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Association of Epidemiological and Hematological Parameters with Repeated Spontaneous Miscarriages During First-Trimester of Pregnancy: A Case Control Study

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ABSTRACT

We aimed to investigate the association of first trimester repeated spontaneous miscarriages with hematological profile, blood group and age. This case-control study was conducted at the laboratories of Zoology department The Women University Multan and the Department of Hematology, Nishter Hospital Multan, from June 2016 to Dec 2019. A total of 500 participants, 300 with spontaneous abortion and 200 healthy pregnant controls in first trimester of pregnancy were included in this study. The data was collected through questionnaire at the time of sampling after obtaining informed consent. ABO and Rh blood group was identified by using Antisera (including Anti-A, Anti-B and Anti-D). The analysis of haematological indices was done by using automated haematological analyzer (Sysmex Co. Japan). The mean maternal age in this study was 30.35±7.94 years for cases and 28.53±6.59 years for controls. Highest frequency of miscarriages 90 (30%) was observed in cases of age group 35-39 while lowest frequency of miscarriages 30 (10%) was observed in age group 20-24. Whereas, highest frequency 94 (47%) and lowest 2(1%) of controls were observed in age group 25-29 and 15-19 respectively. Out of 500 women 438 (87.6%) were Rh positive (including 256 cases and 182 control) and 62 (12.4%) were Rh-negative (including 44 cases and 18 controls). The observed ABO distribution pattern was B>A>O>AB. Blood group B was most frequent (34%) including 18% cases and 16% controls. Percentage of Rh-negative was higher in cases than controls 14.6% vs 9% but ABO and Rh blood groups showed no association with RSM in this population p=0.372; p=0.08 respectively. Haematological evaluation revealed significant decrease in Hb ($p<0.001$), MCV ($p<0.001$) and MCHC ($p=0.006$) values in cases as compared to controls whereas no significant differences found between the case and control groups in terms of RBC, WBCs, HCT, MCH, neutrophils, lymphocytes, and PLT ($p>0.05$). It is concluded that the age groups 35-39 years and significant changes in the values of Hb, MCV and MCHC may be associated with repeated spontaneous miscarriage in this population.

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Key words
Repeated spontaneous miscarriage, Haematological parameters, ABO blood groups, First trimester miscarriage, Mean corpuscular hemoglobin concentration

INTRODUCTION

Worldwide, the most common adverse pregnancy outcome is miscarriage (Andersen *et al*., 2016) occurring in 15–20% of clinically recognized pregnancies (Poorolajal *et al*., 2014). Only half of the reported miscarriages can be clearly explained (Wang *et al*., 2002; Vitzthum *et al*., 2006). The strongest known risk factor for miscarriage is the maternal age. The youngest mothers have slightly elevated risk of miscarriage but rises sharply in older mothers (de La Rochebrochard and Thonneau, 2002). The need for studies regarding prevalence of blood groups is multipurpose, as in addition to their importance in evolution, association to disease and environment is being increasingly sought in modern medicine (Khan *et al*., 2004). The change in haematological profiles is one of the factors affecting pregnancy and its outcome. Therefore, haematological profile is measured all over the world because of its reliability, cost-effectiveness and to estimate general health status of individuals (Akinbami *et al*., 2013). The complete blood count (CBC) is an easily available, simple, and economic test including such parameters as red blood cells (RBCs), hemoglobin (Hb), hematocrite value (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBCs), platelets (PLT), neutrophil count and lymphocytes. Different studies examined the relation of

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CBC parameters with chronic diseases such as cancers and infections (Ege *et al*., 2013; Han *et al*., 2020). Other researchers worked on comparison of haematological parameters in pregnant and non-pregnant women (Elgari, 2013; Azab *et al*., 2017) and found significant results but only few studies are available based on comparison of hematological profile of women with spontaneous miscarriage to the healthy pregnant control group in first trimester (Akdemir *et al*., 2013; Bas *et al*., 2018).

A few case-control studies regarding early miscarriage were conducted in Pakistan, including only some risk factors and different parameters (Khaskheli *et al*., 2010; Jaleel and Khan, 2013) but no work has been done in Southern Punjab regarding hematological parameters in women with repeated miscarriages. To increase the understanding of the risks for early miscarriages, we conducted a case-control study with the aim of finding relationship of clinico-pathological parameters including haematological profile, blood groups and age in first trimester repeated spontaneous miscarriage (RSM).

MATERIALS AND METHODS

A hospital-based case-control study was carried out from June 2016 to December 2019, including 300 cases (women diagnosed with repeated spontaneous abortion) and 200 controls (women with normal pregnancies) visiting local hospitals of Multan (Nishter Hospital, Fatima Jinnah Hospital and Bukhtawar Amin Hospital). The inclusion criteria for cases were the women with at least two consecutive spontaneous abortions and that of controls was the women with at least 2 normal pregnancies and no abortion history. The women who have had only one abortion, ectopic pregnancy, induced abortion, un-recognized pregnancies, known medical illness were excluded from this study.

After obtaining an informed consent from the study participants, the required information was obtained through a questionnaire and blood samples were collected. A total 3-4 ml blood was drawn and placed in EDTA tube for further analysis. Blood groups were identified by using corresponding Antisera. Three drops of blood were taken on a slide and placed on paper labelled with Anti-A, Anti-B and Anti-D Antisera. Then, Anti-A was added on first drop, Anti-B on second and Anti-3 on third drop and mixed to observe agglutination within two minutes (Wazirali *et al*., 2005). The equipment used for analysis of complete blood counts was done by an automatic haematological analyzer (Sysmex Co. Japan). The calculated parameters included RBCs, WBCs, HCT, MCV, MCH, MCHC, PLT, Hb, lymphocytes and neutrophils. Laboratory work was performed at Department of Hematology, Nishter Hospital Multan. Ethical approval of this study was obtained from the research ethics committee of The Women University Multan (Ref. No.: WUM/UREC/0009, dated October 28, 2020).

Data was analyzed by using SPSS (version 20). The data in the text and tables was mentioned in percentages and mean ± standard deviation (SD). For statistical analysis of categorical data Chi square test was used. To compare the hematologic values among cases and controls, independent t-test was applied. In all statistical analyses, p-value of ≤ 0.05 was considered to be statistically significant.

RESULTS

Frequency of case and controls in different age-groups

In this study, the maternal mean age was 30.35±7.94 years for cases and 28.53±6.59 years for controls (Table III). The age of women was distributed into six groups (Table I). The age ranged from 15 to 44 years in both groups. The results showed highest number of abortions in women between 35-39 years old and lowest number of miscarriages observed in age group 20-24. Similarly, highest number of controls was observed in age group 25-29 and lowest number of controls seen in age group from 15-19 (Table I). Age is significantly associated with RSM ($p<0.001$).

Table I. Analysis of age groups in cases and control women (p-value represents results of Chi square test).

Age groups (Years)	Control (%)	Cases (%)	Chi square value	p value
15 - 19	02 (1.0)	43 (14.3)	105.61	<0.001***
20 - 24	41 (20.5)	30 (10.0)		
25 - 29	94 (47.0)	46 (15.3)		
30 - 34	35 (17.5)	61 (20.3)		
35 - 39	25 (12.5)	90 (30.0)		
40 - 44	3 (1.5)	30 (10.0)		

*p ≤0.05, **p ≤0.01, ***p≤0.001, p^{ns}, non-significant.

Distribution of ABO and Rh (Rhesus) blood group system among cases and controls

The clinical and laboratory findings of women with RSM and controls are given in Tables II and III. Among Rh positive women 256 were cases and 182 were controls. Similarly, among Rh negative women there were 44 cases and 18 controls. Blood group A^+ included 72 (24%) case and 58 (29%) control women whereas B^+ 82 (27.33%) cases and 61 (30.05%) controls. AB^+ consisted of 48 (16%) cases and 31 (15.05%) controls and O with 54(18%) cases and 32 (16%) controls. Among Rh^- blood groups A^- included

15 cases (3.0%) and 4 (2.08%) controls, B^- comprised of 20 (6.66%) cases and 7 (3.05%) controls, AB^- blood group 4 cases (1.33%) and 2 controls (1 %) and O^- blood group 5 cases (1.66%) and 5 (2.5%) controls. Statistical analysis showed no association of ABO blood group phenotypes with RSM (p=0.372) (Table II).

Table II. Analysis of ABO blood types in cases and controls (p-value represents results of Chi square test).

Blood groups	Controls (%)	Cases (%)	Total (%)	Chi square value	p value
Rh^+	182(91.0)	256 (85.3)	438 (87.6)	3.321	0.081^{ns}
Rh^-	18 (9.0)	44 (14.6)	62 (12.4)		
A (Total)	62 (29.0)	87 (24.0)	149 (29.8)	7.565	0.372^{ns}
A^+	58 (29.0)	72 (24.0)	130 (26.0)		
A^-	04 (2.0)	15 (5.0)	19 (3.8)		
B (Total)	68 (16.0)	102 (18.0)	170 (34.0)		
B^+	61 (30.0)	82 (27.3)	143 (28.6)		
B^-	07 (3.0)	20 (6.6)	27 (5.4)		
AB (Total)	33 (16.5)	52 (17.3)	85 (17)		
AB^+	31 (15.0)	48 (16.0)	79 (15.8)		
AB^-	02 (1.0)	04 (1.3)	06 (1.2)		
O (Total)	37 (30)	59 (19.6)	96 (19.2)		
O^+	32 (16.0)	54 (18.0)	86 (17.2)		
O^-	5 (2.5)	05 (1.6)	10 (2.0)		

*p ≤0.05, **p ≤ 0.01, ***p≤ 0.001, p^{ns}, non-significant.

Table III. Comparison of demographic and haematological profile of case and control women (p-value represent t test analysis).

Parameters	Controls (n=200)	Cases (n=300)	p value
	Mean ± SD	Mean ± SD	
Age (years)	28.53±6.59	30.35±7.94	0.06^{ns}
RBCs ($x10^6$/ µl)	4.2±0.8	4.3±2.3	0.394^{ns}
Hb (g/dl)	11.9±4.9	10.5±4.1	.001***
HCT%	34.5±12.2	34.8±25.2	0.887^{ns}
MCV (µ3)	75.6±14.7	74.6±9.7	.001***
MCH (pg)	26.7±4.3	26.6±3.9	0.464^{ns}
MCHC (g/dl)	32.8±5.6	31.1±4.3	0.006**
WBCs ($x10^3$/µl)	10.8±5.2	11.0±3.5	0.551^{ns}
Neutrophils%	72.2±36.3	71.1±10.6	0.64^{ns}
Lymphocytes%	23.9±8.8	25.0±10.2	0.162^{ns}
Platelets (10^3/µL)	264.9±79.4	278.1±84.9	0.081^{ns}

*p ≤0.05, **p ≤ 0.01, ***p≤ 0.001, p^{ns}, non-significant.

Overall, in study population the high frequency of women with both Rh negative and Rh positive blood was observed in blood group B followed by A, O and AB. Blood group B+ was the most frequent 143 (28.6%) and AB- was the least frequent 6 (1.2%) (Table II).

Haematological profile of women with RSM and control pregnant women

The comparison of blood count parameters in the case and control women is given in Table III. The results showed significant decrease in Hb (p<0.001), MCV (p<0.001) and MCHC (p=0.006) value in cases than controls, indicating an association with RSM. However, statistically no significant difference was observed between the groups for RBC (p=0.394), WBCs (p=0.551), HCT (p=0.887), MCH (p=0.464), neutrophils (p=0.64), lymphocytes (p=0.162), polys (p=0.178) and PLT (p=0.178).

DISCUSSION

This case control study included epidemiological and hematological profile of women in first trimester with repeated spontaneous miscarriages. A previously conducted study in Karachi, Pakistan reported mean maternal age 27.6±4.9 years in cases and 26.5±4.5 years in controls in women with first trimester repeated miscarriages (Jaleel and Khan, 2013). Our study showed mean maternal age 30.35±7.94 years for cases and 28.53±6.59 years for controls was higher than findings of Jaleel and Khan (2013) but lower than reported by Maconochie *et al*. (2007) 31.9 years for cases and 30.0 years for controls. The current study demonstrated that the maternal age >35 years was risk for RSM. A prospective study among Norwegian population, included 421201 pregnancies and the least miscarriage frequency/ risk (10%) was seen in women 25-29 years old and the risk elevated quickly after 30 years with up to 53% in the women aged 45 and above (Magnus *et al*., 2019). Similar results produced from a previously conducted UK population-based case-control study, below the age of 35 years they found no difference in odds of miscarriage (p= 0.73) but afterward the odds rose sharply with an increase of 75% for mothers aged 35-39 years (Maconochie *et al*., 2007). Another study included both maternal and paternal age and reported increased risk of miscarriage if the age of woman was ≥35 years regardless of the age of the man (de La Rochebrochard and Thonneau, 2002). In Denmark, a nationwide cohort included 369,516 singleton pregnancies and concluded that the risk of adverse pregnancy outcomes increased by several factors, but advanced maternal age makes a high proportion of the overall risk (Frederiksen *et al*., 2018). According to Andersen *et al*. (2000) decades ago, low fertility or high parity was the main cause for

pregnancy in older age women. But now a days there is a delay in childbearing due to social reasons. The risk of SA was 8.9% in 20-24 years old women and 74.7% in those aged 45 years or more (Andersen *et al*., 2000). A possible reason for association of advance age with RSM may be the increasing age is directly related to the number of pregnancies and is sometimes be associated with weakening of uterine function ultimately leads to an increased RSA risk. The quality of the egg and weakening/decrease of environment of the womb favoring implantation are also responsible for the elevated risk of SA (Hu *et al*., 2018).

In present study the blood group B was found non-significantly high in cases than controls (p=0.372). A study carried out in Pune city (India), included couples with repeated abortions and investigated the frequency and incompatibility of ABO blood group phenotypes with repeated abortions. The results indicated the most frequent blood group in women was B (Malekasgar, 2004) is in agreement with results of this study. However, the finding of this study was inconsistent with a study carried out in Al-Nassiriyah City (Iraq), determined the association of ABO blood group and spontaneous abortion. They observed increased number of individuals with blood group A in patients with repeated abortion (Al-Fartosi, 2008). A possible explanation to this inconsistency may be the high frequency of women with both Rh negative and Rh positive blood was in blood group B in this population. A previously conducted study in Pakistan indicated high fertility and survival for mothers with B-positive blood groups (Shami and Sultana, 1980) contrasts with results of current study. As cases in this study were non-significantly more frequent with blood type-B. In this study, we could not compare the blood groups in couples to confirm ABO incompatibility and among maternal blood groups, no group showed association with RSM in this population (p=0.372). Similar results reported by Akdemir *et al.* (2013) as they found no evidence of any group of ABO blood system a risk for recurrent miscarriage (Akdemir *et al*., 2013).

Regarding Rh (D) antigen in this study, the total women lacking Rh antigen in their blood were 12.4%, slightly higher than percentage reported in most of earlier studies conducted in Pakistan, 7.55% in Punjab (Khan *et al*., 2006), 5.25% in Baluchistan (Hussain *et al*., 2001), 7.6% in Sindh (Irshad *et al*., 2020), 10.7% in Bannu (Khan *et al*., 2004). However, frequency of Rh-negative blood in Khyber Pakhtunkhwa 12.44% (Khattak *et al*., 2008) is in agreement with present study.

Although variations occur in different geographical and ethnic groups with respect to ABO blood group distribution but blood group O dominates all, followed by A blood group whereas rarest one is the AB (Alemu *et al*., 2014; Haileamlak *et al*., 2012) which is similar with the results of this study except the predominant phenotype in this study is B. In this study the overall ABO distribution pattern was B>A>O>AB same for Rh^+ and Rh^- (case and controls). Similar to this study B>A>O>AB distribution pattern was also reported from other studies in Bannu, Peshawar and Karachi Pakistan (Khan *et al*., 2004; Nazli *et al*., 2015; Irshad *et al*., 2020). The second most commonest phenotype was blood group A in this study, with frequency of 29.8% that is close to 28.3% as described by a study in Libya (Azab *et al*., 2017) but different from 34% observed in study conducted in Nepal (Pramanik and Pramanik, 2000) where the predominant phenotype was A.

The results of two previously conducted studies by Rehman *et al*. (2015) and Mehmood *et al*. (2005) in Multan showed that the most prevalent phenotype was B with frequency 34.15% and 36.95%, respectively, which is in agreement with present study showing overall frequency 34% of blood group B (Rh^+ and Rh^-). The trend of blood group distribution in both studies was B>O>A>AB, similar to finding of this study but second prevalent phenotype O varies from our findings. The variation may be due to study design as they included both genders and all age groups but we included women of reproductive age only.

Studies assessed the effect of maternal Hb concentrations on subsequent miscarriage produced conflicting results. A study examined the relation of Hb concentrations with an adverse outcome and found that Hb values >13·2 g/dl and <10·4 g/dl both high and low were associated with poor outcome of pregnancy (Murphy *et al*., 1986). Two systematic review and meta-analysis suggested higher risk of poor pregnancy outcomes with low Hb concentration (Sukrat *et al*., 2013; Young *et al*., 2019). In this study significantly low Hb values were obtained in women with RSM are in concordance with these studies but in contrast to work carried out in Srilanka (Abeysena *et al*., 2010) as they found no evidence of association of anemia during pregnancy with any of the adverse pregnancy outcomes. A recent study investigated the relationship between MCHC and recurrent miscarriage and confirmed a link between MCHC and recurrent pregnancy loss (Erdem *et al*., 2020) is in agreement with results of this study. In already published literature MCHC has been proved an independent prognostic factor of acute myocardial infarction. In addition to this, low MCHC level were significantly associated with hospital mortality among patients with acute myocardial infarction (Perlstein *et al*., 2009). Further investigations are needed to evaluate the relation between low MCV and MCHC level as observed in cases of this study during first trimester miscarriages. A case control study conducted in Turkey showed the patient

and control groups were not significantly different for Hb, HCT and MCV values but significantly higher values of WBC, RDW, PLT and PCT were found in patients compared to control group (Aynıoglu *et al*., 2016). Contrary to these results, in present study significant decrease observed in Hb and MCV values and no difference found in WBC and PLT values of women with RSM compared to controls. A cross-sectional study carried out on pregnant women indicated no significant association between platelet count and increase in gestational age (p= 0.296) (Akinbami *et al*., 2013). Another study investigated the relation of mean platelet volume to abortions included three groups (threatened miscarriage, missed abortion and control) they found no statistical difference (p=0.125) between groups (Eroglu *et al*., 2013) are consistent with the results of this study in term of platelets count (p=0.081). A study in Yemen, found significantly higher platelets count among recurrent pregnancy loss patients when compared to the controls (Al-Aghbary *et al*., 2018) is different from results of this study.

Study limitations

We lacked access to paternal data for blood groups to check ABO incompatibility. Despite limitations, this was the first study that evaluated the association between CBC count and RSM in women with repeated miscarriages and healthy control pregnant women in Pakistan.

CONCLUSION

It is concluded that age group from 35-39 years and significant decrease in the values of Hb, MCV and MCHC in cases compared to controls may be the possible risk factors for RSM. We recommend the need of free of cost hemoglobin determination and CBC evaluation in antenatal care centers, especially for pregnant women with history of early miscarriages and in high age-groups in order to reduce risk of RSM.

Author's note

This study is a part of doctoral thesis of Nazia Haider.

IRB approval and ethics statement

University Research Ethics Committee (UREC) in its meeting held on 14-01-2020 has certified the approval of the project entitled "Possible risk factors for repeated spontaneous abortions among local Hospital Population of Multan" submitted by Nazia Haider.

The project proposal certifies that the research proposal fulfils all the necessary requirement of ethics of research on human beings/ or any other living organism.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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