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REASSESSMENT OF COAGULATION DYSFUNCTION AND ITS IMPACT ON CLASSIFICATION OF HEMOPHILIA IN DUHOK CITY

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ABSTRACT

The X-linked inherited coagulation disorders, hemophilia A (Factor VIII deficiency) and hemophilia B (factor IXdeficiency) are the most prevalent coagulation dysfunction. The current study involved 75hemophlic patients who have been assigned into two groups; hemophilia A group consisted of 62 patients and hemophilia B group represented by 13 patients.

Venous blood of participants was withdrawn, routine coagulation (PT and aPTT) and specific clotting function test (FVIII, FIX, FXI and FXII) were investigated. PT and aPTT kit (BIOLAB®, France) were used for the measurements of PT and aPTT times. STA®-DEFICIENT VIII and IX reagent kit with Immuno-depleted plasma for FVIII and FIX assay were used for determination of FVIII and FIX activity, respectively. The procedure followed the manufacturer instructions of each particular test.The present study was investigated blood group in a hemophilic patient also .

The results revealed that hemophilia A patients exceeded the patients of hemophilia B and were represented by 82.7% and17.3% respectively. Furthermore, moderate cases of both types of hemophilia were greater than mild and severe cases. The results of this study indicated no significant correlation between the type of hemophilia from the one side and blood group and age from the other side. Conversely, significant correlation has been found between hemophilia and family history(P=0.001). The present study showed significant correlation between prolonged aPTT and hemophilia (P=0.00), while no-significant correlation has been found between prolonged PT and hemophilia. Finally, no significant correlation has been shown between age and the prolonged aPTT.

KEYWORDS: factor VIII, factor IX, Duhok city, aPTT, types of hemophilia

INTRODUCTION

Hemophilia is a hereditary clotting disorder which is caused by a deficiency of factor VIII (hemophilia A) or IX (hemophilia B). It is a genetic recessive X-linked trait and therefore patients are mostly men. Female family members can be carriers of the disorder, which is characterized by a 25% chance of having a son with hemophilia, and a decreased clotting factor activity level. The severity of the disease is determined by the residual clotting factor activity. Patients with mild hemophilia had a FVIII or FIX clotting activity of >0.05-0.40 IU/ml, patients with moderate hemophilia had 0.01-0.05 IU/ml while in severe hemophilia it was <0.01 IU/ml. Frequent bleeding in joints results into damage of the synovial tissue and arthropathy (Bolton-Maggs and Pasi, 2003).

The diagnosis of hemophilias depends on clinical history of deep bleedings particularly into the joints and muscles, family history of bleeding, and a prolonged APTT. An accurate diagnosis relies on factor assay to show deficiency of Factor VIII or Factor IX(Stachnik, 2010).

Evaluation of an individual with a suspected bleeding disorder includes: platelet count and platelet function analysis (PFA closure times) or bleeding time, activated partial thromboplastin time (aPTT), and prothrombin time (PT). Other tests recently suggested for the assessment of the overall clotting function include the thrombin generation test, thromboe- lastogram and the clot wave form analysis (Nair *et al*., 2010).

Individuals with a history of a lifelong bleeding tendency should have specific coagulation factor assays using (one stage, two stage, or chromogenic assay) performed even if all the coagulation screening tests are in the normal range(Verbruggen*et al*., 2008).

Objectives: Re-evaluate the coagulation parameters including PTT, clotting FVIII, and FIX in patients with hemophilia and reassess the

frequency of mild, moderate and severe hemophilia patients.

MATERIAL AND METHODS

Study design

This cohort retrospective study was conducted in the Scientific Research Center/College of Science-University of Duhok, JIN Pediatric Hematology Oncology Center in Duhok/Kurdistan region /Iraq during the period from July 2018 to February 2019.

Patient groups

In the present study, unrelated individuals previously diagnosed with haemophilia A (62 patients), and hemophilia B (13 patients) were enrolled.They included mild, moderate and severely affected cases who didnot receive any FVIII or FIX concentrate or any other blood products for the last fifteen days, the ages in both categories ranged from 1 year to 46 years. The questionnaire form was filled for each patient.

Phenotypic Assessment

All participants with coagulation disorders who had different medical and surgical complaints and lately diagnosed as hemophiliac were included in this study. A thorough family history reviewed, physical examination, blood group, and laboratory investigations were performed.

Samples collection

After obtaining informed consent from all involved subjects, 5 ml of peripheral blood samples were collected in3.2% trisodium citrate tubes and processed immediately for performing biochemical investigations as per previous literature (Martini *et al*., 2008).

Preparation of Plasma

Patients plasma were prepared to determine PT, aPTT, FVIII and FIX levels according to the literature (Martini *et al*., 2008).

Controls plasma: blood was collected into sodium citrate tubes for the purpose of preparing a pool of at least six freshly collected normal plasmas. This pool of plasmas was utilized as reference plasma.

Laboratory assays

A number of standard laboratory tests were performed at the JIN Pediatric Hematology Oncology Center in Duhok including:

- **Prothrombin Time (PT)**

The PT was used to assess deficiencies or inhibitors of the extrinsic pathway factors (Factor VII) and common pathway factors (Factors X, V, II, Fibrinogen).PT determination kit (BIOLAB®, France) was used. Procedure and preparation of stock and working solutions followed manufacturer instructions, Normal PT (in sec.) usually between 11and 16 seconds (Rudasill *et al*., 2019).

- **Activated Partial Thromboplastin Time (aPTT)**

The aPTT is used to assess deficiencies or inhibitors of the intrinsic pathway factors (Factors XII, XI, IX, VIII) and common pathway factors (Factors X, V, II, Fibrinogen). The measurement and recording of clotting time were performed simultaneously. A normal aPTT is approximately 25-35 seconds. aPTT kit (BIOLAB®, FRANCE) was used for the determination of the intended factor, as with previous experiment, the work strictly followed manufacturer regulation. The normal range of The BIOCK reagent involves recalcification of plasma in the presence of standardized amount of cephalin (platelet substitute) and a factor XII activator (Kaolin). The use of Kaolin minimizes reading time and optimizes the optical detection (Robert and Chazouilleres, 1996).

- **Determining activity of factor VIII (FVIII) and factor IX (FIX)**

STA®-DEFICIENTVIII and IX reagent kit with Immuno-depleted plasma for FVIII and FIX assay were used for determination of FVIII and FIX activity, respectively. SAT Coag Expert® is a software automates laboratory processes such as full auto-verification, repeat/reflex testing, comprehensive Quality Controls (QC) (Martini *et al*., 2008).

- **Statistical analysis**

All data were analyzed by the SPSS (IBM Corporation, New York, NY, USA) statistical package (Version 25.0).Chi-square test for independence has been used to find the P value.

RESULT

The results of this study showed that out of the total of 75 cases of Hemophilia, 62(82.7%) were classified as Hemophilia A (HA) and only 13 (17.3%) were confirmed as Hemophilia B (HB), it is indicated that HA patients bled more frequently than HB patients ($p<0.047$)(Table 1).

The study included 19 (25.3%), and 2 (2.7%) patients with severe hemophilia A and B, respectively. Besides, 36 (48.0%) patients were diagnosed with moderate hemophilia A and 6

(8.0%) patients with moderate hemophilia B. Finally, 7 (9.3%) patients and 5 (6.7%) patients were diagnosed as mild hemophilia A and hemophilia B, respectively (Table 1).

Table (1):- Hemophilia types and degree factor level (DFL).

			Hemophilia types		P-value
			Hemophilia A	Hemophilia B	
DFL	Severe	Frequency	19	2	0.047
		% of Total	25.3%	2.7%	
	Moderate	Frequency	36	6	
		% of Total	48.0%	8.0%	
	Mild	Frequency	7	5	
		% of Total	9.3%	6.7%	
	Total	Frequency	62	13	
		% of Total	82.7%	17.3%	

The distribution of hemophilia patients according to age is shown in table 2. Data showed no significant correlation between types of hemophilia and age; according to our finding, in age < 10 years old there was 22 (29.3%) cases with hemophilia A and 5(6.7%) cases with hemophilia B, while in age group > 10 years old there were 40 (53.3%) subjects with hemophilia A and 8 (10.7%) others with hemophilia B.

Table (2):- the relationship between Hemophilia types and age.

			Hemophilia types		\
			Hemophilia A	Hemophilia B	
Age (years)	< 10	Frequency	22	5	0.539
		% of Total	29.3%	6.7%	
	> 10	Frequency	40	8	
		% of Total	53.3%	10.7%	
Total		Frequency	62	13	
		% of Total	82.7%	17.3%	

*P<0.05

The result of present study shows that the blood group O was more common 31 (41.3%) followed by A 26 (34.7%), and B 18 (24%) (Table 3). The present study did not show a significant variation among the ABO blood groups.

Table (3):- Blood groups and Hemophilia types.

			Blood Groups			Chi-square	P-value
			A	B	O		
Hemophilia types	HemophiliaA	Frequency	24	15	23	1.958	0.376*
		% of Total	32.0%	20.0%	30.7%		
	Hemophilia B	Frequency	2	3	8		
		% of Total	2.7%	4.0%	10.7%		
Total		Frequency	26	18	31		
		% of Total	34.7%	24.0%	41.3%		

Table 4 shows group statistics in relation with hemophilia types, when mother and father were relatives, the number and percentage of patients were 31(41.3%), 9 (12%) for hemophilia A and

B, respectively. While 31(41.3%), 4 (5.3%) were the numbers and percentage of patients of hemophilia A and B when mother and father were nonrelatives. As shown in the same table, hemophilia A patients are equal in number in both groups (Relative and non-relative marriage) while hemophilia B was higher in the case when the mother and father were relatives, this finding supports the idea of the more likely occurrence of the disease as a result of consanguineous marriages. yet it is statistically non-significant.

Family history of hemophilia was positive in more than half of the patients while it was negative in the other patients which was statistically significant as shown in table 4.

Third, a statistic has been made that shows whether the disease was inherited by the mother or the father's side or whether the parents were healthy or both were affected by the disease. For hemophilia A and B, the frequencies and percentages of patients when the disease came from mother side, father side, both sides were intact, both sides were affected were 23 (30.7%),1(1.3%),38(50.7%),0 (0%); 4(5.3%),1(1.3%),7(9.3%),1(1.3%), respectively.

Table (4):- Association between hemophilia types and sociological parameters.

			Hemophilia types		Mean	STD	Chi-square	P-value
			Hemophilia A	Hemophilia B				
Do mother and father are relatives?	YES	Frequency	31	9	1.225	0.423	0.206	1.597
		% of Total	41.3%	12.0%				
	NO	Frequency	31	4	1.114	0.323		
		% of Total	41.3%	5.3%				
Total		Frequency	62	13	1.173	0.381		
		% of Total	82.7%	17.3%				
Family history with disease (members affected in the same family)	With out	Frequency	30	5	1.143	0.355	13.062	0.001
		% of Total	40.0%	6.7%				
	1-4	Frequency	27	2	1.069	0.258		
		% of Total	36.0%	2.7%				
	4-8	Frequency	5	6	1.545	0.522		
		% of Total	6.7%	8.0%				
Total		Frequency	62	13	1.173	0.381		
		% of Total	82.7%	17.3%				
Mother or father side	M. Side	Frequency	23	4	1.148	0.362	6.477	0.091
		% of Total	30.7%	5.3%				
	F. Side	Frequency	1	1	1.550	0.707		
		% of Total	1.3%	1.3%				
	With out	Frequency	38	7	1.155	0.367		
		% of Total	50.7%	9.3%				
	Both	Frequency	0	1	2.000	0.000		
		% of Total	0.0%	1.3%				
Total		Frequency	62	13	1.173	0.381		
		% of Total	82.7%	17.3%				

The current study showed that there was a significant correlation between aPTT values and hemophilia A (P=0.00) (Table 5). It can be found that the highest number of patients is positively correlated to the prolongation of aPTT. The results of the current study were as follows; 19 (25.3%) patients within the 80-90 seconds category of aPTT, then 14 (18.7%) patients within 60-70 seconds, two (2.7%) patients within 40-50 second, and 8 (10.7%) patients for 90-100 seconds and more than 100 seconds. The results showed a weak correlation between degree of aPTT prolongation and hemophilia B. 8 (10.7%) patients had 40-50 seconds test time, which can be considered relatively within the normal range of the test, then 3 (4.0%) patients

fall into category 50- 60 seconds, finally 60-70 seconds group consisted of 2 (2.7%) patients.

Table (5):- Hemophilia types andaPTT,PTlevels.

			Hemophilia types		Mean	STD	Chi-square	P-value
			Hemophilia A	Hemophilia B				
aPTT (sec.)	40-50	Frequency	2	8	1.800	0.422	36.965	0.000
		% of Total	2.7%	10.7%				
	50-60	Frequency	7	3	1.300	0.483		
		% of Total	9.3%	4.0%				
	60-70	Frequency	14	2	1.125	0.342		
		% of Total	18.7%	2.7%				
	70-80	Frequency	4	0	1.000	0.000		
		% of Total	5.3%	0.0%				
	80-90	Frequency	19	0	1.000	0.000		
		% of Total	25.3%	0.0%				
	90-100	Frequency	8	0	1.000	0.000		
		% of Total	10.7%	0.0%				
	More than 100	Frequency	8	0	1.000	0.000		
		% of Total	10.7%	0.0%				
Total		Frequency	62	13	1.173	0.381		
		% of Total	82.7%	17.3%				
PT(sec.)	12-15	Frequency	38	7	1.155	.367	0.284	0.618
		% of Total	50.7%	9.3%				
	15-18	Frequency	24	6	1.120	0.407		
		% of Total	32.0%	8.0%				
Total		Frequency	62	13	1.173	0.381		
		% of Total	82.7%	17.3%				

In order to analyze the distribution of aPTT abnormality in different ages the 75 patients enrolled in this study were divided into two age groups:

1. Children – those that were <10 years oldand

2. Teens and older those that were >10 years old.

48 patients and 27 patients were in each group, respectively. aPTT values showed a tendency for prolongation, significant differences being found between children and Teens and older (P=0.032) (Table 6).In children group the highest triplicate number 6 (8.0%) observed in three categories 50-60 sec, 60-70 sec, and more than 100. The lowest number found incategory 40-50, while category 70-80 did not have any individual. In teens and older it was clear that the largest number 14(18.7%) was in a category 80-90 sec, followed by 10(13.3%) within 60-70 sec, then 2(2.7%) as the smallest number which has more than 100 sec of aPTT prolonged.

Table (6):- Distribution of age groups according to aPTTlevel.

			aPTT(sec.)							Mean	STD	Chi-Square	P – Value
			40-50	50-60	60-70	70-80	80-90	90-100	more than 100				
Age(years)	< 10	Frequency	1	6	6	0	5	3	6	4.300	2.035	13.762	0.032
		% of Total	1.3 %	8.0%	8.0%	0.0%	6.7%	4.0%	8.0%				
	> 10	Frequency	9	4	10	4	14	5	2	3.690	1.812		
		% of Total	12.0 %	5.3%	13.3 %	5.3%	18.7 %	6.7%	2.7%				
Total		Frequency	10	10	16	4	19	8	8	-	-		
		% of Total	13.3 %	13.3 %	21.3 %	5.3%	25.3 %	10.7 %	10.7 %	-	-		

DISCUSSION

The present study revealed that the commonest type was hemophilia A affecting 62(82.7%) patients, followed by hemophilia B affecting 13 (17.3%) patients. This result is matching with most studies such as Al- Zubaidy (2014), who clarified that the ratio of hemophilia A (76.7%) was more than the ratio of hemophilia B (23.3%). The higher mutation rate in hemophilia A than hemophilia B could justify the later finding. The results of the current study showed that the moderate cases were more than the mild and the severe ones.This result is compatible with the results of Al Tonbary*et al*., (2010) who indicated that the moderate presentation represents the majority in 17.2% followed by sever presentationin 4.7%,and Nigam *et al*., 2014 who found that the frequent type of hemophila was moderate group in hemophilia A 87 (38.84%), while our findings differed from these of Al-Beldawi, (2010), and Mannuccio (2018), who proved the severe bleeding cases were more than the moderate cases.

The variability of hemophilia presentation could be attributed to the fact that many patients with mild hemophilia were not registered in hemophilia centers, because the disease may go undiagnosed and be discovered only because of excessive hemorrhage postoperatively or following trauma. Also, many countries with lower income do not provide resources (both personnel and treatment products) for treating hemophilia, so many cases with severe hemophilia often die in childhood or early adult life(Stonebraker *et al*.,2010).

This result is similar to the results of a study done in China in which 23 out of 89 (25.8%) of patients were less than 10 years old and 66 out of 89 (74.2%) older than 10 years (Liu *et al*., 2020).

Rejtő*et al*., (2020), conclude that age has to be considered a minor modification factor, as there is a consistent, but weak, increase in FVIII levels with advancing age. This aspect is important in daily practice, when a diagnosis of non-severe hemophilia A has to be made. Now, the technological advances and the abundance of medical research in this field have led to the possibility of early diagnosis of these diseases.

Blood group and vWF are important determinants of the coagulation factor levels in plasma. The blood group non-O is associated with higher vWF and coagulation factor levels than in blood group O. Individuals with blood group AB have the highest vWF levels, whereas AA, AO, BB, and BO genotypes have intermediate levels (Kamphuisen *et al*.,2001).In Iran, Mansouri Torghhabeh, *et al.,* (2006) found no relation between ABO blood groups and hemophilia. This finding agrees with our study.

The result of the present work is matching with the results of Spiezia *et al*., (2018) who studied 204 patients, 87 (42.6%) of them had O blood group and 117 (57.4%) had non-O blood group and found that blood groups A, B and AB in the study population was 38.7%, 10.8% and 7.9%, respectively. Mirdha and Jena (2016) found that the predominant blood group was O (40.7%) type followed by blood group B (32.7%), A (18%), and AB (8.7%).

On the other hand, findings of Nair *et al*., (2016) show that subjects with hemophilia and O blood type have a significantly higher rate of bleeding episodes compared to subjects with non-O blood type.

The family history has its origins in genealogy and over the past Century has become embedded in clinical practice. Its importance in specialized circumstances is unquestioned but largely untested. Moreover, the relevance of the family history to common diseases, especially in an era of genomic markers that convey risk and the emphasis on "personalized medicine," must be given careful scrutiny (Pyeritz, 2012).

Al-Zubaidy *et al*., (2014) showed that 56.7% of cases were among first cousin marriages, 15% of cases were among close family marriages or tribe and 28.3% were unrelated with family marriages. Kim *et al*., (1988) showed 43% cases of known hemophilia patients had family history and they involved brothers, maternal cousins, uncles, and maternal grandfathers in descending order of frequency, thus these results were similar to the results of the current study.

In another study conducted in Pakistan, the researchers revealed a high rate of Positive family history within hemophilia patients which was about 80% (Khanum *et al.,* 2014).Also, Rosslier *et al.,* (1994) indicated that about two of three cases had inherited genetic mutation.

The increasing prevalence over time could be due to many factors such as increased diagnostic procedures and treatments or due to improved reporting technologies, improvement in the access of care for hemophilia, or due to the effect of migration of patients to areas with better care.

The increasing incidence, in almost all countries, is because that hemophilia is an inherited disease, and there is no known way to prevent it, till now. However, genetic counseling for parents is available (Stonebraker *et al.*,2010).

In the present work, certain samples tested for aPTT fell within the category 40-50 of aPTT and few other samples shown within the 90 seconds and above group when the same test applied because moderate hemophilia cases were more than the sever ones in the current study. It appeared that this detection was suitable for the samples from the moderate–severe patients, rather than severe cases as the diagnosis of severe hemophilia with the one-stage assay may be very difficult unless an appropriate aPTT reagent is used (Verbruggen *et al*., 2008).

This study showed that the degree of aPTT prolongation does not correlate well with the type of hemophilia B; this is attributed tothe differences in the activator or phospholipids used in the reagent (Boweyer*et al*., 2011).

Our findings allowed us to prove that in patients with intrinsic coagulation pathway disorders overall aPTT sensitivity was close to 100 %. Theoretically, when there is a factor deficiency there should be an aPTT prolongation. Since a clotting factor deficiency causes problems in the clotting system it takes longer for the blood to form clots. There is some controversy regarding the differential utility and sensitivity of aPTT assay methods. When aPTT measurement is done in combination with PT measurement (which measures the time of extrinsic and combine coagulation pathway) it indicates to what part of coagulation pathway may be deficient and on what specific factors to focus on(vest, 2017).

Similar outcomes have been reported in the present work to that conducted in In Iranian patients, prolonged aPTT with normal PT is a valuable finding in HA and HB. This is especially true when our patients are males with bleeding(Dorgalaleh *et al*.,2016).

The results of the present study were in line with Sadaria *et al*., (2016), who reported that aPTT was prolonged in all patients with hemophilia A and B. It ranged from 41.6 sec. to 124 sec. besides, Parthiban *et al*.,(2015)found that aPTT was prolonged in all cases (100%) of hemophilia A and B with an average of 88 sec, the later finding is similar to the mean aPTT value of 80 seconds obtained in the present study.

The hemostatic system evolves in a manner that is age-dependent. Plasma concentrations of most pro- and anticoagulant proteins are decreased throughout childhood. However, they provide effective hemostatic balance at a lower level compared with adults. This situation necessitates the generation of several appropriate age-dependent reference ranges to interpret laboratory data in pediatric patients and prevent misclassification of children having defects of factors and inhibitors of the coagulation system. Since there are differences in the hemostatic system among different analyzer and reagent systems.Coagulation laboratories should develop age-related reference ranges specific to their own testing systems for the local population (Li *et al*., 2009).

The results of current study showed similarity with the finding of Arslan, *et al*., (2016), in which significance between the child and adult groups was reanalyzed. A significant difference between the child and adult group was found, and two different reference ranges were determined.

Our results were similar to the finding of Li *et al*., (2009) who showed statistically different between adult and childhood values (p= 0.017) but, it was numerically different because he showed that aPTT values in neonates were longest (48.09 sec), followed by infants aged 1–6 months (45.53 sec). aPTT values in infants aged 6–12 months (39.43 sec) were significantly shorter than those in infants aged 1–6 months (p=0.000).

From the other side, Flanders *et al*.,(2005). found no significant difference between children and adults for the levels of aPTT. Vest, (2017) clarified that the frequency of pathological aPTT results did not differ significantly between children and adults (P=0,372).

The different emerged results in some of the age groups, is likely caused by differences between the methods used (mechanical or optical), population, blood collection method (from the antecubital vein or from the back of the hand using the broken needle technique (Arslan, *et al*., 2016), or may be related to the population and analyzer derived reference intervals. Also, the types of the patients may affect the correlation between the aPTT and age. In the current study, the high range of patients were moderate and mild, rather than the severe because mild and moderate hemophilia may not be diagnosed until adulthood.

In conclusion: The results of this study revealed that there was no discrepancy in the coagulation status of our registered hemophilia patients and that the moderate cases are the commonest. As far as hemophilia types were concerned, uneven distribution of hemophilia was ensued.

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