Estimation of factor VIII ; C and inhibitor among severe hemophilia A patients in Sudan

Dr. Wed elbahar H.Aballah[1], Dr. Maria M. Satti[2], Dr. Mansour M. Mansoor[3], Dr. Fathelrahman M. Hassan[4].

College of medical laboratory science, Sudan University of Science and Technology, Khartoum, Sudan[1+3+4]. College of Medicine, Khartoum University, Khartoum, Sudan[2].
Phone: +249118283984; Email: fathbahar@gmail.com

Abstract: Hemophilia A is an X-linked bleeding disorder resulting from heterogeneous mutations in the factor VIII (FVIII) gene which is lead to absence or decreased function of coagulation factor VIII. About 72 patients regularly come to hemophilia center of Khartoum we find all study group are male 59 patients (81.9%) factor VIII activity is less than 1%, 13 patients (19.1%) factor VIII activity from more than 1 and less than 5%. About 8 patients (11.1%) are positive factor viii inhibitor, 20.8% of the patients hemoglobin is less than 12 g/dl, only two patients are reduced platelets from the normal, all patients are elevated APTT and normal PT.
[Wed elbahar H. Aballah, Maria M. Satti, Mansour M. Mansoor, Fathelrahman M. Hassan. **Estimation of factor VIII ; C and inhibitor among severe hemophilia A patients in Sudan.** *Biomedicine and Nursing* 2016;2(2): 5-7]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 2. doi:10.7537/marsbnj02021602

Key words: factor VIII: C and inhibitor, severe hemophilia A, in Sudan

Introduction

Factor VIII circulates in plasma as a large glycoprotein complexes non-covalently to the giant multimeric adhesive protein von Willebrand factor (vWf) which acts as a carrier for factor VIII both during its secretion and in the general circulation. Sequencing of FVIII cDNA predicted a mature secreted protein consisting of 2332 amino acids with a calculated molecular weight of 265kDa (without carbohydrate). Analysis of the sequence showed very clearly a repeating domain structure A1-A2-B-A3-C1-C2 (*Vehar* et al., 1984). In addition close homology was seen (Figure 2.1) to coagulation factor V (also A1-A2-B-A3-C1-C2, although the B domains are apparently unrelated) and to the plasma protein caeruloplasmin (A1-A2-A3) (Vehar et al., (1984); Koschinsky etal., (1986); Kane etal.,(1986). Hemophilia A (factor VIII deficiency) is the most common hereditary disorder of blood coagulation (Reinhold M, Erhard H, Jonathan G et al, 2007). It is due to the absence or decreased function of coagulation factor VIII, resulting from mutations in the factor VIII gene (Brinkhous KM et al 1975). FVIII gene located on Xq28. The gene is 186 Kb in length and has 26 exons. Mutations described in the FVIII gene are mostly gene rearrangements, point mutations and large deletions and insertions (http://europium.mrc.rpms.ac.uk1) (HAMSTeRS 2012). The diagnosis depend on estimation of factor viii level which is decreased and also elevated of APTT with normal PT and other coagulation profile. Factor viii inhibitor is tested. Hemophilia A is the most common hereditary clotting factor deficiencies. The prevalence is 30-100 per million populations. (Reinhold M, Erhard H, Jonathan G et al, 2007) (Forbes CD Aledort LM, Madhok R 1997)

Material and methods

Sampling

The health care provider uses a needle to take blood from one of the available vein. The blood collects into an airtight container. Patients prepaid that not taken factor VIII concentrate or blood. Collection of samples from relax patient in a very high precaution by needle suitable with vacutainers.

Patients:

Study group is 72 Patients are coming to the clinic of the hemophilia center in Khartoum Teaching Hospital. The patients present varying degrees of severity of the disease. Chosen of the patients according to the severe cases, and the severity depend on the clinical feature of the patients.

Factor VIII assay chromogenic assay:

By biophen VIII:c (ANIARA COMPANY USA)

When activated by thrombin, Factor VIII: C forms an enzymatic complex with Factor IXa, phospholipids and Calcium, which activates Factor X to Factor Xa. BIOPHEN Factor VIII: C is a chromogenic assay for testing the cofactor activity of Factor VIII:C. In presence of a constant amount of Factor IXa, Phospholipids (PLPs) and Calcium, thrombin activated Factor VIII:C forms an enzymatic complex, which activates Factor X, supplied in the assay at a constant concentration and in excess, to Factor Xa. This activity is directly related to the amount of Factor VIII:C., which is the limiting factor in the presence of a constant and in excess amount of Factor IXa. Generated Factor Xa is then exactly measured by its activity on a specific Factor Xa chromogenic substrate (SXa-11). Factor Xa cleaves the substrate and releases pNA. The amount of pNA generated is directly proportional to the Factor Xa activity. Finally, there is a direct relationship between

the amount of Factor VIII:C in the assayed sample and the Factor Xa activity generated, measured by the amount of pNA released, determined by colour development at 405nm (Wagenvoord RJ (1989) (Sir John V Dacie, S. Mitchell Lewis, Barbara J. Bain etal 2006).

Assay protocol:

High range tested plasma and control are assayed at the 1:40 dilution in BSA (reagent 4). And the low range tested plasma and controls are assayed at 1:10 dilution in tris BSA buffer (reagent 4) in micro-well pre-incubated at 37° C introduces:

Calibrated, or diluted test plasma, or control	50 μl
R1: Factor X preincubated at 37° C	50 μl
R2: Factor IX mixture preincubated at 37° C	50 μl
Mix and incubate for 5 minutesnat37° C the	
R3 SXa-11 substrate preincubated at 37° C	50 μl
Mix and incubate for 5 minutes at 37° C exactly	
reaction by introducing:	
Citric acid 20 g/l or 20% acetic acid	50 μl
Mix and measure the absorbance at 405 nm against sample blank.	

Calculations:

Bilogarithmic graph paper for high range or linear for low range and plot on abscissa the factor viii:c concentration(%) and on ordinates the corresponding absorbance (A 405nm).Result expressed as % of factor VIII

Factor VIII inhibitors:

Screened for Factor VIII Inhibitor is by mixing the test plasma with a known amount of Factor VIII. After a 2 hour incubation period at 37°C, the residual Factor VIII activity is determined in a factor VIII assay by chromogenic assay. By comparing the difference in the Factor VIII activity of the patient incubation mixture and a control mixture, the absence or presence of a Factor VIII inhibitor can be demonstrated (Mikaelsson M. et al 2006) (Sir John V Dacie, S. Mitchell Lewis, Barbara J. Bain etal 2006).

Result:

Table 1 Distribution of study population according to their age.

Age(years)	Frequency	Percent(%)
2-10	29	40.3
10 - 20	25	34.7
20 - 40	16	22.2
40 - 60	2	2.8
Total	72	100.0

Table 2 factor VIII activity in study population.

Factor VIII activity %	Frequency	Percent
less than 1	59	81.9
from 1 to 5	13	18.1
Total	72	100.0

Table 3 Distribution of study population according to their Inhibitor.

	Frequency	Percent %
Positive	10	13.8
Negative	62	86.2
Total	72	100.0

Table 4 Residual factor VIII activity after mixing study:

Activity %	**Frequency**	**Percent %**
60 -100	62	86.2
40 - 59	5	6.9
20 – 39	1	1.4
Less than 20	4	5.5

Discussion:

About 72 patients regularly came to hemophilia center with bleeding in their joints, knees, elbows, toes, and sometimes with surgical operations looking for factors VIII concentrates, at this time we collect our blood samples for factor assay, inhibitor, hemoglobin, platelets, PT, and APTT to our study we find clinically all patients are severe hemophilia a suffer from pain of bleeding we take samples inK_2 EDTA for HB, platelets, or CBC and citrated blood for platelet poor plasma to do factor VIII assay and inhibitor. We find all the study group are males (100%), all study group are males, their ages as followed 29 patients (40.3%) less than 10 years, 25 patients (34.7%) between 10-20 years, 16 patients (22.2%) between 20 -40 years and only two patients more than 40 years old. Also we find 51 patients (70.8%) are students, 12 patients (16.7%) are labor, and the rest of patients (12.5%) with no work. In assaying of factor VIII activity 59 patients (81.9%) less than 1%, 13 patients (19.1%) were more than 1 and less than 5% those were(moderate hemophilia A). Residual factor VIII % after mixing study from 60 – 100 were 62 (82.2%) those were negative inhibitor, and 10 patients(13.8%) were positive for factor VIII inhibitors (their residual factor VIII after mixing study were less than 60%) (Sir John V Dacie, S. Mitchell Lewis, Barbara J. Bain etal 2006). While in previous study inhibitor were 22% (Maurizio Margaglione, et al 2008), all patients are normal PT (13-15 seconds) with normal INR, APTT is elevated significantly 22 patients (30.6%) are fall between 45 to 60 seconds, 43 patients (59.7%) fall between 60 to 80 seconds, and 7 patients (9.7%) their APTT more than 80 seconds.

Acknowledgements:

The Hemophilia center Sudan and Khartoum Teaching Hospital.

Corresponding Author Address:
Wed elbahar H. Aballah
College of medical laboratory science
Sudan University of Science and Technology
Khartoum 1116, Sudan; P.O. Box: 407
Phone: +249118283984; Email: fathbahar@gmail.com

References

1. The hemophilia a mutation, structure, test and resource site HAMSTeRS UK 2012.
2. Reinhold M. Erhard H, Jonathan G, Ronald P, MODERN HEMATOLOGY Biology and Clinical Management Second Edition2007 Humana Press Inc. 999 Riverview Drive, Suite 208 Totowa, New Jersey 07512.
3. Brinkhous KM. A short history of hemophilia with some comments on the word "hemophilia". In: Brinkhous KM, Hemker HC, eds. Handbook of Hemophilia. New York, NY: Elsevier Science; 1975:3.
4. Forbes CD, Aledort LM, Madhok R, eds. Hemophilia. London, England: Chapman & Hall; 1997:1-371.
5. Wagenvoord RJ et al. Development of a simple chromogenic factor VIII assay for clinical use. Haemostasis Vol. 19, N°4, 196-204 (1989).
6. Mikaelsson M. et al. Influence of phospholipids on the assessment of factor VIII activity. Haemophilia Vol. 4, N°4, 646-50 (1998).
7. Maurizio Margaglione, Giancarlo Castaman, Massimo Morfini, Angiola Rocino5,Elena Santagostino, Giuseppe Tagariello, Anna Rita Tagliaferri, EzioZanon, Maria Patrizia Bicocchi, Giuseppe Castaldo, Flora Peyvandi, Rosa Santacroce, Francesca Torricelli, Elvira Grandone, Pier Mannuccio Mannucci, (2008) Haematologica, The Italian AICE-Genetics hemophilia A database: results and correlation with clinical phenotype, Vol 93, Issue 5, 722-728 doi:10.3324/haematol.12427 Copyright © 2008 by Ferrata Storti Foundation
8. Mikaelsson M. et al. Assaying the circulating factor VIII activity in haemophilia A patients treated with recombinant factor VIII products. Semin. Thromb. Hemost. Vol 28, N° 3, 257-64 (2002).
9. Sir John V Dacie, S. Mitchell Lewis, Barbara J. Bain and Imelda Bates, Quantitative Measurement of Factor VIII:C Inhibitors Practical Haematology, 10th ed., Copyright © 2006 Churchill Livingstone, 10: 0-443-06660-4.
10. Vehar, G.A., Keyt, B., Eaton, D., Rodriguez, H., O'Brien, D.P., Rotblat, F., Oppermann, H., Keck, R., Wood, W.I., Harkins, R.N., Tuddenham, E.G.D., Lawn, R.-M. And Capon, D.J. (1984) Structure of human factor VIII. Nature 312, 337-342.
11. Kane, W.H. and Davie, E.W. (1986) Cloning of a cDNA coding for human factor V, a blood coagulation factor homologous to factor VIII and ceruloplasmin. Proc. Natl. Acad. Sci. U. S. A. 83, 6800-6804.
12. Koschinsky, M.L., Funk, W.D., van Oost, B.A. and Mac Gillivray, R.T. (1986) Completec DNA sequence of human preceruloplasmin. Proc. Natl. Acad. Sci. U. S. A. 83, 5086-5090. Levinson, B., Kenwrick, S., Lakich, D., Hammonds, G., Jr. and Gitschier, J. (1990) A transcribed gene in an intron of the human factor VIII gene. Genomics 7, 1-11.

4/19/2016