Correlation of Hematologic parameters with factor VIII levels in obligate female carriers of Hemophilia A

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ABSTRACT

Objective: To compare and correlate hematological parameters (complete blood picture, prothrombin time, activated partial thromboplastin time, blood group and factor VIII levels) in obligate female carriers of Hemophilia A with control women.
Study Design: A cross-sectional comparative study
Place and Duration: Department of Pathology, Army Medical College, Rawalpindi from 1st January 2017 1st January 2018.
Methodology: Obligate Female carriers (Group-1) of hemophilia A and control women (Group-2) from all ages were included except those not meeting inclusion criteria. Nine ml of blood was extracted after informed consent from each healthy control and female carrier, and was tested for blood complete picture, PT, APTT, Factor VIII and blood groups.
Results: A total of 66 patients were included and 33 were obligate female carriers and 33 females were included as controls. Mean age of carriers vs controls was statistically insignificant (25+13.1 vs.27+ 11.8, p =0.776). Statistically significant differences were not observed between mean hemoglobin, mean white cell count, mean platelet count and mean prothrombin time of carriers and controls. However mean activated partial thromboplastin time was significantly different between two groups (carriers vs controls, 37.09+5.13 vs 31.8+1.92, p=0.000). Factor VIII levels were lower in carriers than in non-carriers. Levels observed were statistically significant (0.000) and strongly correlated with APTT in carriers vs controls. Blood groups were also statistically significant between carriers and controls (0.004) and showed significant correlation with factor VIII levels in carriers (0.001).
Conclusion: Factor VIII levels were lower and statistically significant between carriers and controls and also showed statistically significant correlations with APTT in hemophiliac carrier females. No correlation was found with age, hemoglobin, white cell count, platelet count, blood grouping and prothrombin time.
Keywords: Hemophilia A, Carrier females, APTT, Blood grouping, Prothrombin time, Factor VIII.

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INTRODUCTION

Haemophilia A, the most common inherited coagulation disorder, affects all races and populations worldwide[1]. Prevelance of the disease differs in each country, according to WFH (world federation of Haemophilia) 500,000 haemophiliacs are present universally[2,3]. In Pakistan, prevalance of haemophilia is 1.6/100,000 male population[4]. Incidence of recessive hereditary disorders is relatively higher due to increased rate of consanguineous marriages in countries like Pakistan and India[5].

Haemophilia A is caused by hereditary deficiency or absence of plasma coagulation factor VIII[6]. Coagulation factor VIII is a large protein synthesized mainly within liver[7,8]. Von-willi brand factor antigen, FVIII carrier molecule and ABO blood groups are known as the major modulators of plasma factor VIII levels[9,10]. The exact mechanisms underlying such variations are still poorly understood[11]. Haemophilia A being an X-linked inherited bleeding disorder, affects mainly male population while females are mostly the carriers of the disease[12]. Hemophiliac carrier females are either obligate/definite or possible carriers. An obligate female carrier is a woman who has more than one affected son or one affected son and one or more of affected relatives in family line, diagnosed by factor VIII level measurements[13].

A female haemophiliac carrier with mutated factor VIII gene shows variable levels of plasma factor VIII ranging from 50-150% of the normal[13]. Hemophiliac carrier females show variable levels but the reasons for this are poorly understood.

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There is lack of information regarding factor FVIII variability and FVIII modulators in Haemophilia A carriers[14].

Laboratory parameters of haemophiliac carriers show variations. Complete blood counts, a baseline investigation done for all haematological disorders and it is normal .Platelet counts and platelet function studies are also normal. Most important and first line screening tests done for inherited coagulation disorders are prothrombin time (PT) and activated partial thromboplastin time (APTT). Both these tests measure activities of coagulation factors involved in extrinsic and intrinsic pathways except factor XIII. Prothrombin time measures factor V, VII, X, fibrinogen and prothrombin activity. APTT is sensitive to deficient plasma levels of factor VIII, prekallikrein (PKK), HMWK, factor XII, factor XI and factor IX. APTT will be prolonged in absence or deficiency of any one of the above mentioned factors. Studies suggest prolonged APTT levels in haemophiliac carriers females[1]. This study was planned to determine blood complete counts, PT, APTT, blood grouping and factor VIII levels in obligate hemophiliac carriers and to compare and correlate with healthy control females. So this study was conducted with an objective to compare and correlate hematological parameters (complete blood picture, prothrombin time, activated partial thromboplastin time, blood group and factor VIII levels) in obligate female carriers with control women.

METHODOLOGY

This cross-sectional comparative study was carried out in department of Pathology, Army Medical College, Rawalpindi in collaboration with department of pathology (Armed Forces Institute Of Pathology (AFIP), Combined Military Hospital Rawalpindi and Haemophilia Treatment Centre, Rawalpindi in one year duration from 1st January 2017 to 1st January 2018.

Female carriers of all ages were included except those who were pregnant or using oral contraceptives or doing aerobic exercises or having chronic inflammation. Study subjects were stratified into two groups: Group-1 and Group-2. The Group-1 (Carrier) comprised of Obligate female carriers meeting inclusion and exclusion criteria's. Group-2 (Control) comprised of healthy females, who served as controls.

Nine (09) ml of blood was extracted after informed consent from each healthy control and female carrier, and was distributed as: 02 ml of blood was transferred to vial containing K_3 EDTA as anticoagulant for complete blood counts. Complete blood counts were generated through Sysmex KX-21[15] after running routine quality control procedures. Five (05) ml of blood was transferred to vial containing 3.2% sodium citrate, 2ml of blood was used for coagulation profile to be carried on water bath, and 3ml of blood was used for factor VIII assays. Factor VIII assays were carried at AFIP on Sysmex CA 1500[16](automated coagulation analyzer) after running routine quality control procedures. Blood grouping was done by routine serological tests using monoclonal anti-A and anti-B antibodies from rest of 02 ml blood.

Operational definition: For purpose of this study, obligate female carrier was defined as a woman who had more than one affected son or one affected son and one or more of affected relatives in family line[13]

Data Analysis: The data was analyzed on Statistical Package for the Social Sciences (SPSS) 22. For qualitative variables, frequency and percentages were calculated. For quantitative variables, mean, standard deviation and 25th, 50th and 75th percentiles were calculated. Qualitative parameters were compared among the groups using the chi-square test. A two-tailed P-value < 0.05 was considered statistically significant. Correlations between continuous variables were compared applying pearsons correlation, Mann-Whitney test or Kruskal-Wallis test. For continuous non normal variables, nonparametric correlation (Spearman's correlation coefficient) was applied. Independent T test was used for comparison between two groups (carriers and controls). One way ANOVA was applied for describing blood grouping.

RESULTS

A total of 66 subjects participated in the study, 33 were female carriers and 33 were healthy females. Female carriers had an age range of 8 years to 50 years with a mean value of 25.9+13.7. Age range of controls was 9 years to 51 years with a mean value of 27.00+11.8. No statistically significant difference was observed between both groups i.e carriers vs controls (25+13.1 vs.27+ 11.8, p =0.776).

Table-I: Laboratory results in both groups (female carriers and controls) (N=66)

Parameters	Carrier females	Controls	p-value
Age, mean+SD	25.9+13.7	27.00+11.8	0.776
Haemoglobin	10.9+ 2.05	11.58+0.92	0.220
TLC	8.23+2.02	8.83+3.42	0.426
Platelets	284.03+57.07	292.75+108.92	0.743
PT	13.21+0.92	13.30+1.62	0.827
APTT	37.09+5.13	31.85+1.92	0.000
FVIII	85.95+33.95	157.65+44.38	0.000

Mean hemoglobin, TLC, platelet count, PT, APTT and factor VIII levels in carriers and controls are shown in table I. Complete blood counts (hemoglobin, white cell count, platelets) and prothrombin time done for carriers and controls were statistically insignificant. Activated partial thromboplastin time was significantly prolonged between carrier females (p=0.000). Factor VIII levels of female obligate carriers were assayed and compared with those of control females. These were significantly lower in carriers compared with control (p=0.000). Mean factor VIII levels for carriers were 85.95+33.95 ranging from 26.1% to 140% of the normal. Mothers of hemophiliacs had values ranging from 26.1% to 140% of normal (mean= 87.6+33.5). Daughters of hemophiliac fathers had mean level 83+37.8 with a minimum level of 33% of the normal and maximum level of 135% of the normal. Factor VIII levels in mothers and daughters of hemophiliacs were statistically not significant (p=0.09). Factor VIII levels of control females had a minimum level 100% of the normal and maximum level of 237% of the normal (mean =157.65+44.3).

Table-II: Correlations of FVIII in female carriers of Haemophilia A and control woman (N=66)

Parameters	Correlations of FVIII in carriers r, p	Correlations of FVIII in controls r, p
Age	0.26 (0.20)	0.22 (0.34)
Haemoglobin	0.26 (0.14)	0.05 (0.82)
TLC	0.15 (0.39)	0.16 (0.48)
Platelets	0.11 (0.52)	0.14 (0.45)
PT	-0.12 (0.47)	0.08 (0.70)
APTT	-0.87 (0.000)	0.15 (0.52)

Correlation of factor VIII levels with laboratory parameters was also determined and is shown in table-II. Factor VIII levels showed no correlation with age, hemoglobin, TLC, platelet count and prothrombin time. APTT was significantly prolonged in obligate female carriers and showed strong negative correlation with factor VIII levels. Blood groups between carriers and controls were also statistically significant (0.04). Most common blood group studied among carriers (51%) was blood group B, (18%) had blood group AB, (19%) were with blood group O and (12%) carriers had blood group A. Among controls (35%) had blood group O, (35%) had blood group B, (20%) had blood group A, (10%) control females had blood group AB.

Blood groups showed a significant correlation with factor VIII levels in carriers (0.001). The distribution of factor VIII according to blood groups in haemophiliac obligate carriers and control females is shown in Table-III.

Table-III: The division of factor VIII levels according to blood groups (in carrier and controls) (N=66)

	A	B	AB	O	p-value
FVIII levels in carriers%, median (IQR	69, (63-105)	95, (76-131)	137, (106-140)	54.54, (37.3-72.6)	0.005
FVIII levels in controls mean +SD	139+28.57	152+54.4	167.7+44.2	190	0.659

DISCUSSION

Factor VIII gene mutations and several other genetic and biological determinants are known to modulate plasma factor VIII activity. ABO blood groups are well known to modulate plasma factor VIII levels in heathy individuals[15,16] as a result of primary effect of ABO locus on VWF: Ag (the carrier molecule of FVIII in circulation)[17] . Individuals with blood group O are known to have lower plasma factor VIII levels. We planned this study to evaluate the influences of age, complete blood picture, PT, APTT and blood grouping on plasma factor VIII levels in hemophiliac carriers and controls in our population.

In our study, age groups between carriers and controls were not significant. In our study, female carriers were comparatively younger with a mean age of 27 years in contrast to Paroskie et al, Plug et al, and et al.,2010 where mean age was 36.8 years ,39 years and 40 years respectively[14,18,19]. Status of relationship of obligate female carriers to haemophiliac patients was mothers 70% and daughters 30% and compared to Ay et al.,2010 where mothers were 52%, daughters 12% and sisters 36%[14].

Laboratory parameters of obligate female Haemophiliac carriers were compared with controls. Hemoglobin, TLC and platelet counts were normal. Mean hemoglobin in carrier females was 10.9 g/dl compared with controls was 12.8 g/dl whereas Paroskie et al[19] gave mean hemoglobin 13.2 g/dl in carrier females and 13.1 g/dl in control women which was also statistically insignificant. Mean white cell count in our obligate female carriers was 8.23 x 10^9/l and platelet count was 284 x 10^9/l comparable to Paroskie et al[19] where platelet count in carriers was 277 x 10^9/l. Prothrombin time was normal both in carriers and controls and APTT was significantly prolonged in carriers and showed strong negative correlation with factor VIII levels. Mean APTT was 37 sec for obligate female carriers. In correspondence to our study, Paroskie et al and Ay et[14] al also showed normal PT and significantly prolonged APTT 33 sec and 36 sec in carrier females compared with normal women, respectively. Similarly, studies done in India also showed prolonged APTT in haemophiliac carrier females[20].

Factor VIII levels measured were significantly lower in obligate female carriers. Mean value of FVIII in our female carriers was 85%, comparable to our study factor VIII level reported by Paroskie et al[19] FVIII level observed was 82.5% in Haemophilia A carriers. FVIII levels reported by Plug et al[18], Ay et al[14], were 60, 74, 55, and 90.4 %in carrier females compared with controls.

Correlation of FVIII with age and laboratory parameters was determined. In correspondence to our study no correlation was found with age and haemoglobin, PT showed weak negative correlation and strong negative correlation was found with APTT[14].

Blood groups were also studied among carriers and controls. Most common blood group studied among carriers was B (51%), (18%) carriers had blood group AB, (19%) with O and (12%) carriers had blood group A whereas, Ay et al.[14] and Paroskie et al[19] reported blood group A (38% and 40%), blood group B (19% and 12%), blood group AB (10% and 5%) and blood group O (31% and 27%) respectively. Blood group typing was consistent with expected frequencies in population in relevant countries.

FVIII levels were lower in blood group O than non-O blood groups in carrier females whereas blood group AB individuals had higher FVIII levels. Similarly, Ay et al.[14] also reported lower factor VIII in blood group O hemophiliac carriers. Contrarily Morange et al and Ay et al described highest FVIII levels in blood group B carriers. ABO blood groups showed a significant correlation with FVIII levels in carrier females. In contrast to our study statistical analysis provided by Ay et al showed significant association of FVIII with ABO blood groups in the control women. The ABO blood groups are known as major determinants of plasma levels of FVIII and VWF in general population. The carrier status and not ABO blood type seemed

to be primary determinant of FVIII levels in carrier females[14]. ABO blood groups are considered as secondary modulators of plasma FVIII levels but genetic factors do exist that influence the variability of FVIII.

CONCLUSION

Factor VIII levels were lower and statistically significant between carriers and controls and also showed statistically significant correlations with APTT in hemophiliac carrier females. No correlation was found with age, hemoglobin, white cell count, platelet count, blood grouping and prothrombin time. Blood groups were statistically significant between carriers and controls and strongly associated with factor VIII levels in carrier females.

Recommendations: Haemophilia A having high mortality and morbidity demands mandatory screening of carriers in developing countries. Our study was aimed to determine screening tests of Haemophiliac carrier females from this study we recommend the need for validation of laboratory findings in a larger group for proper understanding however genetic variations in FVIII gene and several other regulators of plasma FVIII were not included in the study due to certain limitations.

AUTHOR'S CONTRIBUTION

Kiani SN: Conceived idea, Data collection, Manuscript writing.
Basharat M: Data analysis, Manuscript writing

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