# INCIDENCE OF HAEMOPHILIA A AND HAEMOPHILIA B DEFICIENCY AMONG SCHOOL CHILDREN IN CALABAR SOUTH

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## ABSTRACT

Factor viii and factor ix are key factors in the intrinsic clotting cascade and their incidence of deficiencies varies substantially by countries and limited information is available in some regions, as in: Nigeria and Calabar south, cross River State where this study is carried out. The primary objective of this study is to determine the activity of factor viii and factor ix and their deficiencies among school children in Calabar south metropolis. Three government primary schools were selected which includes: government Primary Schools, Myne Avenue, Government Primary School, Anantiglia and government Primary School, Atu. A total of 120 pupils were examined with 40 pupils from each of the primary schools. Out of the 120 pupils examined 74 pupils were male while 46 pupils were females. The age range of tlie pupils was between 4-13 years) 4.5mls of whole blood was collected from each of the pupils through veinepuncture into a trisodium citrate container with 0.5mls volume of trisodium citrate. The samples were spun and the plasma was separated which was then transported to Federal Medical Centre) Owerri where it was analyzed. It was observed that out of the 74 pupils that were male, 56 pupils were within the age range of 4-8years while 18 pupils were within the age range of 9-13years. In the female category 32 pupils were within the age range of 4-8years while 14 pupils were within the range of 9-13years. Out of the 56 male pupils within the age range of 4-

8years, 2 pupils were deficient for factor viii, giving a percentage incidence of 3.5% and giving an overall incidence of 1. 6% out of the 120 pupils, 3 pupils out of the 56 male pupils within the age range of 4-8years were also deficient for factor ix with a percentage incidence of 5.3 %, one female out of the 32pupils within the age range of 4-8years was also deficient for factor ix, with a percentage incidence of 3.1%. This also gave an overall 3.3 % incidence for factor ix deficiency. The results observed from this study will serve as a form of documentation for the prevalence of factor viii and ix deficiencies in this study area.

*Keywords: incidence, haemophilia a, haemophilia b, school children*

## INTRODUCTION

Haemophilia is a group of hereditary genetic disorders that impair the body's ability to control blood clotting or coagulation, which is used' to stop bleeding when a blood vessel is broken. Haemophilia A (clotting factor Viii deficiency) is the most common form lof the disorder, present in about 1 in 5,000-10,000 male births (Wynbrandth *et al*., 2009). Haemophilia B (factor 1x deficiency) occurs in around 1 in about 20,000-34,000 male births (Wynbranth *et al.*, 2009).

Like most recessive sex-linked, x chromosome disorders, haemophilia is more likely to occur in males than females. This is because females have two x chromosomes while males have only one, so the defective gene is guaranteed to manifest in any male who carries it. Females have two x chromosomes and haemophilia is rare, so the chance of a female having two defective copies of the gene is very remote, so females are almost exdusivelv asymptomatic carriers of the disorder, Female carriers can inherit the defective gene from either their mother or father, or it may be a new mutation. Although it is not impossible for a female to have haemophilia, it is unusual: a female with haemoptuua A or B would have to be a daughter of both a male haempphilia C and a female carrier, while the non-sex linked haemophilia C due to coagulant factor xi deficiency which can affect either sex is more common in Jews of Ashkenazi (east European) descent (Whynbrandt et al, 2009) but rare in other population groups.

Advances in molecular biology have enabled more precised diagnosis and reduced the dependence on plasma – derived concentrates, at least in the economically rich countries. Direct identification of the mutation responsible for the factor deficiency in an individual kindred has now superseded the use of restriction fragment length polymorphisms (RELPs). This can remove the uncertainty from earner detection in many cases (Okoroiwu *et al.,* 2014; Ifeanyi *et al.,* 2020; Obeagu and Obeagu, 2015; Nwovu *et al. 2018;* Obeagu *et al. 2021;* Obeagu, 2022) There are numerous different mutations which cause each type of haemophilia. Due to differences in changes to the genes involved, patients with haemophhilia often have some level of a active clotting factor. Individuals with less than 1% active factor are classified as having severe haemophilia, those with 1-5% active factor have moderate haemophilia, and thoseyvith mild haemophilia have between 5-40% of normal levels of clotting factor (Dimitorios et al., 2009).

The study was done to determine the incidence of haemophilia A and haemophilia B deficiency among school children in Calabar South.

**Materials and Method**

**Study Location and Site**

The study was carried out in Calabar south, Cross River State and three different primary schools were selected. The primary schools were: Government Primary School Mayne Avenue, Government Primary School, Anantigha, and Government Primary School Atu. Cross River state with coordinate location of latitude 4.95 North longitude 8.32 East and 99 meters elevation above the sea level having about 461, 796 inhabitants Calabar is made up mainly Efik ethnic group and few other nationalities with high rate of literacy while civil service is their major occupation (Folola *et al*., 2011).

**Study Population and Sample Size**

One hundred and twenty pupils aged 4-13 years attending the schools in Calabar south were screened.

**Sample Size Calculation**

Sample size was calculated using the formula proposed by Daniel (1999)

$$N = \frac{Z^2 p(1-P)}{D^2}$$

where n = sample size

$Z^2$ = statistic for confidence level at 95% i.e. (1.96)

p = expected prevalence or population

D = degree of precision (0.05)

The sample size for this study was calculated based on Hay et al (2008), 8. 4% incidence of FVIIIC inhibitor in severe haemophilia patients.

$$N = \frac{1.96^2 \times 0.084\ (1.00 - 0.084)}{(0.05^2)}$$

== 118 samples

**Informed Consent**

Participant information sheet (PIS) was given to the prospective headmasters/headmistress. After reading and understanding the PIS, questions were asked and proper explanation given. They consented to participate in the study and thereby signing the informed consent form on behalf of the pupils.

**Sample Collection**

Informed consented subjects were sampled. 4.5mls of blood was collected from all the subjects and added into trisodium citrate container, containing 0.5mls of trisodium citrate container, for coagLilation studies (factor VIII assay and factor IX assay). The sample was spun at 3000rpm for 10mins and then the clear plasma was collected into a clean dry plastic container. The test was performed, using, Rayto semi auto coagulation analyzer, RT-2204C model manufactured by Rayti life and analytical sciences Co. Ltd.

**Eligibility Criteria**

Informed consented subjects (pupils attending the various schools used)

**Exclusive Criteria**

Children who were below the age of 4 years and those above the age of 13 years old, also children who refused to give their consent were also excluded.

**Inclusion Criteria**

Children within the age of 4 years to 13 years who gave their consent were Included.

**Factor VIII Assay**

Reagent by Helena Biosciences Europe, Queensway south Gateshead, Tyne and wear, NEll OSD UK REF, 5194, 5185, 5375, 5559, and 5186

**METHOD: modified one stage method by Penner (1979)**

Principles: A dilution of the test plasma is mixed with the factor deficient (viii) plasma and the clot time of the mixture determined. The degree of clot time, correction with the patient plasma is compared to the correction with a reference material, allowing the percentage activity of the patient plasma Oto be determined.

**PROCEDURE**: The APIT reagent and calcium chloride solutions were pre-wrmed to 37°C for 10minutes. The standard curve was prepared by making the following dilutions of the

calibration plasma in Owrens buffer, in plastic test tubes.

| Tubes | Calibration plasma (ml) | Owrens Buffer (ml) | Activity % |
|---|---|---|---|
| 1 | 0.1 | 0.4 | 100 |
| 2 | 0.1 | 0.9 | 50 |
| 3 | 0.1 | 1.9 | 25 |
| 4 | 0.1 | 3.9 | 12.5 |

The various tubes were mixed gently without shaking. The patients and Control samples were prepared by adding 100ml of the plasma (patient or control) into 400ul of owrens buffer and mixed without shaking. The testing was carried out by pipetting 100ul of factor deficient plasma into a siliconized cuvette and 100ul of standard or patient or control plasma dilution was added. The mixture was incubated at $37^{0}$C for 2minutes. 100ul of the prewarmed APTT reagent was added and incubated for 5minutes at 37°C. Then, 100ul of the pre-warmed 0.025M calcium chloride was pepitted while simultaneously starting the stop watch. The clot time for each standard, control or patients dilutions were determined. A graph of percentage (%) activity (on X-axis) versus mean clot time (on Y-axls) for the standard on 2 cycle log-log graph paper was plotted and a straight line graph obtained.

**Factor IX Assay**

Reagent by Helena biosciences, Europe, queensway south, gateshead, Tyne and wear, NEll OSD UK, REF 5194, 5185, 5375, 5559 and 5186.

**Method: Modified One Stage Method by Penner (1979)**

**Procedure**

The APTT reagent and calcium chloride solutions was pre-warmed to 37°c for 10 minutes. The standard curve was prepared by making the following dilutions of the calibration plasma in owren's buffer in plastic tubes.

| Tubes | Calibration plasma (ml) | Owrens Buffer (ml) | Activity % |
|-------|-------------------------|--------------------|------------|
| L | 0.1 | 0.4 | 100 |
| 2 | 0.1 | 0.9 | 50 |
| 3 | 0.1 | 1.9 | 25 |
| 4 | 0.1 | 3.9 | 12.5 |

The various tubes were mixed gently without shaking. The patient and control samples were prepared by adding 100ul of the plasma (patient or control) into 400ul of Owren's buffer and mixed without shaking. The testing were carried out by pipetting 100ul of the factor deficient plasma into a siliconized Cuvette and 100ul of standard or patient or control plasma dilution was added and incubated for 5minutes at 37°(, then 100ulof the pre-warmed 0.025M calcium chloride was pipetted while simultaneously starting the stop watch. The clot time for each of the standard control or 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 2 cycles log-log graph paper was plotted and a straight line graph obtained.

**Statistical Method**

The data obtained were subjected to some statistical analysis such as percentage, the mean (X) standard deviation (SD), standard error of mean (SEM), two-way Anova and Pearson moment of correlation using statistical package for social science (SPSS) version.

**RESULTS**

The results are presented as percentage, mean values and± standard deviation and were

analyzed using two-way ANOVA and Pearson moment of correlation.

Table 1 Shows the percentage incidence of factor viii deficiency with a 3.5% incidence among the male pupils within the age of 4-8yrs, and a 1.6% incidence among the 120 pupils examined.

Table 2 shows the percentage incidence of factor iv deficiency with a 5.3% incidence among the male pupils within the age of 4-8yrs, 3.1% incidence among the female pupils within the age of 4-8yrs and an overall 3.3% incidence of factor ix deficiency out of the 120 pupils examined.

Table 3 shows the summary report of a two-way ANOVA that examined the incidence of factor ix, with no statistical significant interaction between the gender and age on fact. ix $F_{(8,101)} = 0.279$ p=0.971.

Table 4 shows the summary report of a two-way ANOVA that examined the incidence of fact viii, with no statistical interaction between the gender and age on factor viii $F_{(8,101)} = 0.220$, p.0.987.

**Table 1: Percentage Incidence of Factor VIII Deficiency**

| Gender | Age range (years) | No. of Pupils | No. of pupils deficient for (%) factor viii | Percentage |
|--------|-------------------|---------------|---------------------------------------------|------------|
| Male | 4-8 | 56 | 2 | 3.5% |
| | 9-13 | 18 | 0 | 0 |
| Female | 4-8 | 32 | 0 | 0 |
| | 9-13 | 14 | 0 | 0 |
| Total | 4-13 | 120 | 2 | 1.6% |

**Table 2: Percentage Incidence of factor IX deficiency**

| Gender | Agerange (years) | No. of Pupils | No. of pupils deficient for (%) factor viii | Percentage |
|--------|------------------|---------------|---------------------------------------------|------------|
| Male | 4-8 | 56 | 3 | 3.5% |
| | 9-13 | 18 | 0 | 0 |
| Female | 4-8 | 32 | 1 | 0 |
| | 9-13 | 14 | 0 | 0 |
| Total | 4-13 | 120 | 4 | 3.3% |

**Table 3: Analysis of Variance for Factor IX**

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| gender | 11.766 | 1 | 11.766 | .016 | .899 |
| Age | 735.020 | 9 | 81.669 | .112 | .999 |
| Gender * Age | 1632.397 | 8 | 204.050 | .279 | .971 |
| Error | 73842.417 | 101 | 731.113 | | |
| Corrected Total | 76615.592 | 119 | | | |

a. R Squared= .036 (Adjusted R Squared = -.136)

**Table 4: Analysis of Variance for factor VIII**

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| gender | 693.557 | 1 | 693.557 | 1.080 | .301 |
| Age | 2560.718 | 9 | 284.524 | .443 | .908 |
| Gender * Age | 4896.421 | 8 | 612.053 | .953 | .477 |
| Error | 64832.265 | 101 | 641.904 | | |
| Corrected Total | 74651.592 | 119 | | | |

a. R Squared= .132 (Adjusted R Squared = -.023)

## DISCUSSION

Factor viii (antihaemophilic factor) is a key factor of the intrinsic clotting cascade. Normal hemostasis requires at least a quarter (25%) of the factor viii activity. Symptomatic hemophiliacs usually have levels 5% of normal level. Disease severity are categorized as severe if the level is less than 1%, moderate, if the level is between 1 to 5% and mild if it is more than 5%.

The totals in the various categories do not equal 100%, as there is inter-population variability due to the heterogeneity of factor viii gene mutations and inter-laboratory variation in factor viii activity measurement (Franchini *et al., 2008).*

The incidence of congenital haemophilia A is about 1 in 5,000 boys/men, whereas the incidence of congenital haemophilia B is about 1 in 30,000 boys/men in the United State (Soucie *et aI., 2008).* In the United State around 400 babies are born with haemophilia each year and the prevalence of congenital haemophilia is estimated at 18,000 boys/men, most of which are classified as severe (Collins *et aI., 2007).*

In the United Kingdom, the haemophilia society estimates that around 6000 boys/men have haemophilia (Collins *et al., 2007).* Also in Morocco, a study by Khoubilal et al., reveals that a total of 289 patients suffers from haemophilia and this data was obtained from the Morocco National Bureau of Statistics (khoubila *et aI., 2013)* Congenital haemophilia affects all ethnic group and has a worldwide distribution. From table 4.1 which shows the summary report of the percentage that examined the incidence of factor viii deficiency, it was observed that for the 74 male pupils that were examined, 56 pupils were within the age, range of 4-8years while 18 pupils were within the age range of 9-13 years. Out of the 56 pupils which were within the age range of 4-8years, 2 of the pupils were deficient for factor viii, giving a percentage of 3.5%. Factor viii deficiency was not detected among the female, pupils. This also shows a 1.6% incidence of factor viii deficiency out of the 120 pupils that were examined.

Table 2 showed the summary report of the percentage that examined the incidence of factor ix deficiency. Out of the 56 male pupils that were within the age range of 4-8years, 3 of the pupils

were deficiency for factor ix giving a percentage of 5.3%. One female pupil out of the 32 pupils within the age range of 4-8years was also deficient for factor ix, giving a percentage of 3.1%. This also shows a 3.3% incidence of factor ix deficiency out of the 120 pupils that were examined.

The analysis of variance as showed in table 4.3 indicates that there is no statistical significant interaction between the gender and the age on factor ix. This in effect also means that ignoring the age differences, the gender did not influence the deficiency rate of school-children. And the non-significant also shows the mean for the male and female children were similar, although with a little difference of 1.608. The same result was also observed from the analysis of variance of factor viii as shown on table 4.

From the results obtained from this research work, the percentage incidence of factor viii deficiency is 1.6% and that of factor ix deficiency is 3.3%. The percentage incidence of factor viii deficiency observed from this research work is similar to the research work carried out by Fakunle et al., with a 1.64% incidence of factor viii deficiency in live male infants undergoing circumcision in South-West Nigeria (Fakunle *et al.,* 2007).

'The low incidences of factor viii (1.6%) deficiency and factor ix deficiency (3.3%) observed from this research work agrees with the discoveries that haemophilia is a rare hereditary genetic (Wynbrandth *et aI.,* 2009). And it also agrees with the fact that Haemophilia is likely to affect males than females, since it is an x-linked recessive genetic disorder. But from the result obtained, the gender did not influence the mean deficiency rate of factor viii and factor ix activity. A little difference of 1.608 was observed. Although rare cases of girls/women with haemophilia are described such as those seen due to Iyonisation (random inactivation of the normal x chromosome), homozygosity, mosaicism, or Turner's syndrome (Leur *et el.,* 2001*).*

The values obtained from the pupils that were deficient for factor viii and factor ix activity indicates cases of mild diseases since the pupils are above the age of 2 years (Mild disease presents with factor viii activity level of $>5^{\%}$, age presentation of older than 2 years and percentage of sufferers ranging from 15-31%) (Preston *et al.,* 2004).

Many developing nations like Nigeria don't have the infrastructure in place to report on

haemophil'ia statistics.

In the developed countries, people living with the disorder are not restricted from activities unlike in Nigeria were such people are usually restricted to avoid excessive bleeding.

**CONCLUSION**

Little has been documented on the prevalence of Haemophilia A and B in Nigeria, the 1.6% value of factor viii deficiency and 3.3% value of factor ix deficiency observed from this research work, also serves as a source of documentation, since no similar work has yet been carried out in the research area and as such results from this research will be used to implement prevention programs to help reduce complications and improve quality of life**.**

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