ABO Genotype and Risk of Recurrent Pregnancy Loss in Carriers of *FVL* in Palestinian Population

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ABSTRACT---- ***The aim of this study was to define ABO genotypes in the Palestinian population and to assess the impact of ABO blood group genotypes and FV Leiden and we investigated whether predisposition to RPL was higher in non-OO genotype carriers than in OO genotype carriers. The study included 209 subjects suffered from RPL : 85 FV Leiden carriers as case group and 124 as a control group. Genotyping to 5 common alleles of ABO blood groups was performed by allele specific polymerase chain reaction with specific primers (AS-PCR). The results showed that there was no association between non-OO blood group genotypes and the risk of RPL(odds ratio [OR] 1.4, 95% confidence interval [95% CI] 0.74-2.6). Comparison of OO genotype and non-OO genotype carriers between case and control subjects yielded a higher OR in A_1A_1 / A_1A_2 blood group genotypes (OR: 2.2, 95% CI: 0.6- 8.19), followed by A_1O_1 / A_1O_2 (OR:1.6,95% CI: 0.77-3.55) B_1B_1/ B_1O_1/ B_1O_2 (OR: 1.3, 95% CI: 0.6-2.76) and A_1B/A_2B (OR: 1.2, 95% CI: 0.33-4.00) which did not reach statistical significance. The results of ABO genotyping showed no statistically significant difference in the frequency of OO and non-OO genotype carriers between the case group who had FVL and the control group (OR: 1.4, 95% CI : 0.74-2.6).***

Keywords – Recurrent pregnancy loss , factor V Leiden, *ABO* genotypes

1. INTRODUCTION

The *ABO* gene which located on chromosome 9[1] , codes for several glycosyltransferases, α1,3-N-acetyl galactosaminyl-transferase and α 1,3-D- galactosyl transferase that add sugar residues to the H antigen thus forming the A and B antigens respectively [2-4] . The presence of the A and B blood group antigens, expressed on red blood cells and other cells and molecules within the body, has been associated with susceptibility to diseases like cancer, leukemia, cardiovascular disease and risk of both arterial and venous thrombosis. In 1943, Levine had identified ABO incompatibility as a cause of early abortions and stillbirths [5] , many studies have been published reporting the recurrent pregnancy loss due to blood group incompatibility . Recurrent pregnancy loss (RPL) is the syndrome that causes repeated miscarriage, stillbirth, and premature delivery impairing the ability to have a live birth [6]. Most RPL are due to chromosomal abnormalities, and other conditions which may favor the production of spontaneous abortion such as pelvic infections, diabetes, thyroid disease and thrombophilia [7]. Thrombophilia was identified as a major cause of RPL, after chromosomal abnormalities with a rate of up to 40% , especially in the first half of pregnancy [8]. The thrombophilias are a number of prothrombotic factors, which can either be inherited or acquired. The inherited thrombophilias include activated protein C resistance due to factor V Leiden (FVL) mutation, protein S deficiency, protein C deficiency, antithrombin III deficiency, prothrombin mutation and hyperhomocysteinaemia [9,10]. Factor V Leiden (FVL) mutation is the most common cause has been implicated as risk factors of hereditary thrombophilias which in turn can result in placentation. Most studies indicated an increased risk of thrombosis associated with the non-O blood group [11,12].Usually blood group phenotypes are used to study the association between blood group and venous thrombosis. Blood group genotypes may be more informative since genotypes can distinguish between heterozygous and homozygous carriers of *A*, *B* and *O* alleles and between A_1 and A_2 alleles. The association of ABO blood groups and diseases resulting in coagulation impairment and venous thrombus formation was first described by Jick et al [13].

Risk of thrombosis has been shown to be increased with higher levels of vWF and FVIII, and it is through the effect of ABO antigens on vWF clearance that *ABO* genotypes are hypothesized to affect thrombotic risk[14-19]. The ABO blood group has a profound influence on hemostasis, as described by Preston and Barr in 1964 [20].

2. MATERIALS AND METHODS

To study the role of the ABO blood group as a genetic thrombotic risk factor associated with recurrent pregnancy loss. A total of 209 patients suffered from recurrent pregnancy loss recruited from the Islamic University-Genetics Laboratory. After viewing the medical file for all subjects; 124 were negative for *FVL* which used as control group ,while 85 were carriers for *FVL* mutation as case group . The study was approved by the local ethics committee and signed consent was obtained from all participants. Genomic DNA was extracted and purified from whole blood .A four separate-reaction multiplex allele specific polymerase chain reaction (AS-PCR) was used to determine the *ABO* genotypes in both groups , case and control as previously described[21] were take place in University College of Science and Technology .

Data and statistical analysis

Results were interpreted and the *ABO* genotypes were determined according to the collective pattern of PCR products that reported by L. Saqer and F. Sharif (2013).
The odds ratio (OR) and their 95% confidence intervals (95% CI) were calculated.

3. RESULTS

ABO blood group frequency

This study showed that group A was the most frequent blood group : 37.90% versus 44.71% for control and case respectively (Table1).

ABO Alleles and Genotypes

The method used for blood group genotyping differentiates A_1, A_2, O_1, O_2, and B alleles. We identified all possible genotypes except the *cis*-ABO_1 genotype , O_1 was the most frequent allele among studied groups (control = 124 ,case = 85). The distribution of *ABO* genotypes and alleles in the groups studied are shown in Table 2.
The frequencies of the five alleles in the our sample population were : O_1 and O_2 alleles

: 0.399 and 0.177, respectively , while A_1 : 0.161 , A_2: 0.089, B : 0.174 in control group. In case group , the frequencies of A_1, A_2, B, alleles are :0.218 , 0.082 , 0.182 respectively, and 0.259 for both O_1 and O_2 alleles .(Table3)

OO and non-*OO* genotype in study population

The comparison between non- *OO* and *OO* carriers showed that genotypes with *A* and *B* alleles had elevation in *FVL* carriers . OR was 2.2 (95% CI: 0.6-8.19) for A_1A_1 / A_1A_2 genotypes, 1.6 (95% CI: 0.77-3.55) for A_1O_1 / A_1O_2 ,1.3 (95% CI: 0.60-2.76) for B_1B_1/
B_1O_1/ B_1O_2 and 1.20 (95% CI:0.33-4.00) for A_1B/ A_2B . A_2 homozygous (A_2A_2) and A_2O

combinations (A_2O_1 and A_2O_2) had low odds ratio (OR: 0.86, 95% CI: 0.30 - 2.46) with no any statistically significance .(Table4)

4. DISCUSSION

Numerous studies have shown the influence of the ABO blood group on the risk for venous thromboembolic disease; individuals with A, B or AB blood groups are at a higher risk than individuals of blood group O[7,22]. ABO allele O_1 was more frequently in the control group than in the cases. The distribution of blood group alleles in the control group was not significantly different to the distribution within the Palestinian population found by L. Saqer and F. Sharif (2013).

The result of our study suggest that no increased risk estimate for the non *OO* blood group [OR 1.4 95% CI= 0.74-2.60] of RPL compared with *OO* blood group carriers. The analysis of non *OO* carriers genotype showed that the highest frequency genotype among *FVL* carriers and control were: A_1O_1 / A_1O_2 (29.41%) genotype followed by B_1B_1/ B_1O_1/ B_1O_2 (25.88%) . Therefore, the significant risk of pregnancy loss was higher

in the carriers of A_1 alleles than in those carrying *B* alleles in *FVL* carriers . Based on the study results, A_2 allele had the lowest frequency among study population, as demonstrated in table3.

In FV Leiden carriers our data indicate that carriers of blood group alleles A_1 and *B* have low association in risk of early pregnancy loss with OR:1.5 (95% CI: 0.89-2.5) and OR:1.03 (95% CI: 0.5-2.1) respectively . Our results suggest that the combination of non-OO blood group with *FVL* does not significantly increase the risk of RPL. In contrast, our results confirm the significant contribution of *FVL* and early RPL which agreement with other studies shown in table 5 . Some case-control studies did not show an association between *FVL* and RPL [23]. The carrier of *FVL* have small effect on the APC sensitivity and may lead to increase thrombosis risk that result to venous stasis may occur at the end of the first trimester, due to enhanced compliance of the vessel walls by a hormonal effect [23].To our knowledge, this is the first study to assess the effect of *ABO* genotype on RPL in *FVL* carriers.
In conclusion, there is no clear evidence that the *ABO* genotypes have any impact on RPL in carriers of the *FVL* mutation.

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Table 1 : The frequency of ABO blood group among case and control

Blood group	Control (n=124)	Case (n=85)
A	47 (37.90%)	38 (44.71%)
B	32 (25.81%)	22 (25.88%)
AB	8 (6.45%)	5 (5.88%)
O	37 (29.84%)	20 (23.53%)

Table 2: Distribution of *ABO* genotypes .

Blood Group	Genotype	Control N=124	Case N=85
A	A_1A_1	3(2.40%)	2(2.35%)
	A_1O_1	15(12.10%)	12(14.12%)
	A_1O_2	12(9.70%)	13(15.29%)
	A_1A_2	2(1.60%)	4(4.70%)
	A_2A_2	2(1.60%)	2(2.35%)
	A_2O_1	11(8.90%)	2(2.35%)
	A_2O_2	2(1.60%)	3(3.53%)
B	B_1B_1	3(2.40%)	4(4.70%)
	B_1O_1	22(17.7%)	9(10.60%)
	B_1O_2	7(5.60%)	9(10.60%)
AB	A_1B	5(4.00%)	4(4.70%)
	A_2B	3(2.40%)	1(1.18%)
O	O_1O_1	16(12.90%)	5(5.89%)
	O_1O_2	19(15.30%)	11(12.94%)
	O_2O_2	2(1.60%)	4(4.70%)

Table 3: Distribution of *ABO* Alleles

Allele	**Control**	**Case**
A_1	40(0.161)	37(0.218)
A_2	22(0.089)	14(0.082)
B	43(0.174)	31(0.182)
O_1	99(0.399)	44(0.259)
O_2	44(0.177)	44(0.259)

Table 4 : *ABO* genotypes risk for RPL

ABO genotype	No. of case(%) n=85	No. of control(%) n= 124	OR	95% CI	P-value
$O_1O_1/O_1O_2/O_2O_2$	20(23.53)	37(29.84)	1*		
Non- *OO*	65(76.47)	87(70.16)	1.4	0.74-2.60	0.3
A_1A_1 / A_1A_2	6(7.06)	5(4.03)	2.2	0.60-8.19	0.2
A_1O_1 / A_1O_2	25(29.41)	28(22.58)	1.6	0.77-3.55	0.19
A_2A_2/ A_2O_1/ A_2O_2	7(8.24)	15(12.10)	0.86	0.30 - 2.46	0.78
B_1B_1/ B_1O_1/ B_1O_2	22(25.88)	31(25.0)	1.3	0.60-2.76	0.48
A_1B/ A_2B	5(5.88)	8(6.45)	1.20	0.33-4.00	0.8
Allele					
O (O_1 and O_2*)*	88(0.518)	143(0.576)	1*		
Non O (A_1,A_2 and *B)*	82(0.482)	105(0.424)	1.3	0.86-1.90	0.23

*Reference category