PATTERN OF HEREDITARY COAGULATION DISORDERS IN NORTHERN AREAS OF PAKISTAN

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SUMMARY

About five hundred patients with bleeding diathesis were investigated to detect hereditary coagulation disorders. The investigations included, complete blood counts, platelet counts, B.T., C.T, prothrombin time, APTT, thrombin time in all cases and solubility test for factor XIII and factor assay where indicated. A total of fifty cases having hereditary coagulation disorders were detected. The commonest of all was haemophilia (30%) followed by von Willebrand disease (26%) and Christmas disease (14%). The rare autosomal recessive hereditary coagulation disorders together constituted (30%). The factor assays of these patients showed low levels of factor activity thus confirming the significance of the test in the diagnosis of these disorders.

INTRODUCTION

Haemophilia is the commonest hereditary disorder of blood coagulation. The incidence is of the order of 30-100/10^6 population.[1] The defect is an absence or low level of plasma factor VIII. The inheritance is sex-linked, gene for factor VIII located on X^q 2.8.[2] One third of cases may be sporadic and have no family history. Many genetic defects which include point mutations and gene deletions have been identified in haemophilic families by cloning the CDNA for factor VIII.[3] The inheritance and clinical features of factor IX deficiency (Christmas disease) are identical to those of haemophilia A. Specific coagulation factor assays can only distinguish these two disorder. The incidence is one fifth that of haemophilia A.[4] The gene for factor IX is located close to factor VIII gene (X^q 2.6) on X chromosome.[5]

The incidence of von willebrand disease is almost similar to that of haemophilia A one per thousand of population.[6] The inheritance is autosomal recessive with varying expressions. The primary defect is a reduced von willebrand factor which acts as a carrier protein for factor VIII-C and also helps in platelet adhesion.[7] The molecular defects which have been identified include point mutations and major deletions.[8]

Hereditary disorders of other coagulation factors are rare and inheritance is autosomal recessive.[9]

MATERIAL AND METHODS

Bleeding time and Hess's tests were performed on patients while venous blood samples were collected and distributed as follows.

5-10 ml in trisodium citrate (1 ml to 9 ml of blood)

3 ml in EDTA container

3-4 ml in a plain glass test tube.

For control blood was collected from six normal subjects (of different ages and sex) in trisodium citrate. On fresh blood samples complete blood counts, peripheral blood smears clotting time and platelet count were performed. Platelet poor plasma was obtained by centrifugation of citrated sample

(both test and control) at 2000 g for 10 min. Control plasma samples from all the six normal subjects were pooled.

One stage prothrombin time (P.T.) activated partial thromboplastin time (A.P.T.T.), Thrombin time (T.T.) and chemical assay of fibrinogen (if needed) were done on this citrated plasma on the same day. Correction studies, solubility test for factor XIII and factor assay done later (within 2 weeks of sample collection).

Factor assay:- All the above mentioned investigations and factor assay were performed according to the standard WHOmethods.[10] Factor assays were done in all the patients showing abnormalities in the screening tests. Freeze dried factor deficient plasma (of human or bovine) having not more than 1% of the deficient factor but adequate concentration of other factors was used. Three dilutions of both test and standard plasma (1 in 10, 1 in 20 and 1 in 50) were made in barbitone buffered saline.

Assay based on A.P.T.T. (for factor VIII, IX, XI) 0.1 ml of factor VIII :c deficient plasma , 0.1 ml of standard or test dilution, 0.1 ml of platelet substitute (Bell and Alton phospholipid) and 0.1 ml of kaolin were pippeted into a siliconized glass tube and incubated for 10 min. Then 0.1 ml of calcium chloride was added to this mixture and clotting time noted. The procedure was repeated with all the test/ standard plasma dilutions. The clotting times of these mixtures were plotted on a double log paper against concentration of deficient factor.

Factor assay based on prothrombin time (for factor V, VII, X). 0.1 ml of thromboplastin, 0.1 ml of standard test plasma dilution and 0.1 ml of factor deficient plasma were pippeted in siliconized glass tube and incubated at 37°C for 1 min. Then 0.1 ml of 0.025 mmol/l calcium chloride was added and clotting time recorded. The clotting times of both test and standard plasma were plotted on a log-log graph paper against the factor concentration.

RESULTS

About five hundred patients with bleeding diathesis from Northern areas of Pakistan were screened to see the pattern of hereditary coagulation defects.

A total of fifty cases having hereditary coagulation defects were detected.

Table-I shows the pattern of hereditary coagulation disorders. This include 15(30%) cases of Haemophilia A, 13(26%) cases of von willebrand's disease, 7(14%) cases of Christmas disease while the rare autosomal recessive hereditary clotting defects constituted 15(30%) of total 50 cases.

Table-II shows male to female ratio of patients having HCD. As haemophilia and Christmas disease are sex linked hereditary disorders, these were seen exclusively in males. The autosomal recessive HCD with the exception of von willebrand's disease, factor VII deficiency and fibrinogen deficiency also showed an increased M:F ratio.

Table-III shows percentage of patients with positive family history. It is obvious

TABLE - I

DISTRIBUTION PATTERN OF HEREDITARY COAGULATION DISORDER

(n=50)

Hereditary coagulation defect	No	%
Haemophilia A	15	30%
Von willebrand's disease	13	26%
Haemophilia B (Christmas disease)	07	14%
Prothormbin deficiency	06	12%
Factor XIII deficiency	04	08%
Factor VII deficiency	02	04%
Factor XI deficiency	01	02%
Factor V deficiency	01	02%
Fibrinogen deficiency	01	02%

TABLE - II

MALE TO FEMALE (M:F) FATIO OF PATIENT HAVING HEREDITARY COAGULATION DEFECTS

(n=50)

Hereditary coagulation defect	M:F Ratio
Haemophilia	15:0
Von willebrand disease	1:1
Christmas disease	7:1
Prothrombin deficiency	2:1
Factor XIIII deficiency	4:0
Factor VII deficiency	1:1
Factor V deficiency	1:0
Factor XI deficiency	1:0
Fibrinogen deficiency	0:1

that mutation of gene is quite common in case of haemophilias.

Haematological Data:- The platelet count ranged between 150-600 x 10^9/L. The bleeding time was prolonged in all the cases of VWD and Fibrinogen deficiency. Patients having deficiency of factor V, Fibrinogen and Prothrombin showed prolonged clotting time while 9 out of 15 patients of haemophilia had clotting time more than 15 minutes. Prothrombin time was markedly prolonged (>3 min) in all the cases of prothrombin deficiency and fibrinogen deficiency while factor VII and factor V deficient patients showed a moderate prolongation of PT. APTT was prolonged in all the cases of haemophilia VWD and Christmas disease. Patients having deficiency of, Factor XI, Factor V, Prothrombin and Fibrinogen also showed prolongation of APTT. Thrombin time was normal in all the cases except the one with fibrinogen deficiency. In the present study the diagnosis of various hereditary coagulation defects was based on factor assay. The factor assay showed less than 1% factor activity in 9 out of 15 patients of haemophilia and in 4 out of 7 patients of Christmas disease. The level of factor VIII:C was also low in 11 out of 13 cases of VWD. The patients with autosomal recessive disorder showed factor level between 5-15% of normal.

TABLE – III

PERCENTAGE (%) OF PATIENTS HAVING HEREDITARY COAGULATION DEFECT SHOWING POSITIVE CONSANGUINITY

(n=50)

Hereditary coagulation defect	% of cases showing positing consonguinity
Haemophilia	40%
Von willebrand disease	69%
Christmas disease	43%
Prothrombin deficiency	67%
Factor XIII deficiency	25%
Factor VII deficiency	50%
Factor V deficiency	Nil
Factor XI deficiency	Nil
Fibrinogen deficiency	100%

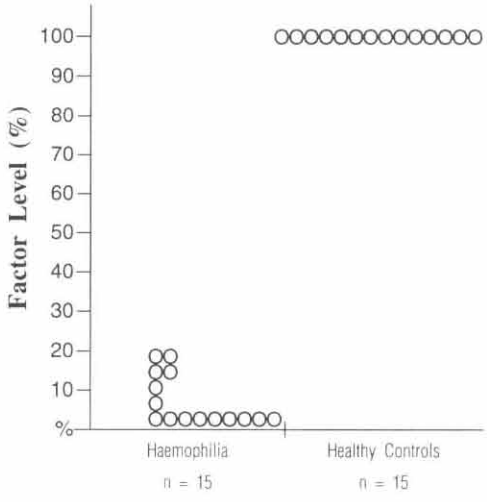

Fig. 1: Comparison of factor level between healthy controls and haemophilic patients. The results were compared using Mann-Whitney U – Test (p 0.0001)

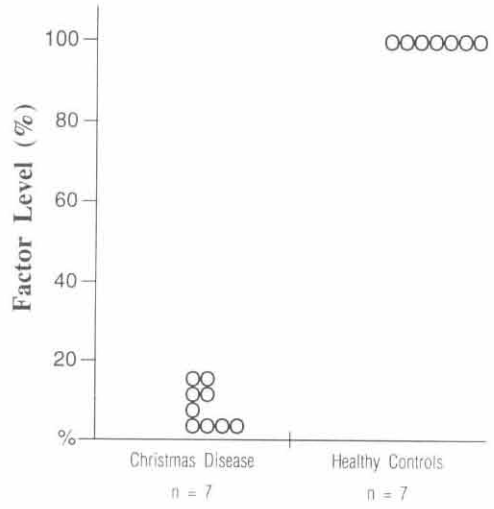

Fig. 2: Comparison of factor level between healthy controls and Christmas disease. The results were compared using Mann-Whitney U – Test (p 0.001)

DISCUSSION

The most prevalent of the hereditary coagulation disorders are haemophilias. Haemophilia A (factor VIII) deficiency accounts for 80-85 % of cases of haemophilia having a worldwide prevalence and occur in all ethnic groups.[11] Von willebrand disease also has a world wide distribution having incidence similar to or even exceed that of haemophilia.[12] The exact incidence of rare autosomal recessive hereditary coagulation disorders is not clearly known.[13]

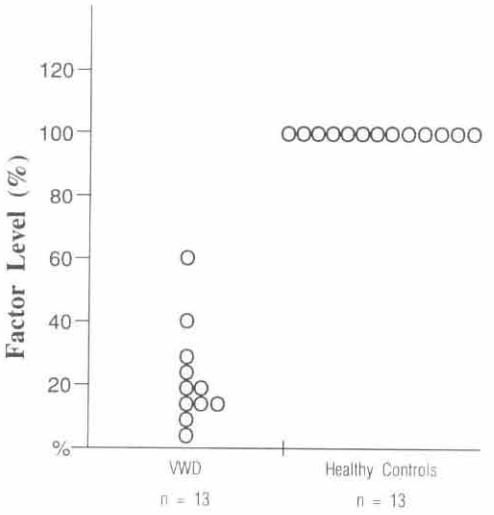

Fig. 3: Comparison of factor VIII: C level between healthy controls and VWD. The results were compared using Mann-Whitney U – Test (p 0.0002)

VWD = Von Willebrand's Disease.

The occurrence of H.C.D. in Pakistan has been known for long time, although the data on the incidence and pattern is limited. The nutritional problems and infections are so common in our country that they mask the magnitude of hereditary disorders. The population of each region of country has a different racial origin, therefore genetic disorders also have an uneven distribution within the country.

The only comparable data is derived from a study in Punjab by Yasmin.[14] According to her, the commonest H.C.D. in Punjabi subjects was Christmas disease. Another study was done by Tanveer in the same year.[15] According to him haemophilia was the commonest H.C.D. followed by VWD and Christmas disease (Personal communication). Factor assay was not done in the suspected HCD patient in both these studies. Only correction studies were performed for diagnosis. Some of the haemophiliacs also showed liacs correction by aged serum and wrongly labelled as Christmas disease.[16] So in HCD factor assay is confirmatory.

The distribution pattern of hereditary coagulation disorders in this study showed the commonest H.C.D. to be haemophilia (30%) followed by VWD (26%) and Christmas disease (14%). The rare autosomal recessive H.C.D. together constituted 30% of the total cases.

The diagnosis of all the patients in this study is based on factor assay. In haemophilia and Christmas disease clinical severity has a definite correlation with factor level.[17] Mild cases have factor level 5-15%, moderate have a level between 1-5% while severe cases have < 2% factor activity.[18] In

this study although more than 50% of patients with haemophilia and Christmas disease were severe (factor level < 1%) (Figs. 1 and 2) but only on patients had clinically severe disease. Patients with VWD also show a variable clinical picture. The commonest type is clinically mild accounting for 70% of all VWD cases,[19] we found 11(84.6%) out of a total 13 patients of VWD (Fig. 3).

Patients having rare coagulation disorder also have a variable clinical picture but there is little correlation between factor level and clinical severity.[20] The differential diagnosis of rest of the cases included in the study in order of occurrence was, I.T.P, Acquired combined factor deficiency, DIC, acute leukaemias, megaloblastic anaemia, Bernard soulier syndrome and one case of Sle inhibitor.

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