the study which relies on previously collected data and raises the possibility of bias in data selection as well as other confounding variables might also affect the results of this study. The study considered CBC test results only but for more understanding of the RPL, specifically one of the important biomarkers CRP and its gene polymorphisms, DNA test results should be included in future studies.

Conclusion

This study examined Kazakh women's CBC blood parameters to assess the hypothesis stating that LGI is one of the contributing factors of RPL. However, the finding of this study established a weak association between inflammation markers such as cholesterol (including LDL-c and HDL-c), CRP, RBC, TSH, and RPL diagnosis, except for neutrophils which is in line with prior studies. Finally, well-designed clinical studies with a larger sample size are needed to gain a more comprehensive understanding of RPL and to identify new RPL biomarkers.

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Ethical Considerations: This study received approval from the Nazarbayev University Institutional Research Ethics Committee (NU-IREC) on 29/09/2022, #599/05092022. All study participants were informed about the study aims, methods, and potential risks and benefits. A consent form was introduced and taken from all study subjects of this research study.

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