

Original Research Article

Association between ABO Blood Groups and Genetic Risk Factors for Thrombosis in Sudanese Women with Recurrent Spontaneous Abortion

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ABSTRACT

Aim: The purpose of this study was to define the association between ABO blood groups and genetic risk factors for thrombosis in Sudanese women with recurrent spontaneous abortion.

Materials & methods: The study is a prospective analytical case control study was conducted in Omdurman Maternity Hospital (Sudan). Hundred Sudanese women who experienced three or more of the adverse pregnancy outcomes during their reproductive age were selected. ABO typing was performed by saline techniques in tubes, slide or micro-plates, by testing the patient's or donor's red cells with Anti-A and Anti-B, and/or the serum or plasma with A cells, B cells and O cells, for detection of factor V Lieden (FVL) prothrombin (FII) and Methylene tetra hydrofolate reductase (MTHFR) mutations used polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Results: women with recurrent miscarriage had blood group O which is the highest no of cases blood group; the next are the women of A blood group with (15%) ($p=0.25$), B (9%) ($p=0.14$) and finally 6% women had blood group AB ($p=0.16$). There is no significant association between A,B, AB and O blood groups and risk factor of thrombosis (FVL, Prothrombin and MTHFR).

Conclusion: we conclude that carriers of O blood group mainly develop thrombosis and recurrent abortion compared with that found in A, B and AB blood group. FV Leiden mutations associated with group O carriers are at an additional thrombotic risk and recurrent abortion.

Keywords: ABO system, F V Leiden, Prothrombin, MTHFR.

INTRODUCTION

The pregnancy associated with hypercoagulability sets a foundation for hemostatic abnormalities during pregnancy and may be associated with pregnancy complications. ^[1] Thrombophilia is considered still a debated problem that may be common in women with

unexplained recurrent pregnancy loss, with prevalence as high as 65% in selected populations. ^[2] The thrombophilias are a number of prothrombotic factors, which can either be inherited or acquired. The influence of thrombophilia in pregnancy is a popular research topic in recurrent miscarriage. The inherited thrombophilias

include activated protein C resistance (95% due to factor V Leiden (FVL) mutation), protein S deficiency, protein C deficiency, antithrombin III deficiency, FII (prothrombin) mutation, hyper homocysteinaemia and methylene tetrahydrofolate reductase MTHFR) gene [3] Factor V Leiden (FVL) and prothrombin (G20210A) mutations are the 2 most common causes have been implicated as risk factors of hereditary thrombophilias which in turn can result in placentalation. [4] ABO gene is located on chromosome 9 and its inheritance is explained by the Mendel and Bernstein three-allele theory. Methods of serologic typing allow the determination of 6 main phenotypes: A, B, A2, A2B, AB, and O. [5] the association of ABO blood groups and diseases resulting in coagulation impairment and venous thrombus formation was first described by Jick et al. [6] Most studies performed to date have generally agreed that non-OO blood group carriers have a higher risk of thrombosis than OO blood group carriers. [7]

Factor V Leiden (FVL) and ABO (H) blood groups are the common influences on hemostasis and retrospective studies have linked FVL with pregnancy complications. [8]

MATERIALS AND METHODS

The study is a prospective analytical case control study was conducted in Omdurman Maternity Hospital (Sudan). Hundred Sudanese women who experienced three or more of the adverse pregnancy outcomes during their reproductive age were selected. The control group included ninety four healthy women who attended the same medical facilities (mean age was 30 ± 4 years) with at least more than 2 normal pregnancies and without any history of adverse pregnancy outcome or recurrent miscarriages.

Exclusion criteria: Woman who had any of the following criteria were excluded from the Study groups: A history of

vascular thrombotic disease, fetal congenital anomalies, fetal chromosomal anomalies, uterine abnormalities, a known causes of the abortion and women known to have the mutation.

Data Collection: The study group data collected using structure questionnaire to collect information about age, parity, medical and obstetric history, smoking, family medical and obstetric history, residency and relative marriage.

Method:

Sample collection: Five ml venous blood was collected from each participant into EDTA and tri-sodium citrate container after consent was obtained from each participant blood Specimens were labeled with patient name, medical record number, date and time of collection, and then sent at room temperature to the laboratory.

ABO Typing: ABO typing was performed by saline techniques in tubes, slide or micro-plates, by testing the patient's or donor's red cells with Anti-A and Anti-B, and/or the serum or plasma with A cells, B cells and O cells. [9]

Genomic DNA isolation from EDTA blood samples was performed by the commercial spin column procedure QiaAmp DNA Mini kit (Qiagen, Hilden, Germany).

Detection of Factor V Leiden gene mutation (G1691A) gene: Extracted genomic DNA was tested for the presence of FVL mutation used polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).8 In brief, a 267-basepair (bp) segment of the factor V gene was amplified used specific forward primer (5'TCA GGC AGG AAC AAC ACC AT-3') and reverse primer 5'GGT TAC TTC AAG GAC AAA ATA CCT GTA AAG CT 3. [10]

Detection of prothrombin gene mutation (G20210) gene: For detection of G20210 prothrombin gene mutation, a 345-bp genomic DNA fragment encompassing a part of the prothrombin gene that contains the mutation was amplified by PCR using

specific primers Forward (5'TCT AGA AAC AGT TGC CTG GC-3') and Reverse primer (5'ATA GCA CTG GGA GCA TTG AAG C-3) [11]

Methylene tetra hydrofolate reductase (MTHFR) Gene Mutations: PCR will be carried out to made millions of copies of a specific DNA fragment consisting of a known functional mutation in the MTHFR gene by used site specific primers Forward (5' TGA AGG AGA AGG TGT CTG CGG GA-3') and

Reverse primers: 5'AGG ACG GTG CGG TGA GAG AGT G -3'. [12]

Data analysis: Data were entered and analyzed by SPSS programmme (version: 17.0). All demographic data of the study population were presented as mean ± SD in the text and Odds Ratio was used for detecting the power of relationship between the determinant and the outcome and 95% confidence interval was calculated. Data were analyzed using the Chi-square test for comparison the prevalence of MTHFR, Prothrombin *gene* and FVL mutation between patients and controls, also Chi- square was used to analyze association between ABO blood group and thrombophilia risk factor (The test considered significant when P.value <0.05)

RESULTS

A total of 100 cases of recurrent spontaneous abortion not exceeding 20 weeks of gestation were observed at the Omdurman maternal Hospital. The maternal blood type was distributed according to time of pregnancy loss as follows: group O shows the most

frequency among repeated time of recurrent pregnancy loss, fallow by group A was frequent in all time from twice to eight times of recurrent abortion, while group B and AB show the lowest frequency according to time of recurrent abortion (Table 1). Maternal age was divided into 5 major groups (17-24,25-29,30-34,35-39 and ≥ 40). Abortions rates among these groups were represented by 38(18.8), 26(12.9%), 39(19.2%), 40(19.8%) and 59(29.2%), respectively. (figure 1) (69%) (p=0.29) of women with recurrent miscarriage had blood group O which is the highest no of cases blood group; the next are the women of A blood group with (15%) (p=0.25), B (9%) (p=0.14) and finally 6% women had blood group AB (p=0.16). The blood group of the cases in this study has been compared with control group 94women the blood group O shows the maximum number in control with (61.7%). B type women stand second with (16%), A type account for (9.6 %) and AB type with (11.7%). According to prevalence of blood group among the test group, this indicates no significant difference between this group and the control group. (Table 2) There is no significant association between A,B, AB and O blood groups and risk factor of thrombosis (FVL, Prothrombin and MTHFR) (Table 1)

Table 1: Comparison of blood group carries in risk for recurrent pregnancy loss

Blood group	Patients N (%)	Controls N (%)	P-value	OR (95%CI)
O	69(69.0)	58(61.7)	0.29	1.38(0.76 to 2.50)
A	15(15.0)	9(9.6)	0.25	1.67(0.69 to 4.02)
B	9(9.0)	15(16.0)	0.14	0.52(0.22 to 1.26)
AB	6(6.0)	11(11.7)	0.16	0.48(0.17 to 1.36)

Table 2: Comparison of blood group with times of recurrent pregnancy loss

Times of recurrent abortion	Blood group				Total N (%)
	O N (%)	A N (%)	B N (%)	AB N (%)	
Twice	4(5.8)	3(20)	1(11.1)	0	8(8.1)
Three times	45(65.2)	4(26.7)	7(77.8)	4(66.7)	60(60.6)
Four times	13(18.8)	4(26.7)	1(11.1)	2(33.3)	20(20.2)
Five times	6(8.7)	1(6.7)	0	0	7(7.1)
Six times	0	1(6.7)	0	0	1(1.0)
Seven times	0	1(6.7)	0	0	1(1.0)
Eight times	1(1.4)	1(6.7)	0	0	2(2.0)

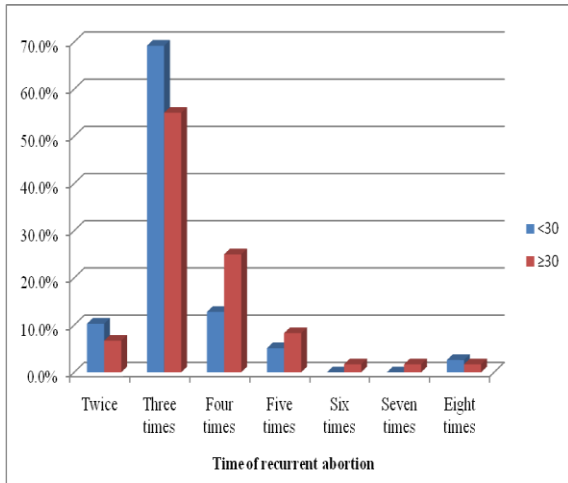


Figure 1: Distribution of time of recurrent pregnancy loss according to age group

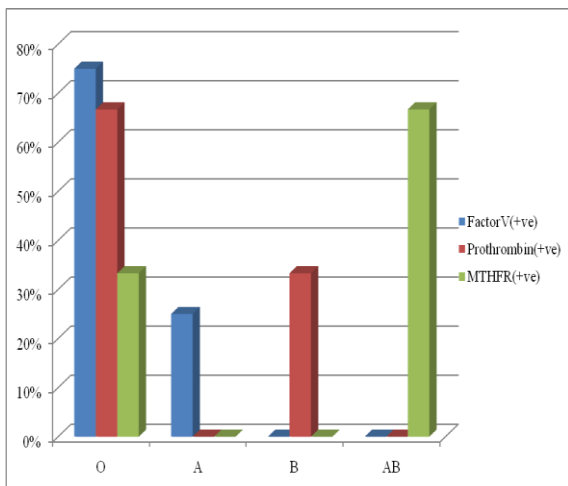


Figure 2: Frequency of Factor V, Prothrombin and MTHFR mutation in relation to the blood group

DISCUSSION

Spontaneous abortion is the most common complication of pregnancy, exploring the relation between ABO blood group, Factor V Leiden, Prothrombin and methylene gene mutation with recurrent miscarriages is a challenge. This is due to the fact that recurrent miscarriages are with multiple etiologies, where genetic factors are considered one of those etiologies. Advances in molecular genetics technology provide an accurate and reliable tool to precisely study the genetic abnormalities associated with many diseases. Several studies identified thrombophilia as the principal cause of recurrent pregnancy loss.

In our study group O shows the most frequency among repeated time of

recurrent pregnancy loss, follow by group A was frequent in all time from twice to eight times of recurrent abortion, while group B and AB show the lowest frequency according to time of recurrent abortion this result was agreed with other studies on a considerable number of women affected by miscarriage showed that the mothers with blood group O and fetus with blood group B could cause spontaneous abortion. [13] The association of ABO blood groups and diseases resulting in coagulation impairment and venous thrombus formation was first described by Jick et al. [14] The results of the present study indicate that there was no significant association between A, B, AB and O blood group and thrombosis risk factor in women with recurrent spontaneous abortion. As group O has greatest distribution in this study so the most existed mutations of FV, prothrombin and MTHFR associated with group O, accordingly group O associated with an increased risk factors for thrombophilia and recurrent abortion, this results were disagreed with most studies performed to date have generally agreed that non-OO blood group carriers have a higher risk of thrombosis than OO blood group carriers (4-7). Wu et al [15] Combination of O blood group and FV Leiden mutation carrier state is a risk factor for the development of thrombosis.

In present study we found strong combination between MTHFR mutation and AB blood group. Isolated methylenetetrahydrofolate reductase C677T mutation is not a risk factor for thrombosis unless being associated with some other genetic or acquired risk factor, as demonstrated in our study as well as in other related studies. [16]

The most common inherited prothrombotic risk factors with significant impact on the development of thrombosis are mutations of the genes encoding proteins involved in the coagulation cascade, such as FV Leiden mutation and G20210A mutation of the prothrombin

gene. [17] In addition to coagulation factors, mutation of the methylenetetrahydrofolate reductase gene, known to influence the development of atherosclerosis and vascular diseases, has also been extensively investigated. [18] It should be noted that the thrombogenic impact of homozygosity for methylenetetrahydrofolate reductase C677T mutation is only pronounced in case of its association with mild or moderate hyperhomocysteinemia and the genetic mutations mentioned above. [19] Our study has some limitations, such as the relatively small number of patients and controls, due to the non-existence or low frequency of prothrombotic carriers among participants.

CONCLUSION

The main conclusion of this study is that carriers of O blood group have a mainly association predisposition to develop thrombosis and recurrent abortion compared with that found in A, B and AB blood group. FV Leiden mutation carriers are at an additional thrombotic risk and recurrent abortion, higher in O blood group carriers. ABO blood grouping in blood donors could produce valuable results for use in epidemiologic and anthropologic studies in our population and which could serve as a basis for future research on the association between ABO system and various diseases.

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Conflict of interests: Authors declare that this study has no conflict with their interests.

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