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Transabdominal Chorionic Villous Sampling in Nigeria: Correlation between Number of Cases and other Variables

Le Prelevement Des Villosites Choriales Par Voie Trans-Abdominale Au Nigeria : Correlation Entre Le Nombre De Cas Et Les Autres Variables

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ABSTRACT

BACKGROUND: **transabdominal chorionic villous sampling is generally preferred to the transvaginal approach. The procedure may, however, be associated with complcations due to a number of factors.**

OBJECTIVES: **to review the relationship between the number of cases and other variables in transabdominal chorionic villous sampling and also to identify the complications associated with the procedure.**

METHODS: **two hundred and twenty-six cases of trans-abdominal chorionic sampling performed by a single operator were reviewed. Pearson's correlation coefficient was used to individually analyze the relationship between numbers of samplings performed and other procedure-related variables such as the number of needle aspirations, gestational age, percentage diagnosed at first attempt and weight of sample. The complications reported during procedure and pregnancy was also reviewed.**

RESULTS: **prenatal diagnosis of sickle cell disease which associated for 95% of the cases was the most frequent (95.1%) indication for chorionic villous sampling. There was a statistically significant negative correlation between number of cases and needle aspirations ($p < 0.05$) and between increasing number of number of cases and gestational age ($p>0.05$). Also statistically significant negative correlations were obtained between number of aspirations and weight of sample ($r= –0.9$, $p<0.05$) and between gestational age and weight of sample ($r = –0.89$, $p<0.05$). Correlation between number of cases and weight of sample was highly positive ($r =+ 0.9$). Reported complications included abortion and hypertension.**

CONCLUSIONS: **There is a strong relationship between of number of cases of transabdominal chorionic villous samplings performed and other procedure variables. WAJM 2011; 30(1): 57–61.**

Keywords: Chorionic villous sampling, transabdominal, complications, pre-natal diagnosis.

RÉSUMÉ

CONTEXTE: *La voie trans-abdominale est généralement préférée à la voie vaginale pour le prélèvement des villosités choriales. Toutefois, la procédure peut être associée à des complications en raison d'un certain nombre de facteurs.*

OBJECTIFS: *Examiner la corrélation entre le nombre de prélèvements de villosités choriales par voie trans-abdominale et les autres variables et identifier les complications liées à la technique.*

METHODES: *Deux cent vingt six cas de prélèvements de villosités choriales par voie trans-abdominale, réalisés par un seul opérateur ont été étudiés. Le coefficient de corrélation de Pearson a été utilisé pour analyser individuellement la relation entre le nombre de prélèvements effectués et les autres variables liées à la technique, tels que le nombre d'aspiration à l'aiguille, l'âge gestationnel, le pourcentage de diagnostic dés la première tentative et le poids de l'échantillon. Les complications rencontrées lors de la procédure et pendant la grossesse ont été également étudiées.*

RÉSULTATS: *Le diagnostic prénatal de la drépanocytose était l'indication la plus fréquente pour le prélèvent des villosités choriales (95,1%). Il y avait une corrélation négative statistiquement significative entre le nombre de cas et les aspirations à l'aiguille ($p <0,05$) et entre un nombre élevé de prélèvements et l'âge gestationnel ($p>0,05$).*

Aussi, des corrélations négatives statistiquement significatives étaient notées entre le nombre d'aspirations et le poids de l'échantillon ($r = -0,9$, $p <0,05$) et entre l'âge gestationnel et le poids de l'échantillon ($r = -0,89$, $p <0,05$). Il existait une forte corrélation entre le nombre de cas et le poids de l'échantillon ($r =+ 0,9$). L'avortement et l'hypertension étaient inclus dans les complications liées à la procédure.

CONCLUSION: *Il existe une forte corrélation entre le nombre de cas de prélèvements de villosités choriales par voie trans-abdominale et les autres variables de la procédure. WAJM 2011; 30 (1): 57–61.*

Mots-Cles: *Villosites Choriales, Voie Trans-Abdominale, Les Complications, Diagnostic Prenatal*

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Abbreviations: CVS, Chorionic villous sampling; **RFLP,** Restriction Length Fragment Polymorphism; **SMS,** Short Memory Messages; **SNTR,** Short Nucleotide Tandem Repeat;

INTRODUCTION

Chorionic villous sampling (CVS) was introduced as the most feasible method for the control of genetic diseases in Nigeria in 1987, with two methods, namely transabdominal and transvaginal approach as being in common use.[1] Of the two, the transabdominal approach has gained better acceptance, mainly because it has lesser risk of abortions, transmission of infection to the foetus and also allows for multiple numbers of aspirations, with cleaner tissue yield.[2–5] Studies have shown that it is preferred by most women over the lithotomy position, which is considered embarrassing.[5, 6] It is however not completely free of complications.

The experience of the operator is considered to influence the safety and success of medical procedures.[7] In Nigeria as in many other developing countries, majority of women are concerned about the safety of the CVS procedure, especially the possibilities of morbidities and mortality that may be associated with it.[8] This is because the procedure was only recently available and consequently may be associated with a higher risk of complications based on perceived poor experience of the operators as a result of the few number of cases performed.

Previous studies and reports on the procedure (which included both transcervical ad transabdominal routes) were constrained by the limited number of cases performed. However, with increasing number of cases and all procedures being transabdominal since 2005, it is important to review experience on the possible relationship between increasing number of samplings performed and other procedure-related variables. This report will also serve as an audit of the CVS service and as a basis or reference for other centres in Nigeria.

SUBJECTS, MATERIALS, AND METHODS

The data on 226 consecutive transabdominal chorionic villous samplings done between 1st January 2005 and 30th December 2008 were retrieved for analysis.

A signed informed consent was obtained from all the patients after counseling on the steps in the procedure and possible risks. The study was conducted only on those who voluntarily wished to have prenatal testing conducted on an ongoing pregnancy, mainly because of the history of a genetic abnormality in a member of the family and would wish to avoid it. Exclusion criteria included unresolved vaginal bleeding, threatened abortion and parental haemoglobin electrophoresis pattern of AA.

All cases were performed from 11 weeks by a consultant obstetrician, with subspecialization in foetal medicine, and who had conducted transabdominal CVS under supervision on over 50 on-going pregnancies before first attempt unsupervised in both local and foreign training institutions. The 226 procedures were performed at High Rocks Prenatal Diagnosis Centre, Ilorin, Nigeria.

Chorionic Villous Sampling Procedure

The standard procedures of transabdominal chorionic villous sampling were followed in every case. These included pre and post procedure counseling, transabdominal ultrasound scan for confirmation of gestational age, placenta localisation, and determination of area of maximum placental thickness. The biopsy path was chosen based on these parameters. Local infiltration of the biopsy path was done using between 5-10 mls of 1% Xylocaine solution. Under continuous ultrasound guidance, the aspiration trocar and cannula (16–18mm) were carefully introduced through the anterior abdominal wall, the uterine muscle and finally into the placental tissue. A 'give sign' confirmed entry into the placenta. The assistant then took over the scan probe, ensuring that the tip was always in view. The trocar was withdrawn and replaced with a longer aspiration needle and stylet (18–20mm) and carefully guided further into the placenta tissue before the stylet was removed. A 10–20 ml syringe, preloaded with about 2–4mls of either Heparin or EDTA was attached to one end of the connection tubes which was also attached to the aspiration needle at the other end and a negative pressure created. While maintaining continuous ultrasound guidance, the continuum of syringe, connection tube and aspiration needle and cannula was withdrawn under negative pressure to obtain the villi. The retrieved product was flushed into the petrish dish containing either Heparin or EDTA for further processing.

Samples were analyzed under standard laboratory procedures. The steps in analysis included DNA extraction using boiling method, polymerase chain reaction analysis and either Restriction Length Fragment Polymorphism (RFLP) or Short Nucleotide Tandem Repeat (SNTR) analysis.

The minimum gestational age for sampling was 11 weeks, based on ultrasound scan measurements. Steps in sampling were adequately documented in operation notes and follow-up information added as pregnancy continued. The pregnancies were followed up by regular phone and mail contacts with the primary physician, as well as phone calls, short memory messages (SMS) and internet mail messages between the centre and the women. The complications experienced during the procedure and pregnancy were also obtained from the operation notes, the supervising physician or the patients. In the study, hypertension was defined as one measurement of diastolic blood pressure of >110mmHg or more or two measurements of diastolic blood pressure of ≥ 90mmHg 4 hours or more apart.[9]

Data Management and Statistical Analysis

Analysis conducted on the data included the number of sampling per year, number of aspirations per sampling session, the mean weight of sample obtained, the number of successful samplings at first attempt and the mean gestational age.

In each case, data were entered into the computer and final analysis was done using the Epi info version 6.1 soft ware package. Proportions and percentages were used for categorical data. Correlations between number of cases and variables as well as between variables were individually determined using the Pearson's Product Moment and calculated linear correlation coefficient considered statistically significant at $p < 0.05$.

RESULTS

Two hundred and twenty-six women underwent transabdominal chorionic villous sampling during the period of study. The main indications were sickle cell disease in 215 (95.1%) cases and, chromosomal abnormality in 11 (4.9%). The results are summarised in Table 1.

Table 1: Results of Perceived Indication for Chorionic Villous Samples

Results	Number (%)
HbAA	38 (16.8)
HbAS	115 (50.9)
HbSC	2 (0.9)
HbAC	3 (1.3)
Chromosomal Complement	4 (1.8)

Table 2 shows the trends in the number of cases and other variables from 2005–2008. The number of procedures performed each year increased progressively. The mean number of needle aspirations reduced in each succeeding year. The mean weight of sample however increased in succeeding years, being 25mg and 35mg in 2007 and 40mg in 2008. The number of women who were successfully sampled at first attempt rose from 18 (85.7%) in 2005 to 87 (100%) in 2008.

Table 3 shows the inter-relationships between the different variables in the 226 transabdominal chorionic villous samplings. There was a negative relationship between number of cases performed and number of uterine needle aspirations, which was statistically significant ($p<0.05$). A strong direct but not significant relationship was found between number of cases and weight of sample obtained. There was an insignificant negative correlation between number of cases and percentage successfully diagnosed at first attempt ($r = -0.7, p > 0.05$). The first 3 (75%) cases who were repeated were due to inadequate sample, while the last case was due to failure of DNA amplification. On the whole, 94% successful sampling was achieved in the first 50 cases and became 100% in the following 50 cases and beyond. A statistically significant negative correlation was found between number of aspirations and weight of samples obtained ($p < 0.05$). The mean gestational age at the time of sampling was 15 weeks in 2005 and decreased to 12 weeks in 2008. This had a strong indirect correlation with the weight of sample ($r = -0.9$, $p < 0.05$). Multiple aspirations were done in 53 cases, with a decreasing frequency as the number of cases increased.

Table 2: Distribution of Villous Samplings and other Variables by Year of Study

Year	N(%) Samplings	Mean Values				
		Aspiration	Sample Weight (mg)	GA (Weeks)	1st Attempt (%)	> 3 Aspiration
2005	21 (9.0)	5	25	15.0	85.7	16
2006	45 (22.9)	3.5	30	12.8	98.1	18
2007	73 (31.1)	2.0	35	13.0	100.0	14
2008	87 (37.0)	1.5	40	12.0	100.0	15

GA, Gestational age; 1st attempt, percentage diagnosed at first attempt.

Table 3: Inter-relationship between some Variables in Chorionic Villous Sampling

Correlates	r	p
Number of Cases v Number of Aspiration	–1.0	<0.05
Number of Needle Aspiration v Sample Weight	–0.9	<0.05
Number of Cases v Sample Weight	+0.9	>0.05
Number of Cases v Gestational Age	–0.13	>0.05
Number of Cases v Percent Diagnosed at First Attempt	–0.7	>0.05
Gestational Age v Sample Weight	0.89	<0.05

The complications reported during the procedure and pregnancies were few. Two (0.9%) cases of abortion were recorded, with one among cases with multiple aspirations and the other among cases without multiple aspirations. Both were within the first 50 samplings. Hypertension was recorded in 2 (0.9%) cases and in one of these, there was associated intrauterine growth retardation. They were all severe hypertension occurring after 28 weeks and without any background risk factor for hypertensive disorder.

DISCUSSION

Chorionic villous sampling is not a commonly utilized method of invasive prenatal diagnosis in Nigeria and many developing African countries. The transabdominal method that has been shown to have a better outcome compared with the transcervical method has been the method of choice in Nigeria since 2005. One of the major constraints however to its widespread availability is that of lack of consistent manpower to ensure that the procedure is associated with minimal complication, especially that of abortion. It has been reported that the centralisation and restriction of chorionic villous sampling to a limited number of experienced operators within centres, is likely to have a positive influence on safety and success of the procedure.[7] This positive influence might be related to the fact that better skill is acquired after performing several numbers of the same procedure over several years. The number of cases performed therefore becomes an important consideration. Lack of awareness of the availability of service among health workers and the populace was identified as a cause of the low number of cases in the initial years.[10] In addition to this however, poor education and socio cultural inhibition also have adverse influence on the utilization of health services.[11] This trend is shown in the study to have gradually improved over the years.

This study shows significant relationship between number of cases performed and the mean number of needle aspirations required to obtain adequate sample. This observation confirms the expectations of the learning curve, where

proficiency improves with increasing number of cases performed. Two factors that further support the learning curve are the finding of significant negative correlations between number of aspirations and the weight of sample obtained as well as the number of cases performed and the weight of sample obtained. The extrapolation from these findings is that the likelihood to obtain adequate sample increases with fewer aspirations by a skilled operator. In addition to the factor of the operator, other factors that improve safety and good outcome include the presence of a competent assistant and a scan with high resolution. The correlation between the number of cases and gestational age was negative and statistically insignificant. The initial poor utilization was due to dearth of information among health workers who are expected to provide informed medical advice to the population.[10] The improving trend in patronage could be the evidence that both the medical community and the populace were now better informed. Also worthy of note is the positive correlation between number of cases performed and the percentage diagnosed at first sampling attempt. This is also statistically insignificant. It was reported that laboratory failure of DNA amplification was responsible for about a quarter of those who had a repeat sampling rather than solely operator's influence.[3]

The women were followed up after the procedure till delivery. This was achieved by regular contact with the primary physician, as well as direct information obtained from the women thorough telephone call and mail messages. While the former can be largely relied upon, the former methods have few limitations. In spite of these limitations, it is still accepted as an invaluable means of patient's monitoring after surgical services.[12]

Abortion which was considered the most significant problem following CVS was observed to reduce significantly over the years from the study. This could be explained in part by the reduction over the years in the number of needle aspirations required to obtain adequate sample, the aseptic measures that was always maintained during the procedure and the possible tamponading effect of the abdominal wall muscles in cases of iatrogenic rupture of amniotic membranes. We were conscious of the number of needle aspirations based on the reports of studies which associated a significant increase in abortion rate after 3 aspirations.[13] The abortions that were recorded in our centre were in the earlier period of the study, when the mean number of aspirations was highest.

The association between CVS and hypertensive disorder of pregnancy remains unresolved. Some studies suggest an association between the procedure and severe hypertension.[14, 15] We recorded two cases of hypertension. Both were severe, with one proceeding to term, while the second case was complicated by intrauterine growth retardation. Although constrained by absence of a control group, nevertheless the absence of any background risk factor for hypertension in these patients makes the report worthy of note and may suggest an association between the procedure and hypertension in these cases. The possible mechanism theorised is disruption of the placenta with focal haemorrhage and subsequent inflammation, which is postulated to inhibit the spiral arteriolar widening, contributing to reduced placental perfusion and initiating a cascade of additional effects that include oxidative stress and endothelial cell dysfunction.[16] Statistical significance of the observation in this study can only be clarified in a more extensive study.

A previous study in our centre recommended a minimum gestational age of 11 weeks for sampling.[3] One of the major advantages of this is the reduced risk of congenital limb defect. Interestingly, this morbidity was not encountered even in the early phase of the service, when women above 14 weeks were also sampled. Although sampling above 14 weeks is discouraged because of obvious disadvantages, they were however many of cases above this gestational age period performed. The reason for this is the fact the awareness about procedures for genetic diagnosis was only gradually becoming enhanced women. It is anticipated based on findings from local studies that majority would present in future at an earlier gestation.[8]

The major constraints to making firmer scientific conclusions from this study are the sample size and absence of a control group to demonstrate statistical significance of the complications observed. Being a relatively new service and the low patronage, especially of innovative health services among women in many developing countries, the small sample size is not unexpected. It is known that small number of participants poses challenge in making firm scientific conclusions, however a suggestion has also been made that scarcity of data should be no obstacle to the introduction of a likelihood ratio.[17] The study however presents the local experiences since the introduction of transabdominal chorionic villous sampling. It is expected that firmer conclusions will be made in the next few years, when appreciation of prenatal diagnosis services would have improved and participation enhanced in Nigeria.

REFERENCES

1. WHO Proposal for feasibility study on the control of sickle cell disease in Africa. Report of a WHO informal consultation group. WHO document. WHO/HDP. SCD/87.3.Geneva.1987.
2. Horwell DH, Loeffler FE, Coleman DV. Assessment of transcervical aspiration technique for chorionic villus biopsy in first trimester of pregnancy. *Br J Obs Gynae.*1983; **90:** 196–198.
3. Oloyede AO Akinde JA. Evaluation of early Complications of Chorionic Villus Sampling. *Emirates Medical Journal.* 2004; **22:** 225–230.
4. Jackson JG, Wapner RJ. Risks of Chorionic Villus Sampling. *Clinical Obstetrics Gynaecology.*1987; **1:**513–531.
5. Church JK, Golberg JG, Wohlferd MM, Goldbus MS. Comparison of transcervical and transabdominal chorionic villus sampling loss rate in nine thousand cases from a single centre. *Am J Obs Gynae.* 1995; **173:**1277– 1282.
6. Lilford RJ. Invasive diagnostic procedures. In: Lilford R.J (Ed). Prenatal Diagnosis and Prognosis, 1st Ed. Butterworths & Co Ltd, London. 1990; 208–225.
7. Wijnberger LD, van der- Schouw YT, Christiaens GC. Learning in Medicine: Chorionic Villous Sampling. Prenatal Diagnosis. 2000; **20:** 241–246.
8. Oloyede OAO, Oyedele RA. Women's attitude to prenatal screening services

for congenital abnormalities in Nigeria. *Journal of Obstetrics and Gynaecology.*2008; **28:** 406–407.

9. Davey DA, Mac Gallivray I. The classification and definition of the hypertensive disorders of pregnancy. *Clin Exp Hypertens.* 1986; **86:** 97–133.
10. Oloyede OAO, Akinde JA, Emuveyan EE, Odusoga OL, Lamina MA, Ibidapo MO. Prenatal Diagnosis: Appraisal of Awareness and Utilization among Health practitioners in Southwestern, Nigeria. *Nigerian Journal of Clinical Practice.* 2003; **5:** 41–47.
11. Salako AA, Oloyede OAO, Odusoga OL. Factors Influencing Non-Utilization of Maternity Care Services in Sagamu, South Western Nigeria. *Tropical Journal of Obstetrics and Gynaecology.* 2006; **23:** 48–53.
12. Mbah N. Telephone use for surgical follow up in a developing country. *Nigerian Journal of Clinical Practice.* 2007; **10:** 266.
13. Williams J. 3rd; Wang BB, Rubin CH, Aiken-Hunting D. Chorionic Villous Sampling: Experience with 3016 cases performed by a single operator. *Obstetetrics and Gynecol.* 1992; **80:** 1023–9.
14. Adusumalli C, Han S, Beckham M, Bartholomew J, Williams III. Chorionic villous sampling and risk for hypertensive disorders of pregnancy. The American College of Obstetricians and Gynaecologists. 2007; **196:** 591.el-591.e7.
15. Richard K. Silver, Douglas Wilson, John Philip, Elizabeth A. *et al.* Late First Trimester Placental Disruption and Subsequent Gestational Hypertension/ Preeclampsia. The American College of Obstetricians and Gynecologists. 2005; **105:** 587–592.
16. Wilson ML, Goodwin TM, Pan VL, Ingles S.A. Molecular epidemiology of Pre-eclampsia. Obstetrics and Gynecology. *Survey.* 2003; **58:** 39–66.
17. Larsen SO, Christiansen M, Norgaard-Perdeson B. Inclusion of serum Markers measurements from a previous pregnancy improves Down's syndrome screening performance. Prenatal Diagnosis. 1998; **18:** 607–612.