ORIGINAL ARTICLE

JAK 2 MUTATION IN RECURRENT FOETAL LOSS

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Background: The frequency of recurrent fetal loss (RFL) is around 1% of the total pregnancy. Workup is recommended in patients after three consecutive fetal losses. A multitude of chromosomal, environmental, structural immunological and hematological factors can result in RFL. Frequency of pregnancy loss is increased in MPD patients carrying JAK2 V617F mutation. In fact, JAK2 mutation is an independent risk factor for pregnancy complication and fetal loss. This study was conducted to assess the role of the JAK 2 mutation in recurrent pregnancy loss.
Methods: This was a case control study conducted in Armed Forces Institute of Pathology, Rawalpindi. Using un-matched case-control situation a sample size of 216 cases and 216 controls was calculated using the WHO sample size calculator with assumption of 95% confidence interval, 1:1 ratio of cases to control, expected proportion of mutation as 0.10% and 1.06% in control and cases respectively. DNA analyses were performed and results were recorded done by kit method. The data was entered and analyzed using SPSS version 18.0. Odds ratio was calculated to assess the association between JAK2 V617F mutation and recurrent fetal loss.
Results: Three out of 216 cases were positive for JAK 2 mutation while one control had positive JAK 2 mutation. The prevalence of JAK2 mutation in cases of RFL was 1.38% (95% C.I 0.58–2.17%). The odds ratio for JAK 2 mutation in cases and controls was 3.028 (95% C.I of 0.28–76.13) and a *p*-value of 0.623. Conclusion: JAK2 positive females are 3.03 times more at risk of having RFL as compared to JAK2 negative pregnant females. JAK2 mutation testing may be recommended for inclusion in the workup to manage RFL

Keywords: Recurrent Fetal Loss; JAK 2 mutation; JAK 2; Abortion

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INTRODUCTION

Recurrent foetal loss (RFL) is defined as loss of three or more consecutive pregnancies in the first trimester.[1] Although foetal loss is the commonest complication of pregnancy the prevalence of three consecutive pregnancy losses in women is not more than 1% of the total pregnancies.[2] It is a rare problem if we look at the prevalent figures. But if we look at the social and psychological burden associated with this problem; it has a significant effect on the population.

It is generally agreed that a workup for possible causes of RFL is indicated in most patients after three consecutive foetal losses and is reasonable in some women over the age 30–35 years with two foetal losses.

Pregnancy involves many factors that govern the development and viability of the foetus and an alteration in any of these factors can result in loss of pregnancy. A multitude of chromosomal, environmental, structural immunological and haematological factors can result in RFL.[3,4] The meta-analysis performed for the identification of the aetiology of RFL showed that the myeloproliferative disorders (MPD) contribute significantly to its pathogenesis. Pregnancy loss is the established complication in patients with either ET or PV. Its incident is increased in MPD patients carrying JAK2 V617F mutation. In fact, some studies show that JAK2 mutation is an independent risk factor for pregnancy complication and foetal loss.[5] A few studies have been done to assess the relationship of recurrent foetal loss with JAK 2 mutation but no such study is available on the population in Pakistan.

This case control study was planned with an objective to assess the role of the JAK 2 mutation in recurrent pregnancy loss.

MATERIAL AND METHODS

It was a case control Study done in Armed Forces Institute of Pathology, Rawalpindi. Its objective was to study association of JAK 2 Mutation and recurrent foetal loss. Sample size was calculated by using the statistical package Epi info version 3.4. Using un-matched case-control situation a sample size of 216 cases and 216 controls have been achieved. The following parameters were used for calculation of sample size.

- Confidence Interval=95%
- Not ill/ill ratio=1:1
- Expected proportion of exposure in not ill=0.10%
- Percent exposure among ill=1.06%

Five millilitres (5 ml) venous blood was collected in tubes containing Ethylene-diamine-tetra-acetic acid (EDTA). The haemoglobin, platelet count and DNA analysis were performed and results were recorded. Samples will be analysed for haemoglobin and platelets on Haematology Analyzer Sysmex KX-21 and DNA extraction from whole blood is done by kit method (Gentra USA).

First RBCs were lysed and removed. Then proteins were precipitated and DNA was extracted from supernatant by precipitation and hydration. This extracted DNA was stored at -20 °C. PCR was performed on this DNA.

The PCR was done in 25µl reaction mixture containing 2.5 µl of 10 x PCR buffer, $MgCl_2$ 25 mM, dNTPs 200 mM, primers (10 pmol each), Taq DNA polymerase 5U/µl and template DNA (about 100 ng).
Primer used: a common reverse primer
(5-CTGAATAGTCCTACAGTGTTTTCAGTTTCA)
and a forward specific primer
(5-AGCATTTGGTTTTAAATTATGGAGTATATT)
and a forward control primer
(5-ATCTATAGTCATGCTGAAATAGGAGAAAAG).

The amplified products were subjected to polyacrylamide gel electrophoresis. Gel was prepared by mixing 10 ml acrylamide (6%) with 100 µl ammonium per sulphate (APS) solution and 20 µl tetramethyl ethylene diamine (TEMED). It was allowed to stand for 15–20 minutes. The enzyme digested amplified products were loaded on the gel withLoading dye (1mg bromophenol in 40% sucrose solution). Electrophoresis was performed for 20 minutes at 200V.

Gel was stained by silver nitrate for 10–15 minutes. Then washed with distilled water and counter stained with sodium hydroxide formalin for 2–3 minutes. Gel was dried in a gel drier for 20 minutes. Known positive and negative controls for the mutations were included in each batch to validate the methodology of DNA testing.

The data was entered and analysed using SPSS version 18.0. Mean±SD was calculated for quantitative variables. Frequencies, percentages and graphs were prepared for qualitative variables.
Odds ratio was calculated to assess the association between JAK2 V617F mutation and recurrent foetal loss, Chi-square test was applied to ascertain the strength of association and *p*-value <0.05 was considered significantly associated with an increased risk of pregnancy loss.

RESULTS

Cases and Controls were compared for history of abortion, consanguinity, parity status and mean live births in their families. Both the groups had a statistically significant difference in terms of their family abortion history, parity status and mean live births. The difference in the cases and controls in terms of consanguinity was not statistically significant.

The mean Hb level in the study subjects was 12.29±1.314 g/dl and ranged from 8.30 g/dl to 16 g/dl. In cases the mean Hb level was 12.22±1.33 g/dl and 12.35 g/dl±1.29 in controls.

The mean platelet count in the study population was 304.61±85.14 $x10^9$/L and ranged from 151 and 542 $x10^9$/L. In cases the mean platelet count was 307.66 $x10^9$/L while it was 301.56 $x10^9$/L in controls.

The cases and controls were compared in respect of JAK2 mutation as shown in the table-3, Three out of 216 cases were positive for JAK 2 mutation while one control had positive JAK 2 mutation. The prevalence of JAK2 mutation in cases of RFL was 1.38% with 95% C.I of 0.58–2.17%.The odds ratio for JAK 2 mutation in cases and controls was 3.028 with 95% C.I of 0.28–76.13 and a *p*-value of 0.623

Table-1: Basic Characteristic of both the cases and controls

Characteristic		Cases (%)	Controls (%)	*p*-value
Age (Mean± SD)		29.12±4.54	28.64±4.48	0.264
Education level	No Education	61 (28.2)	50 (23.1)	0.454
	Primary	49 (22.7)	45 (20.8)	
	Middle	45 (20.8)	59 (27.3)	
	College	53 (24.5)	51 (23.6)	
	University	8 (3.7)	11 (5.1)	
Occupation	House wife	158 (73.1)	145 (67.1)	0.302
	Office Work	48 (22.2)	62 (28.7)	
	Self employed	10 (4.6)	9 (4.2)	
Residence	Urban	143 (66.2)	136 (63)	0.546
	Rural	73 (33.8)	80 (37)	
Socieo- economic status	Upper Class	37 (17.1)	33 (15.3)	0.102
	Middle Class	135 (62.5)	154 (71.3)	
	Lower Class	44 (20.4)	29 (13.4)	

Table-2: Comparison of mean haemoglobin and platelet count in cases and controls

Characteristic	Cases	Controls	*p*-value
Haemoglobin (g/dl)	12.22±1.33	12.35±1.29	0.317
Platelet count (x10^9/L)	307.66±88.19	301.56±82.07	0.458

Table-3: Odds ratio of JAK 2 mutation in cases and controls

JAK2 Mutation		Cases	Controls	Total	Odds ratio for JAK2 mutation	*p*-value
	Yes	3	1	4	3.03	0.623
	No	213	215	428		
		216	216	432		

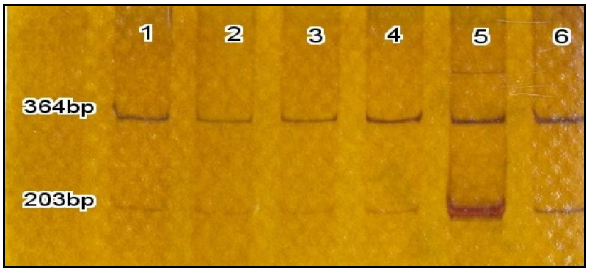

Figure-1: Silver Nitrate stained gel showing JAK 2 mutation

The PCR internal control is represented by a 364 bp fragment whereas the mutation is represented by a 203bp fragment. The lanes 1, 2, 3, 4 & 6 show negative results while lane 5 shows positive result.

DISCUSSION

The V617F mutation of the Janus Kinase 2 gene is an acquired mutation and its relationship with MPDs is well established. The prevalence of JAK2 mutation in general population is estimated to be in the range of 0.2–1.2.[6,7] Different studies have reported prevalence of JAK2 mutation in general population ranging from 0.2–1.2%.[6,7] The prevalence of JAK2 mutation in the control group in our study was 0.42. It was in the range of the JAK2 mutation in normal population as in other studies. Although the participants in our study were free of any MPD and as there is a higher prevalence of JAK2 mutation in MPDs[8] so we expect a lower prevalence in the control group as compared to the general population but the results were contrary to our expectations. This may be attributed to a smaller sample size in our study.

In addition to JAK2 mutation both the cases and controls were compared for some other factors such as consanguinity and family history of abortion. The effect of consanguinity on foetal loss and mortality and morbidity of offspring is the subject of controversy. In our study we investigated the cases and controls for consanguinity, our study results showed that consanguinity is not a factor which affects the morbidity of the offspring. The results were in consistence with some other studies such as a retrospective cross sectional study on 469 couples with foetal loss by ISA, A.R in which 237 (50.53%) couples had consanguineous union and 232 (49.47%) of couples had no consanguinity.[9] In another study the maternal characteristics and obstetric outcome of 92 Qatari women in a consanguineous relationship and with an obstetrical history of three or more early pregnancy losses were compared with those of 92 non-consanguineous women from the same population and with the same obstetrical history, matched for maternal age. The retrospective investigation showed no difference in the rate of previous pregnancy loss and maternal disorders. The prospective study showed no difference in the rate of subsequent pregnancy loss and the median gestational age and foetal weight at delivery in ongoing pregnancies.[10]

Specific factors in a couple's history may influence the recurrence risk following RFL.[11] Any couple who has suffered a miscarriage may have a definable and treatable cause for pregnancy loss. However, pregnancy loss is a common event occurring in up to 30% of all pregnancies. The decision to pursue a workup for pregnancy loss therefore becomes an individual one based on the couple's age, the number of miscarriages they have had, a family history of recurrent miscarriages, or known risk factor for recurrent pregnancy loss. Usually an evaluation is started after two pregnancy losses.[12–14] In our study we investigated both the cases and controls for history of abortion in their family. The results showed that a significant difference exists in the family history of abortion in cases and controls, with cases having a greater frequency of abortion in their families which implies that those women having a family history of abortion may need to be worked up for recurrent pregnancy loss. This finding cannot be linked to JAK2 mutation because it is an acquired mutation[8] but it does points to some familial causes for RFL.

The prevalence of JAK2 mutation in the cases of recurrent foetal loss in our study was 1.38%. Other studies have reported the prevalence of JAK2 mutation in females having recurrent foetal loss as 1.06%[15] while the prevalence of JAK2 mutation in females with MPDs has been reported to be up to 49%[5].

In our study we compared the prevalence of JAK2 mutation in cases and controls, i.e., in women having recurrent foetal loss and those without any foetal loss. Females having recurrent foetal loss were found to be having greater prevalence of JAK2 mutation, i.e., 1.38% as compared to controls (0.42%). The odds ratio of JAK2 mutation in cases and controls was 3.028. The results of this study imply that those having JAK2 mutation are 3.028 times more at risk of having RFL. The results are statistically non-significant but the odds of developing recurrent foetal loss in JAK2 mutation results are consistent with some previous studies such as reported by Passamonti *et al*[5] in which JAK2 positive patients had an odds ratio of 2.02 (95% CI: 1.1–3.8) of developing complications in comparison with JAK2 negative patients indicating that patients carrying the JAK2 mutation have higher risk of developing pregnancy complications. Similarly, Mercier *et al*[15] detected JAK2 V617F mutation in few women; however, the mutation occurred more frequently in patients with pregnancy loss (1.06%) than in control subjects (0.20%). The mutation was significantly associated with the risk of foetal loss, which occurred in 23 case subjects as compared with 5 control subjects (odds ratio, 4.63; 95% confidence interval:1.76–12.2; p=0.002).[15] The results being non-significant points to the need of conducting further studies involving participants form multiple centres with a larger sample size. On the other hand, Gangat, N in his study reported that no association exists between JAK2 mutation and pregnancy loss.[16] The study reports that pregnancy loss was similar between JAK2V617F-positive (4 of 10 pregnancies) and JAK2V617F-negative (4 of 10 pregnancies) patients (p>0.9). Furthermore, among 5 cases of 3 consecutive miscarriages, 4 were JAK2 V617F-negative.[16]

CONCLUSIONS

Prevalence of JAK2 mutation in cases of RFL was 1.38%, while it was 0.42% in woman without RFL in our study participants which shows JAK2 positive females are 3.03 times more at risk of having RFL as compared to JAK2 negative participants of our study. Further studies, with larger sample size and at multiple centres, are needed to prove the significance and its generalizability to the population at large. JAK2 mutation testing may be recommended for inclusion in the workup of RFL cases and research may be directed towards development of JAK2 mutation inhibitors to prevent the physical, social and psychological consequences of RFL.

AUTHORS' CONTRIBUTION

RI: Write-up, data collection, concept. UF & MZH: Review, Write-up, Analysis. SM: Write-up, review

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