International Journal of Academic Health and Medical Research (IJAHMR)
ISSN: 2643-9824
Vol. 5 Issue 8, August - 2021, Pages: 12-17

Prevalence of Antibody Inhibitors among Sudanese patients with Hemophilia

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***Abstract**: Hemophilia is an inherited Bleeding coagulopathy in which coagulation factor deficiencies demonstrate-linked inheritance, Factor VIII and factor XI replacement therapy is effective for hemophilia A and B unless a patient develops an alloantibody (inhibitor) against exogenous FVIII and FXI. Which is the most significant treatment complication for them, associated with considerable morbidity and decreased quality of life. The study aimed to study the prevalence of exogenous alloantibody inhibitors against FVIII and FXI amongst patients with hemophilia A and B in Kordofanian states. A descriptive cross-sectional Carried out in the period from October to December 2019. Informed consent was taken from each patient, then the structured questionnaire was filled, and a blood sample was collected to prepare (PPP) which was tested thru APTT afterward, it was screened for inhibitory antibodies. No one reported to has inhibitors of investigated hemophilic patients amongst a total of 30 hemophiliac patients, aged between (94-33) Years, moreover amidst them, 25(83.3%) belong Hemophilia A, while 5(16.7%) with hemophilia B.also,15 (50%) have mild hemophilia, 12(40%)moderate and 3(10%) severe disease.19(63.3%) suffer from joints complication, they aged between (11-20) years.24(80%) of patients with family history. This study concludes that there are no inhibitors developed in those hemophilia patients, although most of them have developed Hemarthrosis. Finally, the establishment of a local specialized center is recommended to supply hemophiliac patients with treatment and knowledge*.

Keywords: Hemophilia, Antibody Inhibitors, Bleeding coagulopathy

Introduction

Hemophilia is an inherited Bleeding coagulopathy in which coagulation factor deficiencies demonstrate X-linked inheritance. [1, 11, 12].When factor VIII is the deficient factor, the disease is called hemophilia A or classical hemophilia and when factor IX is deficient & Factor XI deficiency is type C. [1, 9]. The overall incidence of hemophilia is approximately 1 in 5000 males. [9] Approximately 80% of the patients have factor VIII deficiency and 20% are deficient in factor IX. In approximately 30% of the affected individuals, there is no positive family history of the disease [1, 9]

The manifestations of the disease are clinically indistinguishable and occur in mild, moderate, and severe forms. Patients with severe hemophilia A will manifest early bleeding manifestations such as circumcision bleeds or umbilical cord bleeding**.** The gastrointestinal tract, the kidneys (hematuria), or gums or in hematomas. [2, 10]

The screening tests are used in the diagnosis of hemophilia A and most cases of hemophilia B, PT is normal and PTT is prolonged [12]. However, a specific assay of factor IX coagulant activity is required for a definitive diagnosis. [14, 15]The reaction of factor VIII with its inhibitor is time-dependent both in vitro and in vivo, an observation relevant to measurement and clinical treatment [16, 17]. An abnormal mixing test is not specific for individual factor inhibitors because lupus anticoagulants show the same phenomenon. [18] As a quantitative method**,** the Bethesda assay and the Nijmegen assay are based on the principle of measurement of inactivation of FVIII [19].Treatment of hemophilia involves not only replacement therapy for the deficient factor, but an overall approach to the patient and his family concerning education, psychosocial care, and periodical dental and orthopedic evaluation [13].Two-factor concentrates are available, plasma-derived (pdf iii/IX) and recombinant factor (VIII/IX) [3, 13]. Inhibitors are antibodies directed against hemophilia treatment products, which interfere with their function. Their persistence may increase morbidity and mortality. [4] Some factors may increase the risk for developing inhibitors [2] [6, 7] as shown in figure (1), [29].

Hemophilia patients with inhibitors are at increased risk for joint disease and other complications from bleeding and reduced quality of life. The present study aimed to study the prevalence of exogenous alloantibody inhibitors against FVIII and FXI amongst patients with hemophilia A and B in Kordofanian states. To the best of our knowledge, no previous study was done on that concern, so this study may fill such gap.

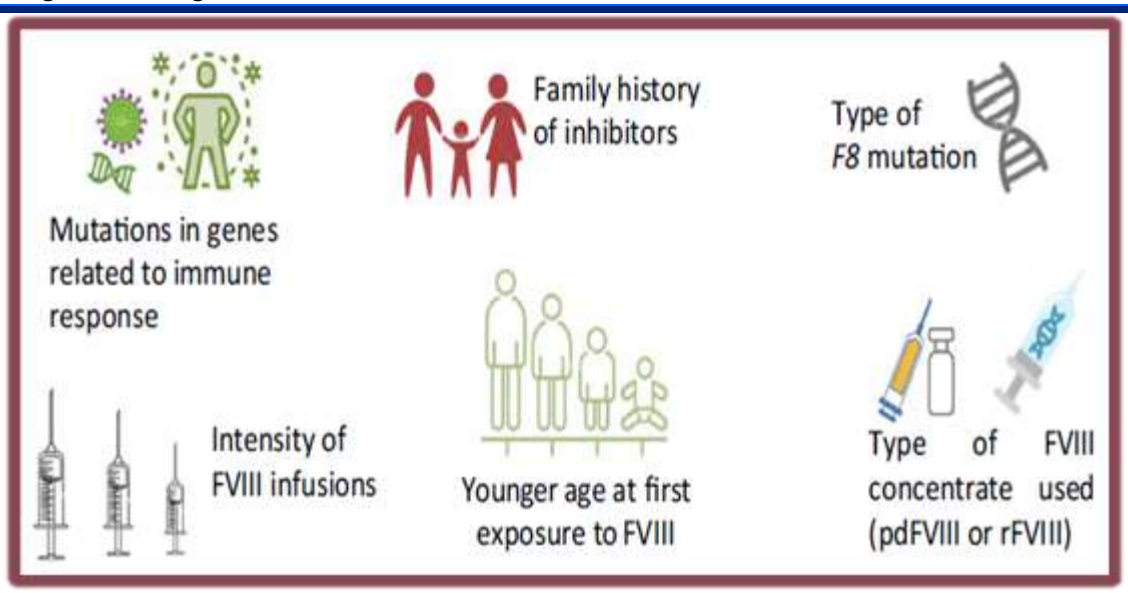

Figure (1): *Risk factors for inhibitor development amidst hemophiliac patients [29]*.

Materials and Method

Study design & Setting

This study is a descriptive cross-sectional study, conducted in Kordofanian states, in the period between the 10th October and the 25th December 2019.

Informed consent was obtained from the patients before enrollment; on the other hand, it was taken from parents of children patients. A total of 30 Hemophilic patients were included in this study, who have not received any form of factor replacement therapy at least for the 72 hours, other hemophilic under therapy were excluded.

Sample collection

Data were collected via a structured interview questionnaire that includes questions about patients demographics (Age, Gender, Residence) besides Clinical features (Type of hemophilia, Severity of the disease, Age at first diagnosed, Duration and dose of factor, Treatment by others than factor concentrate, Complications, Family history), in addition to Laboratory findings(Result of inhibitors screening and their type if present: Time-dependent Immediate), that filled by direct interviewing 2.5 ml of blood sample was collected in trisodium citrate anticoagulant (9 volumes of blood to 1 volume of trisodium citrate), then hard centrifugation at 3000g for 15 minutes was performed to prepared (PPP) for each patient.

Laboratory investigation

APTT

Normal plasma and test plasma are incubated at 37ºC for 1-2 hours, both separately and as a 50:50 mixture. The APTT was then determined on the normal plasma, test plasma, and incubated mixture,

as well as on a mixture prepared from equal volumes of test and normal plasma after separate incubation (immediate mix). The degree of correction of the APTT of each mixture is compared.

Poor correction in the mixture prepared after separate incubation is suggestive of an immediate-acting inhibitor. Poor correction in the incubated mixture is suggestive of a time-dependent inhibitor.

Screening for inhibitors

A three plastic tubes: A, B, C. were prepared 0.5ml of normal plasma was pipetted into tube A, 0.5ml of test plasma into tube B, and 0.2ml of each of normal and test plasma into tube C.

Then all tubes were incubated for 1-2 hours at 37c. Afterward, 50:50 mix from tubes A and B were prepared. Labeled in tube D, which was served as an immediate mix.

APTT was performed in duplicate on A, C, D, and B (In that order).

Results and interpretation

Table (1): Example of a clotting factor inhibitor screen based on APTT
Results and interpretation

Sample	1	2	3
A: normal plasma	40	40	40
B: test plasma	90	90	90
C:Test+normal(incubated after mixing together)	45	70	70
D:Test+normal(incubated separately before mixing)	45	48	70

Sample 1: a plasma with an intrinsic defect but no inhibitor.
Sample 2: a plasma containing a time-dependent inhibitor.
Sample 3: a plasma containing an immediate-acting inhibitor.
Patients who were found positive inhibitor screening were further evaluated for quantities assay (Bethesda assay).

Result

A total of 30 hemophiliac patients are included in the study. They aged between 4-33 years (Figure 2). Moreover, 20(66.7%) of patients are residents of north Kordofanian states, 5(16.7%) of west Kordofanian, while 5(16.7%) of south Kordofanian states, as demonstrated in (Table 1).On another hand, 25(83.3%) of Hemophilia patients are previously diagnosed with type A while 5(16.7%) with hemophilia B. Also, 15 (50%) of patients classified to have mild hemophilia, 12(40%) moderate, and 3(10%) with severe disease. (56.8%) diagnosed in the period of (1-5) years of birth. (Figure 4).19(63.3%) of patients with hemophilia, aged from (11-20) years suffer from joints complications. while 11(36.7%) never complain about such complications.24 (80%) of patients have a family history of the disease, whereas 6(20%) never have. A total of thirty (100%) of hemophiliac patients are negative to inhibitors and 0(0%) are positive, no one amongst them never developed inhibitors after factor replacement therapy.

Table (1): Distribution of patients according to residence

Residence area	Frequency	Percent
North kordofan	20	66.7%
West kordofan	5	16.7%
South kordofan	5	16.7%
Total	30	100%

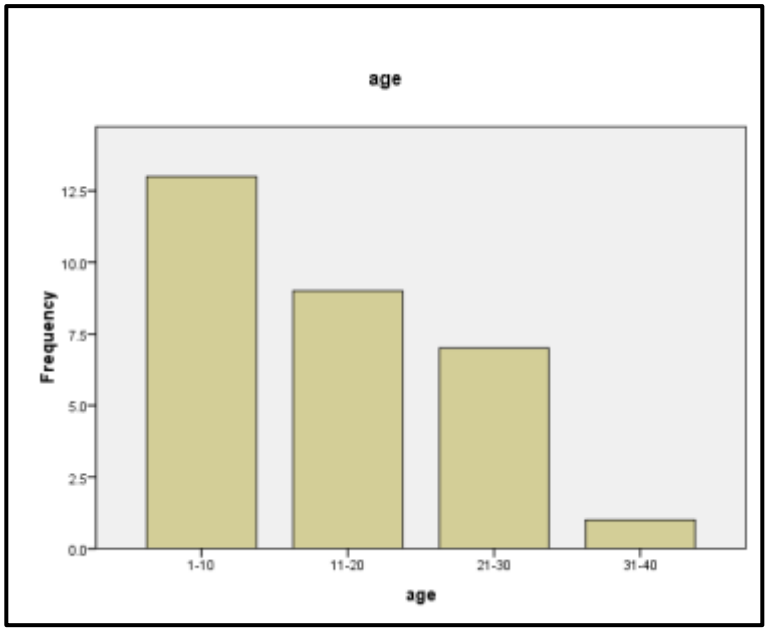

Figure (2): distribution of patients according to age.

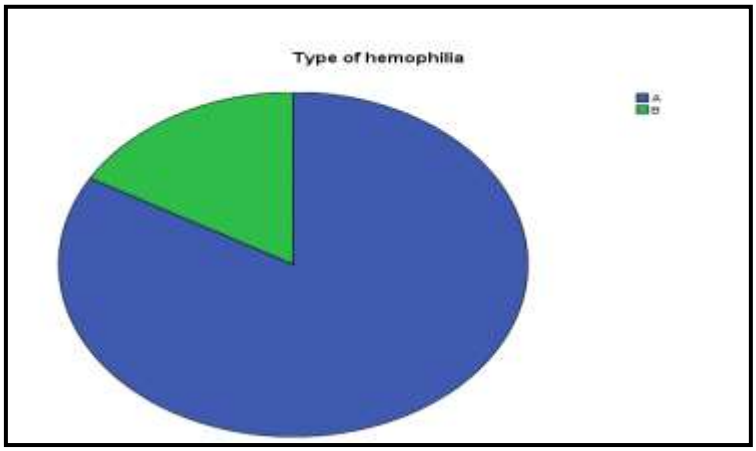

Figure (3): Distribution of patients according to type of hemophilia.

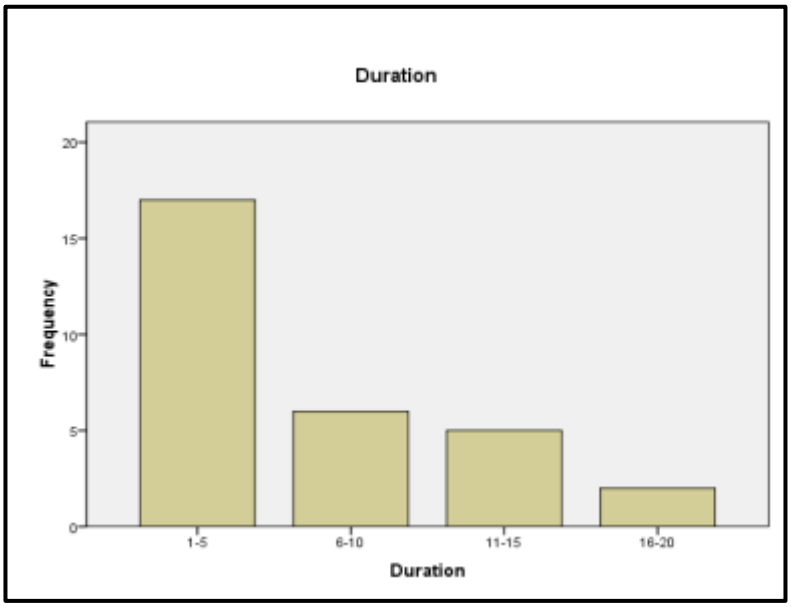

Figure (4): Distribution of patients according to disease duration (years) before investigation.

Discussion

The development of inhibitors remains one of the most serious complications of replacement therapy in hemophilia patients. The inhibitor keeps the treatment from working which makes it more difficult to stop a bleeding episode. This study was designed to access the prevalence of inhibitors among patients with hemophilia in Kordofanian states in the period 10th October to 25th December 2019. All thirty patients were included in this study aged between 4-33 years.25 (80.3%) of the patients suffering from hemophilia A, which is about four times as common as hemophilia B [19]. (50%) of the patients belong to mild hemophilia followed by moderate (40%), and lastly severe (10%). This is consistent with a Study done by Abdelrhman et al in Sudan 2004 that stated mild hemophilia is dominating (42%) followed by severe (33%)of patients. [20]Current findings revealed that (56.8%) of the patients

with hemophilia are diagnosed within (1-5) years of birth. This accords with the literature in the United States, which reported that most people with hemophilia are diagnosed at a very young age. Based on CDC data, the median age at diagnosis is 36 months for people with mild hemophilia 8 months for those with moderate hemophilia, and 1 month for those with severe hemophilia. **[**21]The instant study noted that the most common sites of bleeding are the joints and muscles therefore (63.3%) of patients suffer from joints complications. All patients in this study used coagulation factors only during bleeding and no one of them is following a physiotherapy program. They are aged between (11-20) years and represented about 57.8%. This is supported by Al Momen et al s' (Saudi Arabia 2008-2011) which noted that 48.5% of patients suffering from chronic joints disability. [22]

The current study, showing that the majority (80%) of affected patients have a family history of the disease, and (20%) with no family history, which is contradictory to Turgeons' literature, that recorded negative family history occurs in 30%. [8]No patient in this study presented inhibitors formation. The most prediction of inhibitor development is a genetic mutation, family history of inhibitors, and ethnicity[24].Age at first treatment and the intensity of the first-factor exposure is postulated to be a risk factor for inhibitor formation, [25] the majority of inhibitors have been reported to develop during childhood, at an average age of 12 years, in persons with severe hemophilia A, inhibitor development occurred at an average age of between 1 and 2 years, and after an average of 9-12 treatment. [5]Additionally, inhibitors development are highly dependent on disease severity: it is estimated that between 20% and 50% of patients with severe hemophilia A develop inhibitors compared with approximately 3% of those with mild to moderate disease. [26] Inhibitors are most commonly encountered in people with severe hemophilia which is comprised of the lowermost category here. This may be one reason explain why our population has not reported developing inhibitors. A study was done by Salih, (Sudan, 2007) for 342patients with hemophilia A and 34 patients belong to hemophilia B with mild or moderate disease, inhibitors are not detected in those patients. The researchers explain this by no severe hemophiliac disease situation exists, or that patient's die early due to the remoteness of some areas or lack of factor concentrates. This is in parallel with the present results. [27]In another study performed by Mohammed (Sudan, 2011),80 patients with hemophilia A 17(21%) are found to have inhibitors. which not detected in any of 14 patients with mild hemophilia, present in 9 of 27(33%) patients with moderate and 70f 17(41%) patients with severe disease. This is not inconsistent with the present study. [28]

Conclusion

The results of the present study demonstrated that patients with hemophilia in Kordofanian states did not exhibit favorable practice in some fields of prevention of disease complication. Therefore; such patients should be provided with adequate information to prevent the lifelong and fatal complications of the disease. Amongst all participated patients, there are no inhibitors never developed, however, the majority of them had haemarthrosis. The establishment of specialized centers, supplies patients with treatments, training, and education to achieve comprehensive hemophilia care are the main advantages of comprehensive treatment.

Recommendation

Further study with a larger sample size should be established to be more representative of hemophiliac patients in Kordofanian states.

Abbreviation

Factor VIII: Factor eight.
Factor IX: Factor nine.
Factor: Factor eleven.
PT: Prothrombin time.
APTT: Activated Partial thromboplastin time.

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