Evaluation of Factor VIII and Factor IX Activity among Primary School Children in Owerri, Imo State, Nigeria

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors ILO, EIO, LNE, AMI and CCNV designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BCU, CNA, UOC, CIO, AOS, EFC, BII and COA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The values of factor VIII (FVIII) and factor IX (FIX) were evaluated among some primary school children in Owerri zone Imo State. The aim was to assess deficiency state or reduction in activity among these children. A total of two hundred and ten (210) venous blood samples were collected from primary school pupils whose parents consented to, and whose answers to the distributed questionnaires suggested symptoms of haemophilia. The samples were collected from pupils between the ages of five and thirteen years, and from different primary schools to represent different areas of Owerri (Works Layout Area, Nekede Area, Trans Egbu Area, and Akwukuma Area). Samples were preserved using trisodium citrate anticoagulant and transported to the haematology unit of the Federal Medical Centre Owerri within 3 hours for analysis which was done using Rayto semi auto coagulation analyzer RT 2204C with its normal ranges for factor VIII and IX activities as (50% - 200% and 70% - 200%) respectively. Out of the 210 samples collected, 16(7.6%) have <50% of factor VIII activity and 14(6.7%) have <70% of factor IX activity. Akwakuma area produced highest occurrence of factor VIII deficiency (14.8%, 8 pupils) while Works Layout and Trans Egbu Areas produced the least incidence (3.8%, 2 pupils) each. Factor IX deficiency was most prevalent at Trans Egbu Area (11.5%) and least at Works Layout (0.0%). Six children between the ages of 8 and 7 years had the highest incidence of FVIII deficiency (23.1%), while eight pupils between the ages of 11-13 years showed the highest incidence of FIX deficiency. Eight Females were found to have the highest incidence of both FVIII and FIX deficiencies (8.2% for both defects), while the males presented a lower incidence of the same defects (7.1%, 8 pupils respectively). The mean levels of FVIII and FIX in all the pupils evaluated are 78.61 and 89.98 respectively while the standard deviation of the results from the mean are 2.584 and 5.4%, 6 pupils respectively).

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SAMPLES

The samples were collected from pupils between the ages of five and thirteen years, and from different primary schools to represent different areas of Owerri (Works Layout Area, Nekede Area, Trans Egbu Area, and Akwukuma Area). Samples were preserved using trisodium citrate anticoagulant and transported to the haematology unit of the Federal Medical Centre Owerri within 3 hours for analysis which was done using Rayto semi auto coagulation analyzer RT 2204C with its normal ranges for factor VIII and IX activities as (50% - 200% and 70% - 200%) respectively. Out of the 210 samples collected, 16(7.6%) have <50% of factor VIII activity and 14(6.7%) have <70% of factor IX activity. Akwakuma area produced highest occurrence of factor VIII deficiency (14.8%, 8 pupils) while Works Layout and Trans Egbu Areas produced the least incidence (3.8%, 2 pupils) each. Factor IX deficiency was most prevalent at Trans Egbu Area (11.5%) and least at Works Layout (0.0%). Six children between the ages of 8 and 7 years had the highest incidence of FVIII deficiency (23.1%), while eight pupils between the ages of 11-13 years showed the highest incidence of FIX deficiency. Eight Females were found to have the highest incidence of both FVIII and FIX deficiencies (8.2% for both defects), while the males presented a lower incidence of the same defects (7.1%, 8 pupils respectively). The mean levels of FVIII and FIX in all the pupils evaluated are 78.61 and 89.98 respectively while the standard deviation of the results from the mean are 2.584 and 5.4% (6 pupils respectively). Among these children, a total of 263 (127 males and 136 females) were evaluated. The mean levels of FVIII and FIX in all the pupils evaluated are 78.61 and 89.98 respectively while the standard deviation of the results from the mean are 2.584 and 5.4% (6 pupils respectively). These data show that haemophilia A and B exist in Owerri and considering the danger it portends to lives of the citizenry, Government should provide facilities in our hospitals to take care of the affected pupils to ensure a healthy society.

Keywords: Factor viii; factor ix; primary school; children.

1. INTRODUCTION

Haemophilia is the congenital deficiency of an essential blood clotting protein Fviii or factor ix [1]. Deficiency in factor viii is known as haemophilia type A and deficiency in fix is known as haemophilia type B.

Factor IX plays a key role in hemostasis: it is a vitamin K dependent glycoprotein which is activated through the intrinsic pathway as well as the extrinsic pathway [2].

Factor IX, when activated by FXa or FVIIa-tissue factor, converts FX into FXa and this eventually leads to the formation of a fibrin clot. This conversion is accelerated by the presence of the non enzymatic co factor VIIIa, calcium ions, and a phospholipid membrane [3].

In healthy individuals, FIX activity and antigen levels vary between 50% and 200% of that pooled normal plasma [4].

Haemophilia A and B are clinically indistinguishable from each other and occur in mild, moderate and severe forms (with plasma factor levels of 6-30%, 2-5% and 1% or less respectively). Inherited as X-linked traits, haemophilia A and B are prevalent in the general population of approximately 1 in 10,000 and 1 in 50,000, with no significant racial difference [5].

Blood is usually maintained in a fluid state, without evidence of bleeding or clotting [6]. The presence of an X-linked pattern of inheritance of a bleeding diathesis in families, referred to as haemophilia, has been recognized for hundreds of years [5].

Deficiency in a clotting factor means that the complex cascade of reactions to recognize and stop internal or external bleeding by forming a firm clot is very inefficient or cannot be completed. A male child receives an x-chromosomes from his mother and Y-chromosomes from his father. If the X-chromosomes have the mutated gene, he will have haemophilia. If a female child receives a mutated X-chromosome from the other parent, the normal X-chromosome will dominate and she will be able to produce sufficient amounts of clotting factor. She will not have haemophilia, but will be a carrier of the haemophilia trait. Some females who are carriers have low levels of clotting factor and can experience bleeding.
The majority of patients of haemophilia treatment centers are male [1,7]. The most common types are carried as a sex-linked genes with females carrying the trait and disease manifestations almost always in males occasionally, woman carrying the trait for haemophilia A or B have bleeding manifestation themselves, probably as a result of non random inactivation of their X-chromosomes and over expression of the X-chromosomes coding for haemophilia.

The study was done to evaluate factor VIII and IX activity among primary school children in Owerri, Imo State.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was done in Owerri, Owerri is in Imo State, located at the south eastern part of Nigeria. It is bounded in the south by Rivers State, in the North and East by Abia State and in the West by Anambra State.

2.2 Study Population

The population under study was school children. A total of two hundred and ten (210) venous blood samples were collected from primary school pupils from the selected schools in Owerri who were between the ages of five (5) and thirteen (13) years, selected from the pupils whose parents gave consent and whose answer to questionnaire suggested symptoms of haemophilia.

2.3 Sample Collection

Clinical features of inherited bleeding disorder particularly fairly history of excessive bleeding, history of blood in the urine or stool and others were determined with the aid of questionnaire.

4.5mls of venous blood was collected from all the subjects and added into trisodium citrate container, containing 0.5mls of trisodium Citrate, for coagulation studies (factor viii assay and factor ix assay). The samples were spun at 3000.rpm for 10mins and then the clear plasma was collected into a clean dry plastic container. And transported to the haematology laboratory unit of Federal Medical Centre Owerri within three hours of collection for analysis, using Ray to semi auto coagulation analyzer, RT-2204C model manufactured by Rayti life and analytical sciences Co. Ltd and reagent by Helena biosciences, Europe, Queensway south, gateshead, Tyne and wear, NEII OD UK, REF 5194, 5185, 5375, 5559 and 5186.

2.4 Factor Viili Assay

The factor viii assay was done using the modified one stage method by Penner [8]. The APTT reagent and calcium chloride solutions which were stored at 2°C were pre-warmed to 37°C for 10 minutes. The standard curve was prepared by making dilutions of the calibration plasma in owrens buffer in plastic test tubes. The various tubes were mixed gently without shaking.

The patients and control samples were prepared by adding 100μl of each of the plasmas (patient and control) into two different test tubes containing 400 μl of owners buffer and mixed without shaking.

The testing was carried out by pipetting 100 μl of factor viii deficient plasma into three different siliconized cuvettes, a 100 μl of the standard reagent was added into one of the cuvettes, while another 100 μl each of the patients and control plasma dilutions was added into the other two cuvettes respectively. The mixtures were incubated at 37°C for 2 minutes. Again, 100 μl of the pre-warmed APTT reagent was added to each and incubated for 5 minutes at 37°C. Then, 1001-1 of the pre-warmed 0.025M Calcium Chloride was pipetted and added to each cuvette while simultaneously starting the stop watch. The clot time for each of the standard, control and patients dilutions were determined. A graph of percentage (%) activity (on x-axis) versus mean clot time (on y-axis) for the standard on standard graph paper was plotted and a straight line graph obtained. The patient's activity was traced from graph using the mean clot time. Activity less than 50% was considered factor VIII deficient which could result to haemophilia A [8].

2.5 Factor Ix Assay

The factor ix assay was also done using modified one stage method by Penner [8]. The APTT reagent and calcium chloride solutions which were stored at 2°C were pre-warmed to 37°C for 10 minutes. The standard curve was prepared by making dilutions of the calibration plasma in owren's buffer in plastic tubes. The various tubes were mixed gently without shaking.
The testing was carried out by pipetting 100 μl of factor IX deficient plasma into three different siliconized cuvettes, a 100 μl of the standard reagent was added into one of the cuvettes, while another 100 μl each of the patients and control plasma dilutions was added into the other two cuvettes respectively. The mixtures were incubated at 37°C for 2 minutes. Again, 100μl of the pre-warmed APTT reagent was added to each and incubated for 5 minutes at 37°C. Then, 1001 μl of the pre-warmed 0.025M Calcium Chloride was pipetted and added to each cuvette while simultaneously starting the stop watch. The clot time for each of the standard, control and patient dilutions were determined. A graph of percentage (%) activity (on the x-axis) versus mean clot time (on the y-axis) for the standard on standard graph paper was plotted and a straight line graph obtained. The patient's activity was traced from the graph using the mean clot time. Activity less than 70% was considered to be factor IX deficient which could result to a haemophilia B [8].

2.6 Statistical Analysis

Data obtained was subjected to statistical analysis using chi-square to test goodness of fit and independence. Percentage was used to determine proportion.

3. RESULTS

The results of two hundred and ten (210) venous blood samples investigated for both factor VIII and factor IX levels are as follows:

Table 1 shows the distribution of factor VIII and factors IX activities according to age-group. The age-group 5-7 had the highest of factor VIII deficiency (23.1%), followed by Age-group 8-10 with 6(5.9%) and the least was Age-group 11-13 years 4(4.9%). Factor IX deficiency occurred most among the age-group 11-13 years with 8(9.8%) followed by age-group 8-10 with 6(5.9%) and the least was Age-group 5-7 with 0(0.0%).

Table 2, shows the distribution of factor VIII and IX activities according to sex. Incidence of factor VIII deficiency was higher in female (8.2%) than in male (7.1%). That of factor IX deficiency was also higher in female (8.2%) than in male 6(5.4%).

The overall incidence of factor VIII deficiency in Owerri is 16(7.6%) and that of factor IX deficiency is 14(6.7%). This incidence varies from one area of Owerri to the other; According to area, the incidence of factor VIII deficiency are 8(14.8%) in Akwakuma, 4(7.7%) in Nekede and 2(3.8%) in both Works Layout and Trans Egbu. That of factor IX deficiency are 6(11.5%) for Trans Egbu, 6(11.1%) for Akwakuma, 2(3.8%) for Nekede and 0(0.0%) for Works Layout as shown in Table 3.

4. DISCUSSION

This study was done primarily to evaluate the levels of factor VIII and IX activities so as to assess the occurrence of factor VIII and IX deficiency among primary school pupils in Owerri. The study is also to determine the levels of haemophilia A and haemophilia B in Owerri as well as to determine the area that has the highest occurrence. Much has been written in recent years about haemophilia disease but not much work has been done to ascertain its existence on them, in this part of the world.

Table 1. Distribution of factor VIII and IX activities according to age-group

| Age group in years | Mean Age | No. of samples Collected | Factor VIII Activity | Factor IX Activity |
|--------------------|----------|--------------------------|---------------------|-------------------|
| 5-7                | 6        | 26                       | (623.1%) 20         | (65.9%) 96        |
| 8-10               | 9        | 102                      | (65.9%) 98         | (65.9%) 96        |
| 11-13              | 12       | 82                       | (44.9%) 78         | (83.8%) 96        |
| Total              | 210      | 100                      | 16(7.6%) 194       | 14(6.7%) 196      |

Table 2. Distribution of factor VIII and factor IX activities according to sex

| Sex     | No. of samples Collected | Factor VIII Activity | Factor IX Activity |
|---------|-------------------------|----------------------|-------------------|
|         |                         | <50%                 | >50%              |
| Male    | 112                     | 8(7.1%)              | 104               |
| Female  | 98                      | 8(8.2%)              | 90                |
| Total   | 210                     | 16(7.6%)             | 194               |

238
Table 3. Distribution of factor VIII and factor IX activities according to area

| Area          | No. of samples Collected | Factor VIII Activity | Factor IX Activity |
|--------------|--------------------------|----------------------|--------------------|
|              |                          | <50%     | >50%   | <70%   | >70%   |
| Works Layout | 52                       | 2(3.8)   | 50     | 0(0.0) | 52     |
| Nekede       | 52                       | 4(7.7)   | 48     | 2(3.8) | 50     |
| Trans Egbu   | 52                       | 2(3.8)   | 50     | 6(11.5)| 46     |
| Akwakuma     | 54                       | 8(14.8)  | 46     | 6(11.1)| 48     |
| Total        | 210                      | 16(7.6)  | 194    | 14(6.7)| 196    |

Haemophilia is the congenital deficiency of an essential blood clotting factor VIII or factor IX. Deficiency in a clotting factor means that the complex cascade of reactions to recognize and stop internal or external bleeding by forming a firm clot is very inefficient or cannot be completed Butler et al. [1]. Deficiency in factor VIII is known as haemophilia type "A" and deficiency in factor IX is known as haemophilia type B.

The age group (5-7 years) has the highest incidence of factor VIII deficiency 6(23.1%). This could be as a result of the fact that this age group engages less in exercise than any other group.

The age group (11-13) produced the highest incidence of factor IX deficiency 8(9.8%). While the age group (5-7) produced the least 0(0.0%). This is in contrast with Kulkani et al. [9]. Who said that an age-and puberty - related (testosterone induced) give rise to factor ix levels, with an amelioration in bleeding symptoms, which occurs in patients with factor ix leyden. This shift may be attributed to gene hereditary.

The incidence of factor VIII and IX deficiencies in the female pupils; 8(8.2%) each, is higher than in male pupils 8(7.1%) and 6(5.4%) respectively. This rise in female population may be attributed to the fact that females are asymptomatic carriers, hence do not suffer from the disease, while males suffer from the disease. This makes it possible for the males to die more of the disease than female thereby reducing the population of the males with the deficiencies especially in this lower-income part of the world. This is in agreement with skinner [10] who attributed the lower prevalence in lower-income countries to little or no treatment due to lower economic funds in lower-income countries.

This is in agreement with Quadros et al. [13]. Who stated that the disorder is found in all ethnic groups and does not have a specific ethnic or geographic distribution.

Ethnic differences in polymorphisms close to or in the factor X gene he said are important because they provide linkage data when identifying carriers, particularly when the mutation is known or for identification of "denovo mutation".

The result of this study shows that the reduction in factor VIII activity which could result to haemophilia 'A' is 16(7.6%) and that of factor IX is 14(6.7%). This is in agreement with Fakunle et al [11] who got 8.4% incidence of FVIIIC deficiency in live male infants undergoing circumcision in South West Nigeria.

The low incidence of factor VIII and IX deficiencies recorded is this work 7.6% and 6.7% is in agreement with the discoveries of with Evatt [12] who agreed that there is lower prevalence in lower-income countries, but attributed it to insufficient reporting of these data due to the lack of access to health care and improved diagnostic capabilities. Skinner [10], in another study of 56, 762 diagnosed prevalent cases of haemophilia 'A' and 'B' in 2012 in the nine major markets (US, France, Germany, Italy, Spain, UK, Japan, Argentina and China), 30% of these cases he said occured in the US.

According to Area, Akwakuma area has the highest incidence of haemophilia "A" 8(14.8%) followed by Nekede area 4(7.7%) while Trans Egbu has the highest incidence of haemophilia B' 6(11.5%) followed by Akwakuma 6(11.1%). This could be because members of related families always found themselves in a particular school, either because of proximity or because of interest they have in the school.

This is in agreement with Funnel and Crossley [14], who stated that factor IX deficiency, is 4-6 times less prevalent than
factor VIII deficiency even though they said they are similar, with haemophilia B being less severe[15-18].

This study has several limitations among which is that because of the state ministry of education's refusal of permission to collect samples from pupils, the samples were collected from only private primary schools that gave their consent, hence creating an opportunity to collect samples from relations with related genes. Another is that the status of the parents of the pupils was not confirmed to know if they are inherited from the parents. Considering these limitations, some might argue that the prevalence indicated in this study is not a true representation of the actual prevalence in Owerri.

However, haemophilia certainly has become a hot topic in Nigeria, Imo State and Owerri not excluded. It therefore becomes imperative that more research be carried out in this part of the world to concretize the findings of this study.

5. CONCLUSION

In conclusion, this research has confirmed that hemophilia, a congenital deficiency of an essential blood clotting factor VIII or factor IX exist in Owerri zone. Also that the age group (5-7 years) has the highest prevalence of hemophilia ‘A’ while age group (11-13 years) has the highest prevalence of hemophilia ‘B’. This work also proved that females are asymptomatic carriers while males suffer the disease.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Written consent was obtained from the parents of each of the pupils before samples were collected from them.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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