Sindh Univ. Res. Jour. (Sci. Ser.) Vol. 48 (1) 85-88 (2016)

SINDH UNIVERSITY RESEARCH JOURNAL (SCIENCE SERIES)

Clinical Severity and Hematological parameters Investigation of Hemophilia A patients from some cities of Sindh, Pakistan

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Received 19th June 2015 and Revised 13th January 2016

Abstract: Hemophilia A is a serious inherited as an X-linked bleeding disorder, which is caused by a deficiency or complete absence of factor VIII activity. In the developing countries it is reported that around 80% of thepatients were affected with hemophilia. In Pakistan the disease was seen in all parts of the country because marriages in blood relatives. The aim of this study to collect the data of Hemophillia A and to give basic awareness to the society about Hemophiliacs by involving government to provide diagnostic and health care facilities. This study was also designed for investigation, correlation of some necessary and related blood parameters from Hemophilia A patients in some cities of Sindh, Pakistan. 28 hemophilia A families were selected from Hyderabad and its adjacent areas. A questionnaire (22 questions) was filled for the collection of complete history of patients. On the basis of collected information about hemophilia A patients family charts/ pedigrees were drawn. Spreading of hemophilia A also categories and observed on the basis of marriages within or outside families, ethnic and different age groups and occurrence in different cities. The Coagulation, hematological and biochemical parameters were also assessed to determine the correlation with hemophilia A patients. It was noted that 71% marriages were within family and belonged to four different ethnic groups. Seven different cities Hyderabad and its adjacent areas were also observed the average of Hyderabad patients were higher as compared to other cities. The reported data in this study exhibited some correlation and some variation in the blood parameters results in different age group, ethnic groups etc.

Keywords: Hemophilia, APTT (Activated partial thromboplastin time), PT (Prothrombin time), blood coagulation factor (F-VIII), CBC (complete blood count), total protein, Albumin/Globulin ratio,

1. INTRODUCTION

Hemophilia is an inherited bleeding disorder which passed down through the family and person's blood is affected and then blood is missing the important proteins therefore in this disease blood does not clot correctly, the body has trouble stopping itself from bleeding (Raabe, 2008). Hemophilia diseases are recessive and X-linked, recognized and identified into two types Hemophilia A and Hemophilia B. Both are caused by decreased level of blood clotting protein FVIII and FIX in plasma respectively. Virtually 85% of the entire hemophiliac have factor VIII deficiency (Hemophilia A), and the remaining 15% exhibit factor IX deficiency (Hemophilia B) (White, *et al.,* 2001).In both Hemophilia A and B merely males are affected but it can't never be transmitted from affected males to their sons, while females are carrier. In case of carrier females there is a chance of having either affected son or carrier daughter. On the molecular basis affected females can be analyzed as inactivation of one normal X-chromosome and Turner syndrome (XO karyo type), in which dominant mutation in FVIII and FIX genes resulting abnormal synthesis of FVIII and FIX correspondingly (Pruthi, 2005).

The gene of factor VIII is located on chromosome and comprises no less than 26 exons separated by 25 non coding introns and about 0.1% is the complete gene constitutes of the chromosome (Wood, *et al.,*1984) (Toole, *et al.,* 1984).If there is any change occur in the DNA code of gene (mutations), then F8 gene have been confirmed to decrease either the expression or the activity of FVIII protein and cause Hemophilia A (Antonarakis, *et al.,* 1995)., FVIII circulates in plasma of healthy individuals in trace amount (100 to 200ng ml^{-1}) (McGlynn, *et al.,* 1996). Decreased activity of FVIII gene caused by mutation results in loss of clotting activity and therefore to unchecked bleeding into joints, muscles or inner organs. Recurrent episode of bleeding into joints results in chronic synovitis (Ghosh, 2004).

In blood coagulation the role of factor VIII is to accelerate the cleavage rate of factor X by activated factor IX. In this reaction of multi component, the enzyme and the substrate are concentrated on a surface of phospholipids, decreasing the K_m (Michaelis constant) by several orders of magnitude. Dramatically factor VIII increases the maximal velocity of the

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reaction, accelerating it many thousand folds. (Van-Dieijen, *et al.,* 1981) (Mann, *et al.,* 1990).

Major symptoms of hemophilia is spontaneous internal bleeding and in which bleeding is common from these body organs, joints, knees, ankles, elbows, muscles and soft tissues. This may be caused due to injury, but it can begin spontaneously in severe hemophilia. Spontaneous mutations reported about ⅓ of all hemophiliacs. Hemarthrosis, in the absence of major trauma Deep muscle hematomas intracranial bleeding is occurs. After initial bleeding the prolonged emission is stop following are tooth extractions, mouth injury, or circumcision, prolonged bleeding or renewed bleeding following are surgery or trauma unexplained GI (gastrointestinal tract) bleeding or hematuria, menorrhagia, and especially at menarche, excessive bruising, prolonged nosebleeds, especially recurrent and bilateral, subcutaneous hematomas and especially with firm. Sona, *et al.,* 2010).

Hemophilia A is tenacious and familiar inherited bleeding disorder that postures considerable social and financial trouble in society (WHO/WFH meeting, 1991). Amongst 191 members of different countries of the World Health Organization, 143 were developing countries, and utmost of the countries were within: Asia, Africa and South America (World Health Organization 1998). According to 2009 Global Survey of the World Federation of Hemophilia (WHF), 153,329 individuals worldwide affected with hemophilia among them hemophilia A is the more frequent in contrast to Hemophilia B. The worldwide prevalence of Hemophilia A and B is 115,204 and 24,038 respectively (World Federation of Hemophilia 2009). Hemophilia A occurs in about 80% to 85% of all hemophilia cases. All ethnic and racial groups appear to be equally affected (Montgomery, *et al.,* 2009). The clinically symptoms of Hemophilia A and hemophilia B are alike, even though type A is 4 to 6 times more common than type B (Lozier, *et al.,* 2005).

The survey of 1999, 2006 and 2007 by world federation of hemophilia (WFH), It is reported that prevalence (per 100 000 males) was determined from the reported number of patients with hemophilia A in various countries from 1998–2006 divided by its male population in the relevant year (Stonebraker, *et al.,* 2010), In Pakistan it is estimated that around 10,000 cases of hemophilia A and 2000 cases of Hemophilia B are registered in different Hemophilia treatment centers. In Punjab 5200 males are affected with Hemophilia A and 1000 to 1300 with Hemophilia B (Majroh, *et al.,* 2002).

2. MATERIAL AND METHODS

Collection of Family History and blood sample**:**

Theaffected families with Hemophilia A were belongs to Hyderabad and adjunct areas/cities such as Jamshoro, Mirpurkhas, Kotri, Nosheroferoz, Sanghar, Dadu, and Matiari. The history and blood samples were collected from Fatimid Foundation Blood Bank and Hematological Center Hyderabad. A detail question naire was formulatedto investigate complete history of patients. The information obtained from the patients on the basis of their age, gender, blood group, ethnic group, marital status. Furthermorethe information was categorized with the number of family members affected, cousins'marriages, age of diagnosis, sign and symptoms of other diseases.On the basis of collected information, about hemophilia A patients family charts/ pedigrees were also drawn but not reported here.

Amongst the 28 hemophilia A affected families, 46 individuals were selected for the collection of blood samples, for the estimation of APTT (Activated partial thromboplastin time), PT (Prothrombin time), F-VIII, total protein, albumin, Albumin/Globulin ratio, CBC (complete blood count).

Blood samples in gel tube were vortexed for 4–5 times, allowed to clot and then centrifuged at 10,000 rpm for 10 minutes, transferred the serum in clean eppendorf tubes and kept at 4°C, were used for the estimation of Protein A/G Ratio. While 3.2% sodium citrate containing whole blood samples (1 part sodium citrate solution with 9 parts venous blood) were vortexed for 1-2 times and centrifuge at 10,000 rpm for 10 minutes, plasma was separated and was used for coagulation studies. For complete blood count whole blood sample was prepared by the addition of EDTA K_2.

Determination of Factor VIII :

Blood coagulation factor (FVIII) test was determined by Siemens Company method.

Determination of Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT):

The APTT and PT tests were determined by Automatic Analyzer, CA-50, Sysmex Company.

INR (International Normalized Ratio):

INR=Rwhere R=Clotting time of patient
Clotting time of mean normal

Determination of Complete Blood Count (CBC):

The complete blood count (CBC) was analyzed by automated analyzer Nihon Kohden model # K-6318.

Estimation of Serum Total Protein,Albumin and Albumin/Globulin Ratio:

The Total Protein and Albumincontents ofhemophiliaA patients were determined by usingMERCK kit method.

The serum globulinconcentration was determined by using following formula.

Globulin = Total Protein – Albumin

The serum Albumin/globulin (A/G) ratio was determined by using following formula:

Albumin ÷ Globulin = A/G ratio

3. RESULT

The aim of this reported work to collect the data of bleeding disorders and to give basic awareness to the society about Hemophiliacs by involving government to provide diagnostic and health care facilities. The purpose of this work not only to send missives to messes also erudite to the patients, their families and health care sectors for betterment of health care.This study was designed for investigation, correlation of blood coagulation, hematologicaltests and biochemical assay of Hemophilia A patients in Hyderabad city and its nearby areas. There is very little reports on correlation of given parameter.

In this study 46 Hemophilia A patients from 28 families were collected for investigation of their historical background such as marriages within or outside families, ethnic groups, percentage of occurrence in different areas. On the basis of their collected information family history or pedigrees were also drawn but not reported here. While blood samples of 46 patients were collected for analysis of some different related parameters.

It was noted that 71% marriages were within families with first or second cousin or with blood relatives (Siblings) while 29% marriages were outside the families they were not the blood relatives as represented in **(Fig.1)**. The present study also revealed that the blood relatives were a carrier of hemophilia A due to marriages between two carriers which may lead the chance of hemophilic disease appearance.

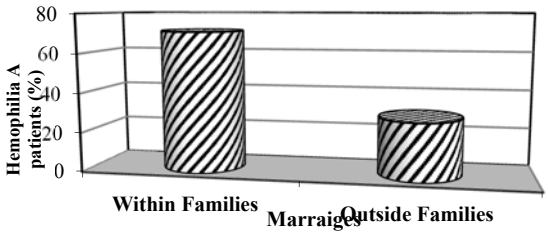

Fig-1: Average (%) Marriages of Hemophilia A between blood relatives and outside families

The Hemophilia A patients and their families were collected from different geographical regions of Sindh (Hyderabad and its adjacent areas/cities). They belonged to 64.28% from Hyderabad, 10.71% from Kotri, and 3.57% each from Jamshoro, Sanghar, Dadu, Tando Mohammad Khan, Mirpurkhas, Nosheroferoz and Matiari as depicted in **(Fig-2)**. According to collected Data the percentage of Hyderabad patients were higher as compared to other cities because Hyderabad is a metropolitan city of different ethnic groups. In present throughout study 28 under investigated families were mainly belonged to four different ethnic groups: Sindhi, Urdu, Pathan and Memon. Among them 61% were Sindhi, 29% were belong to Urdu families, 7% Pathan and 3% Memon as exhibited in **(Fig-3)**.

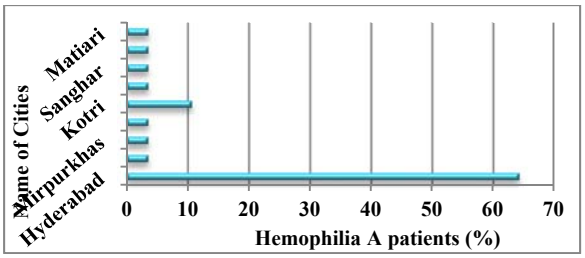

Fig-2: Prevalence (%)of Hemophilia A patients in different cities of Sindh.

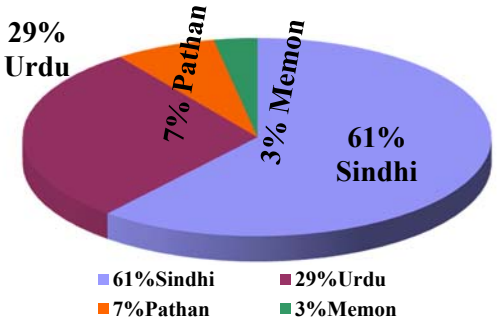

Fig-3: Average (%) of Hemophilia A patients in different ethnic groups.

White *et al.,* also classified Hemophilia A patients as Severe form–factor level <0.01 IU/mL (<1% of normal); Spontaneous bleeding, predominantly in joints and muscles. Moderate form–factor level 0.01 to 0.05 IU/mL (1-5% of normal); Occasional spontaneous bleeding, severe bleeding with trauma, surgery.While Mild form–factor level >0.05 to 0.40 IU/mL (>5 to 40% of normal). 28 hemophilia A disorder affected families out of them 46 individual patients were categorized into three groups with different age as children (3 to 12 years), teen age (13 to 19 years) and adult (20 to 42 years). The blood samples were collected at the time of blood transfusion for the determination of coagulation, biochemical tests and CBC.

4. DISCUSSION

Hemophilia A is enduring and familiar inherited bleeding disorder that poses serious social and financial trouble in society. The chief health priorities of the developing countries are nutrition, hygiene, prevention of contagious diseases, family planning and provision of fundamental health facilities and a very small amount of assets is spent on diseases like hemophilia. The lack of data makes it hard to exactly represent the conditions

regarding the epidemiology, diagnosis and treatment of hemophilia Mohsin, *et al.,* 2010).

5. CONCLUSION

On the basis of reported data, it is concluded that the FVIII level in Hemophilia A patients were 25% Severe, 52% Moderate and 17% Mild. While APTT value in children, teenage and adult was noted to be prolonged but PT was normal. Complete Blood Count (CBC) was also checked from collected whole blood sample of hemophilia A patients. RBC count was recorded normal in children and adult but slightly decreased in teenage group whilst WBC and PLT were found in normal range. The absolute indices parameters were also performed showing that the Hb and MCHC level in all three age groups were low in comparison to normal reference range. HCT level was normal in adult but decreased in children and teenage group. MCV noted to be normal in all three age group and MCH level was low in comparison to reference range. According to the results, it was also noted that total protein is lowered in children, teenage and adult while A/G ratio was normal in all three age group.

AKNOWLEDGEMENT

The authors would like to sincere gratitude and thanks to Fatimid Foundation Blood Bank and Hematological Center, Hyderabad, Pakistan for collection of Hemophilia A affected families' history and blood samples and also helpful suggestions of their doctors and to the University of Sindh, Jamshoro, Pakistan for support in this research.

REFERENCES:

Antonarakis, S. E., H. H. Kazazian, and G. D. Tuddenham, (1995). Molecular etiology of Factor VIII deficiency in hemophilia. Hum. Mutat. 5: 1–22.

Ghosh, K., (2004). Management of hemophilia and its complications in developing countries. Clin. Lab. Haem. 26: 243–251.

Lozier, J. N., and C. M. Kessler, (2005). Clinical aspects and therapy of hemophilia. In: Hoffman R, Benz EJ Jr, Shattil SJ, et al, eds. Hematology: Basic Principles and Practice. 4th ed. Philadelphia, PA: Elsevier Churchill Livingstone. 2047-2069.

Majroh, M. A., and N. Fatima, (2002). Status of thalassemia and hemophilia services in existing health care delivery system of the Punjab. Ann. King Edward Med Coll. 8: 39-42.

Mann, K. G., M. E. Nesheim, W. R. Church, P. Haley, and S. Krishnaswamy, (1990). Surface-dependent reactions of the vitamin K-dependent enzyme complexes. Blood. 76: 1-16.

McGlynn, L. K., C. R. Mueller, M. Begbie, C. R. Notley, and D. Lillicrap, (1996). Role of the liver-enriched transcription factor hepatocyte nuclear factor-1 in transcriptional regulation of the factor VIII gene. Mol. Cell. Biol. 16: 1936–1945.

Raabe, M., (2008). Genes and disease hemophilia. Chelsea House, An important InfoBase publishing, New York. 9.

Mohsin, S., T. Saeed, S. Hussain, S. Mahmood, S. Sohail, and W. Sami, (2010). Clinical Manifestations and Complications of Haemophilia A in Pakistan. Ann. Pak. Inst. Med. Sci. 6(3): 168-171.

Montgomery, R. R., J. C. Gill, and J. D. Paola, (2009). Hemophilia and von Willebr and disease. In: Orkin SH, Nathan DG, et al, eds. Nathan and Oski's Hematology of Infancy and Childhood. 7th ed. Philadelphia, PA: Saunders Elsevier. 1487-1524.

Pruthi., R. K. (2005). Hemophilia: A Practical Approach to Genetic Testing. Mayo Clin Proc. 80(11): 1485-1499.

Sona, P. S., and C. M. Lingam, (2010). Hemophilia an Overview. Int. J. Pharm. Sci. 5:18-26.

Stonebraker J. S., P. H. B. Bolton-Maggs, J. M. Soucie, I. Walker, and M. Brooker, (2010), A study of variations in the reported hemophilia A prevalence around the world. Hemophilia, 16: 20–32.

Toole, J. J., J. L. Knopf, J. M. Wozney, L. A. Sultzman, J. L. Buecker, D. D. Pittman, R. J. Kaufman, E. Brown, C. Shoemaker, E. C. Orr, G. W. Amphlett, W. B. Foster, M. L. Coe, G. J. Knutson, D. N. Fass, and R. M. Hewick, (1984). Molecular cloning of a cDNA encoding human antihaemophilic factor. Nature. 312: 342–347.

Van-Dieijen, G., G. Tans, J. Rosing, and H. C. Hemker, (1981). The role of phospholipid and factor Vita in the activation of bovine factor X. J. Biol. Chem; 256: 3433-3442.

White, G. C., F. R. Rosendaal, L. M. Aledort, J. M. Luscher, C. Rothschild, and J. andIngerslev, (2001). Definations in hemophilia. Thromb. Haemost. 85: 560-575.

Wood, W. I., D. J, Capon, C. C. Simonsen, D. L. Eaton, J. Gitchier, B. Keyt, P. H. Seeburg, D. H. Smith, P. Hollingshead, K. L. Wion, and E. Delwart, (1984). Expression of active human factor VIII from recombinant DNA clones. Nature. 312: 330-337.

World Health Organization (WHO). (1998). The world health report: Geneva: WHO. 1-232.