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Does recurrent pregnancy loss have an inflammatory background?

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Abstract

Although several pathophysiological mechanisms are defined in etiology recurrent pregnancy loss, still causes of half of the cases haven't revealed yet. It is reported that inflammatory processes take place in the etiology of the disease. In our study, we aimed to reveal the relationship between recurrent pregnancy loss with white blood cell count (WBC), C-reactive protein (CRP) and ferritin levels. We included our study 90 pregnant women having recurrent miscarriage history and 101 pregnant women without recurrent miscarriages, 191 patients in total. Maternal and gestational age, height, weight, body mass index (BMI), gravidity, parity, abortion and living children count and WBC, CRP and ferritin levels of these pregnant were evaluated retrospectively. According to outcomes, while the age ($p = 0.01$; $p<0.05$), gravidity ($p = 0.00$; $p<0.01$) and abortion counts ($p = 0.004$; $p<0.01$) of the study group were found significantly to be higher than that of the control group, weight measurement of them was significantly lower than that of the control group ($p = 0.04$; $p <0.05$). Height and BMI measurements, parity and living children counts of the groups showed no statistically significant difference ($p>0.05$). While WBC levels of the study group was found to be lower ($p=0.045$, $p<0,05$) than that of control group, there was no significant difference regarding ferritin and CRP levels ($p> 0.05$). In our study, WBC, CRP and ferritin parameters did not indicate the inflammatory background in recurrent pregnancy loss. We think that further prospective randomized controlled studies are required regarding these parameters.

Keywords: C-reactive protein, ferritin, recurrent pregnancy loss, white blood cell count

1. Introduction

Clinically diagnosed miscarriage accounts for 15 to 25% of all pregnancies and most of them occur under 10 weeks due to chromosomal numerical abnormality (1). The Practice Committee of the American Society for Reproductive Medicine (ASRM) defines recurrent pregnancy loss as two or more clinical miscarriage (2). Less than 5% of women experience two consecutive pregnancy losses while 1% of them experience three consecutive pregnancy losses (1). Those with miscarriage are more likely to have miscarriage again (3). Many pathological mechanisms such as uterine anomalies, endocrine and metabolic problems, genetic anomalies, acquired or congenital thrombophilia and autoimmune diseases have been defined in the etiology of recurrent pregnancy loss. Unfortunately, the underlying pathological mechanism cannot be revealed in half of the patients (4).

An inflammatory microenvironment is needed for successful embryo implantation (5). However, when the inflammatory response is more than necessary, it causes pregnancy complications such as recurrent pregnancy loss, preeclampsia and preterm birth (6). In recurrent pregnancy loss, the immune response is handled in two ways: immune suppression and immune tolerance. Antigens expressed in fetal or placental tissues stimulate the alloimmune response. In contrast, T helper 1 and T helper 2 cells play an important role in immune tolerance and rejection response. The dominance of T helper 2 cells is important in the continuation of normal pregnancy (4). However, if T helper 1 dominance exists and there is an increase in the number and cytotoxicity of Natural Killer (NK) cells responsible for the relative excess of proinflammatory cytokines such as Tumor necrosing factor (TNF) α, Interleukin (IL) 1, 6 in blood and endometrium and reconstruction of vessels and trophoblasts, we may encounter adverse pregnancy outcomes such as recurrent pregnancy loss (7-9).

Ferritin is a protein that stores iron and is actually not involved in transport, and it is widely used to determine the iron status in the body. In inflammatory processes, serum ferritin levels increase due to ferritin release from the destructed cells. As a result of this mechanism, the high ferritin value is actually an indicator of an inflammatory process (10).

C- Reactive protein is a marker released from other cells such as hepatocytes and trophoblasts, indicating low-grade chronic inflammatory response (11). CRP level may increase

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in inflammatory conditions, cancer, asthma, diabetes, cardiovascular diseases as well as in adverse pregnancy outcomes (12-15). Although it increases slightly in the first four weeks of pregnancy compared to normal (16), it is found at higher levels in cases such as preeclampsia, preterm labor, intrauterine growth retardation (15, 17, 18).

Based on this information, in our study, we aimed to investigate the role of ferritin, CRP and White Blood cell Count (WBC), which are some of the inflammatory markers that can be detected easily and cheaply in blood, in the etiology of recurrent pregnancy loss.

2. Materials and methods

191 patients who applied to Samsun Gynecology and Obstetrics Hospital and Health Sciences University Samsun Training and Research Hospital Gynecology and Obstetrics Department between December 2016 and January 2020 were included in the study. Ninety patients who had 5-17 weeks gestational aged (median nine weeks) pregnancy and an intact gestational sac within the heart beats of the fetus could not be obtained by transvaginal ultrasonography and had two consecutive pregnancy loss between 7-10 weeks, included in the recurrent pregnancy loss group. Those with uterine abnormality, chromosome abnormality, thyroid dysfunction, toxoplasma, rubella, cytomegalovirus and herpes virus infection, diabetes, hypertension and autoimmune disease were excluded from the study. 101 women with a live, healthy pregnancy that gestational age matched (5-19 weeks median nine weeks) with the study group and having no miscarriage history were included in the control group. The study was planned as a retrospective cross-sectional study. Maternal and gestational age, height, weight, body mass index (BMI), gravidity, parity, abortion and living children count, ferritin, CRP and WBC levels of participants were obtained from previous hospital records and evaluated.

The ethical committee approval of Health Sciences University Samsun Training and Research Hospital Medical Specialization Training Board, dated 27/05/2020 and No GOKA 2020/7/28 was obtained for conducting the research. In addition, the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. Sample size was determined for α: 0.05 and ß: 0.80 by a biostatistics specialist in 19 Mayıs University. NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used when evaluating the study data. The suitability of quantitative data for normal distribution was tested by Kolmogorov-Smirnov, Shapiro-Wilk test and graphical evaluations. Student's t-test was used for comparing two groups of normally distributed quantitative data, and Mann Whitney U test was used for two-group comparisons of non-normally distributed data. Significance was considered at least $p<0.05$.

3. Results

The total age of 191 cases varies between 17 and 44, with an average of 29.03±0.87 years. Weight measurements of the cases ranged from 41 to 110 kg, with an average of 69.20±1.18 kg; their height varied between 1.45 and 1.92 m, with an average of 1.59 ± 0.09 m; BMI measurements ranged from 17.07 to 40.18 kg/m^2, with an average of 25.55 ± 4.17 kg/m^2. The gestational weeks of the cases ranged from 5 to 19 weeks, with an average of 9.52 ± 0.42 weeks. Gravidity counts range from 1 to 10, and the median was three pregnancies; the parity counts ranged from 0 to 6, the median was 1; the number of abortions ranged from 0 to 5, the median was 2 and the number of living children ranged from 0 to 5, the median was 1.

As shown in Table 1, while the ages ($p = 0.01$; $p <0.05$), gravidity ($p = 0.00$; $p <0.01$) and abortion counts ($p = 0.004$; $p <0.01$) of the study group were found significantly to be higher than that of the control group, weight measurement of them was significantly lower than that of the control group ($p = 0.04$; $p <0.05$). Height and BMI measurements, parity and living children counts of the groups showed no statistically significant difference ($p>0.05$). While WBC levels of the study group was found to be lower ($p=0.045$, $p<0.05$) than that of control group, there was no statistically significant difference between groups regarding ferritin and CRP levels ($p> 0.05$) is presented in Table 2.

4. Discussion

In almost half of cases of recurrent pregnancy loss, the underlying cause cannot be fully revealed despite all the examinations (19). For most of the cases in this category, autoimmune responses such as antiphospholipid antibodies, antinuclear antibodies, antithyroglobulin antibodies, the formation of antimicrosomal antibodies, and cellular immunological causes, including increased NK cell count and cytotoxicity and increased T helper 1/T helper 2 ratio were blamed (20).

According to the results of our study, there is no statistically significant difference in CRP levels between patients with recurrent pregnancy loss and the control group. In a prospective study by Sarı et al. (21), CRP and Growth Differentiation Factor 15 values were compared between healthy control group (including 45 pregnants) and repetitive pregnancy loss group (including 45 patients) with the same demographic characteristics. These two values were found statistically significantly higher in the recurrent pregnancy loss group. In another study, serum CRP levels and CRP gene polymorphisms were compared between the groups in a retrospective case control study including 275 recurrent pregnancy loss and 290 healthy control groups conducted by Ahmed et al. (22).

Table 1. Evaluation of demographic features by groups

		Total (n=191)	Recurrent pregnancy Loss (n=90)	Live pregnancy (n=101)	p
Age (years)	Min-Max (median)	17-44 (29)	17-44 (31)	19-42 (27)	**[a]0.01***
	mean±SD	29.03±0.87	30.26±1.37	27.93±1.07	
Weight (kg)	Min-Max (median)	41-110 (68)	41-96 (65)	45-110 (70)	**[a]0.04***
	mean±SD	69.20±1.18	67.20±2.97	70.99±2.57	
Height (m)	Min-Max (median)	1.45-1.92 (1.43)	1.5-1.8 (1.6)	1.4-1.9 (1.6)	**[a]0.187**
	mean±SD	1.59±0.09	1.60±0.05	1.59±0.10	
BMI (kg/m^2)	Min-Max (median)	17.07-40.18 (25.11)	17.06-36.13 (25.3)	18.72-40.18 (25.2)	**[a]0.11**
	mean±SD	25.55±4.17	25.80±4.27	24.40±4.07	
Gravidity	Min-Max (median)	1-10 (3)	2-8 (3)	1-10 (2)	**[b]0.000****
	mean±SD	2.99±1.73	3.53±1.33	2.50±1.90	
Parity	Min-Max (median)	0-6 (1)	0-3 (1)	0-6 (1)	**[b]0.13**
	mean±SD	0.95±1.14	0.83±1.83	1.05±0.22	
Abortion	Min-Max (median)	1-5 (1)	2-8 (3)	0-0 (0)	**[b]0.004****
	mean±SD	1.41±1.40	3.53±1.33	0	
Living children count	Min-Max (median)	0-5 (1)	0-5 (1)	0-5 (1)	**[b]0.917**
	mean±SD	0.98±1.08	1.03±1.16	0.97±1.06	

[a]; Student t Test, [b]; Mann Whitney U Test, **; p<0.01, *; p<0.05, BMI; Body Mass Index, Min; Minimum, Max; Maximum, SD; Standard deviation

Table 2. Evaluation of laboratory findings by groups

		Total (n=191)	Recurrent Pregnancy Loss (n=90)	Live pregnancy (n=101)	p
Ferritin (µg/L)	Min-Max (median)	3.9-162.7 (24.7)	4.1-147.2 (22.7)	3.9-162.7 (26.2)	**[b]0.340**
	mean±SD	34.10±29.12	30.83±26.09	35.36±30.17	
WBC (x10^3/µL)	Min-Max (median)	1.2-16.5 (8.9)	3.6-14.5 (8.8)	1.2-16.5 (9)	**[a]0.045***
	mean±SD	9.06±2.25	8.62±2.17	9.21±2.26	
CRP (mg/L)	Min-Max (median)	0.2-57.1 (5.5)	0.3-57.1 (4.8)	0.2-33.2 (5.7)	**[b]0.633**
	mean±SD	6.89±6.92	7.90±9.96	6.52±5.41	

[a]; Student t Test, [b]; Mann Whitney U Test, **; p<0.01, *; p<0.05, BMI; Body Mass Index, Min; Minimum, Max; Maximum, SD; Standard deviation

According to the results of this study, serum CRP values were found to be statistically significantly higher in patients with recurrent pregnancy loss compared to the control group, and it was demonstrated that this elevation was observed in those carrying the rs2794520 T allele. In addition, they stated that some CRP gene variants increase the risk of recurrent pregnancy loss without causing an increase in CRP levels. In a recent retrospective study by Weghofer et al. (23), preconceptional CRP values and genetic examination results of conception material of 100 infertile patients with missed abortion were evaluated. CRP values of individuals with euploid material were higher than those with aneuploid material. This finding has been interpreted that inflammatory process takes place in the etiology of euploid pregnancy loss more than those with aneuploid one. In our study, we consider that serum CRP values might be affected by inequality of such factors as socioeconomic status, dietary carbohydrate intake and smoking (24) between groups as a result of retrospective design of the study. Also possible existence of CRP gene polymorphism (22) in our study population might have influence on present results. Complete blood count parameters such as WBC change in parallel with the increase in T helper 1 and granulocyte count and decrease in T helper 2 and monocyte count throughout pregnancy (25). Macrophages and monocytes stimulate extravillous trophoblast invasion, spiral artery forming and the onset of delivery. However, dysregulation in these cells can lead to complications such as preeclampsia and preterm labor (26). Polymorphonuclear leukocytes stimulate tissue remodeling and angiogenesis at the site of infection and secrete defensin. When this infection occurs in decidua, it triggers endometritis, which causes recurrent pregnancy loss (27). In the study conducted by Baş et al. (28) including 325 women who had miscarriage and 245 given term birth, the whole blood parameters at the 6th gestational week were evaluated. They found that women having miscarriage had higher inflammatory markers such as neutrophil count, lymphocyte count and neutrophil lymphocyte ratio. As a result, they stated that these parameters could be used to predict the possibility of miscarriage in a pregnant woman. In addition, a retrospective study comprised of 120 patients with recurrent pregnancy loss and 120 healthy pregnants conducted by Yılmaz et al., inter-groups comparison was performed in terms of complete blood count parameters. While no statistically significant difference was found between the groups in terms of hemoglobin, platelet, Mean Corpuscular Volume (MCV) and WBC values, they found Mean Platelet Volume (MPV) values significantly higher in the recurrent

pregnancy loss group (29). Unlike both studies, we found WBC values to be lower in the study group. WBC value alone may not be a strong parameter in demonstrating inflammation in recurrent pregnancy loss. Moreover, although there are studies in the literature demonstrating intense inflammatory reaction and cell activation in the decidua of patients with recurrent or sporadic miscarriage (30, 31), the reflection of this inflammatory reaction in peripheral blood could not be seen in our study population.

Ferritin is used as an acute phase reactant showing inflammation (32, 33). In the literature, we have not found any studies demonstrating the relation between ferritin levels and recurrent pregnancy loss. In a cross-sectional study conducted by Gou et al. (34), 20 non-pregnant women, 27 pregnants at first trimester, 38 pregnants at second trimester and 36 were evaluated in terms of iron parameters. Ferritin levels of patients with spontaneous miscarriage were found to be statistically significantly higher than that of other groups. However, no statistically significant difference was found between recurrent pregnancy loss and control group in the results of our study.

The limitations of the study are being single center based and could not being controlled some parameters such iron replacement, dietary carbohydrate intake and socioeconomic status that could affect the outcomes of the study as a result of retrospective design. However, this is the first study evaluating the ferritin as an acute phase reactant in recurrent pregnancy loss.

Recurrent pregnancy loss is a condition that deeply wounds women and their families after every miscarriage. Unfortunately, although the etiology of only half of the disease is known, immune disorders are responsible for the vast majority of the unknown part. As a result of our study, WBC, CRP and ferritin parameters did not indicate the inflammatory etiology of recurrent pregnancy loss. Besides, we think that further prospective randomized controlled studies are required regarding these parameters.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

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