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ORIGINAL ARTICLE

DNA Extraction from Chorionic Villi for Prenatal Diagnosis of Foetal Haemoglobin Genotype

Extraction de l'ADN à partir de villosités choriales pour le diagnostic prénatal du génotype hémoglobine fœtale

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ABSTRACT

BACKGROUND: **Extraction of DNA from the chorionic villi is the first major step in the molecular determination of foetal haemoglobin genotype. There are few reports on DNA extraction from the chorionic villi. A desired method should be simple to conduct, reliable and cost effective.**

OBJECTIVE: **The aim of the study was to demonstrate that boiling-based protocol for DNA extraction can be used for the prenatal determination of foetal haemoglobin genotype in a low resource setting.**

METHODS: **Chorionic villi obtained from 10 women in transabdominal procedures were treated with trypsin. Prepared solution 1, (0.5gm NaOH, 250mls distilled H_20 at pH 8.0) was added to the sample and boiled. Finally prepared solution 2 (6.05gm TrisHCl and 250 mls of distilled H_20 at pH 7.5) was added. Extracted DNA was amplified using PCR, run on gel electrophoresis and gel imager for determination of foetal genotype.**

RESULTS: **DNA was successfully extracted and foetal genotype determined in all cases. The mean wet weight of sample was 25 mg with twice aspirations in 50% of women. Foetal genotypes were AA in five, AS in four and SS in one. No case of repeat extraction on account of inability to obtain a genotype was reported.**

CONCLUSION: **Boiling-based method is a rapid and reliable way to extract DNA from the chorionic villi. WAJM 2011; 30(6): 400–403.**

Keywords: **DNA Extraction, Chorionic villi, Prenatal diagnosis, Fetal Haemoglobin genotype.**

RÉSUMÉ

CONTEXTE: L'extraction de l'ADN des villosités choriales est la première étape importante dans la détermination du génotype moléculaire d'hémoglobine fœtale. Il ya peu de rapports sur l'extraction d'ADN à partir des villosités choriales. Une méthode souhaitée devrait être simple à réaliser, fiable et rentable.

OBJECTIF: L'objectif de l'étude était de démontrer que le protocole d'ébullition basé sur l'extraction d'ADN peut être utilisé pour la détermination prénatale du génotype hémoglobine fœtale dans un cadre de ressources faibles.

MÉTHODES: villosités choriales obtenus à partir de 10 femmes dans les procédures par voie transabdominale ont été traités avec de la trypsine. Une solution préparée, (0.5gm NaOH, 250mls H20 distillée à pH 8,0) a été ajouté à l'échantillon et bouillie. Enfin une solution préparée 2 (6.05gm TrisHCl et 250 ml d'eau distillée H20 à pH 7,5) a été ajouté. L'ADN extrait a été amplifié par PCR, exécutez électrophorèse sur gel et d'un imageur de gel pour la détermination du génotype du fœtus.

RÉSULTATS: L'ADN a été extrait avec succès et le génotype du fœtus déterminée dans tous les cas. Le poids frais moyen de l'échantillon était de 25 mg deux fois avec les aspirations de 50% des femmes. Génotypes fœtales étaient AA dans cinq ans, que dans quatre et SS dans un. Aucun cas de l'extraction de répétition en raison de l'impossibilité d'obtenir un génotype n'a été signalé.

CONCLUSION: ébullition à base de méthode est un moyen rapide et fiable pour extraire l'ADN des villosités choriales. **WAJM 2011; 30 (6): 400–403.**

Mots-clés: Extraction d'ADN, des villosités choriales, le diagnostic prénatal, le génotype hémoglobine fœtale.

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Abbreviations: CVS, Chorionic villous sampling; **DNA,** Deoxyribonucleic acid; **PCR,** Polymerase chain reaction.

INTRODUCTION

DNA extraction is fundamental to most applications of molecular biology including the prenatal diagnosis of sickle cell anaemia and other genetic diseases. It is the first laboratory step after the sample is dissected and cleaned of visible maternal contaminants through serial washing with EDTA solution and manual dissection of visible blood clots. The aim is to obtain quality DNA that is suitable for further processing involving PCR analysis, gel electrophoresis and gel imaging using ultraviolet transillumination as well as for DNA sequencing.

There are many published works on DNA extraction from plant and animal tissues.[1–4] However, the extraction of DNA from the chorionic villi has some peculiarities. First the chorionic villi are rare sources of DNA which are not directly obtained from the mother. Second, the procedure for obtaining the sample is invasive and associated with risk of procedure-related abortion.[5–7] The third peculiarity is that the weight of villi obtainable is usually very small compared with other sources of DNA. The placenta is a relatively small organ, especially at the gestational age at which the procedure is conducted. It is however amplified using the PCR technology to obtain multiple copies of the DNA.

Protocols and publications on DNA extraction from the chorionic villi are few. In all the protocols, there are few steps that are compulsory and others that are optional. In some of the protocols, the steps and reagents involved are multiple and occasionally hazardous. The overall objective however is to obtain satisfactory DNA that can be used for further analysis. Phenol and chloroform are the standard solvents used for DNA extraction from the chorionic villi.[8–10] Although the method is reported to yield higher quantity of DNA compared with other protocols, its major drawbacks include toxicity, more laborious steps and costlier reagents.[8, 11] The salting method described by Miller has also been widely used for DNA extraction from the chorionic villi.[12] It utilizes high salt concentrations to remove proteins. Several modifications of this original protocol have been used in order to obtain a higher and purer yield of DNA.[11] Other reported protocols include the promega method, SDS-potassium-acetate method and sonicaid method.[13–16] Generally, most of these protocols take several hours to days to complete and involve reagents that are more costly.[11,13] The challenges associated with the earlier protocols necessitate the design of alternative protocols that would utilise readily accessible reagents (cheap), with few steps (simple), but that would equally produce satisfactory DNA yield (reliable). A preferred protocol should be completed within a short period.

The objective of this study was to demonstrate the application of boiling-based method of DNA extraction as a simple, rapid conducted and reliable method of DNA extraction. Such a technique should be useful in low resource settings.

SUBJECTS, MATERIAL, AND METHODS

Pregnant women who fulfilled the inclusion and exclusion criteria were randomly selected at aprenatal diagnosis clinic. They all had routine preliminary abdominal ultrasound scan to confirm pregnancy, number and viability of foetus(es) and to also determine the accurate gestational age.

All the women were counseled before and after chorionic villous sampling and laboratory analysis. Counselling was usually non directional, because the decision to continue or terminate a foetus with HbSS was made by the couple. In addition to the routine counseling, however, requests were made for participation in the study, from the women who eventually participated. Anonymity was guaranteed, as well as non interference in management if and when they decided to opt out of study. All the participating women signed ta informed consent form. They were also counseled to report to their physician, any observed vaginal bleeding, passage of water per vaginam, fever or abdominal pain.

Study Site

The study was conducted at High Rocks Fetal Medicine and Genetic Diagnosis Centres, in Lagos, Nigeria. The facility undertakes prenatal diagnosis of genetic foetal abnormalities under the supervision of a consultant obstetrician, assisted by genetic counselors and laboratory technologists with additional training in aspects of genetic analysis. All the extraction procedures were done at the centre.

Inclusion and Exclusion Criteria

The inclusion criterion was presence of a viable pregnancy in a couple with at least one S gene in either individual. Excluded were cases of unresolved vaginal bleeding, pelvic infection, pregnancy less than 11weeks and elecctrophorsen pattern of Hb AA in one of the parents.[17]

Chorionic Villous Sampling (CVS)

Routine abdominal ultrasound scanning was done at the commencement of the procedure, to determine best path of needle introduction based on the location of the placenta. After the scan, the woman was placed in a supine position, cleaned and draped, with hibitane and spirit. The biopsy path was infiltrated with 1% xylocaine under ultrasound guide. Biopsy was carried out by introducing the aspiration needle (trocar) carefully through the layers of the skin, uterus and the placenta tissue. The stylet was removed and replaced with a narrower bore needle (cannula), connected to a 10–20 ml syringe that was filled with 2–4ml of normal saline. Aspiration of villi was done under negative pressure created by the syringe. The retrieved villi were flushed into the petrish dish containing EDTA and examined under low power inverted microscope for confirmation. A pair of dissecting forceps was used to manually remove suspected maternal tissue contaminants, until a clean sample was obtained. The final clean samples were compared with a photographic documentation of a reference standard, to get the wet weight.[18] In either normal saline or EDTA solution, this final sample was transferred to the genetic laboratory for further analysis.

DNA Extraction Protocol

DNA extraction was carried out using the boiling-based protocol. The steps were as follows: 300µml of Trypsin added to 20mg of chorionic villi and centrifuged at 5000 rpm for 4 minutes. The supernatant was discarded leaving

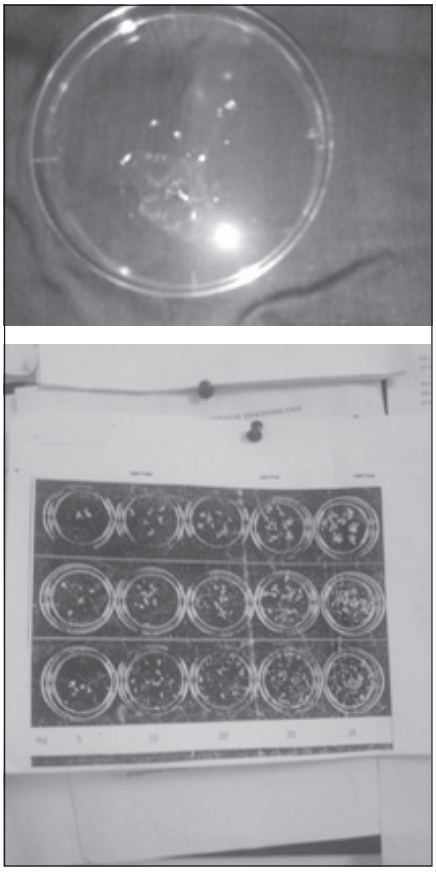

Fig. 1: Pre-wasted aspirated chorionic villi (upper panel) and reference standard of chorionic villi (lower panel).

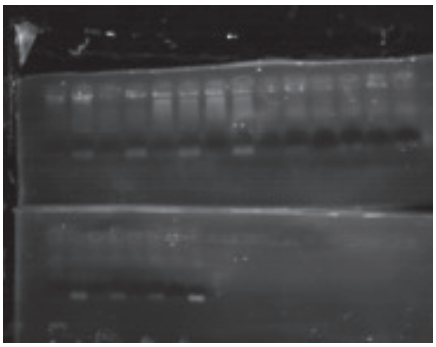

Fig. 2: Gel band of Haemoglobin Genotype AS.

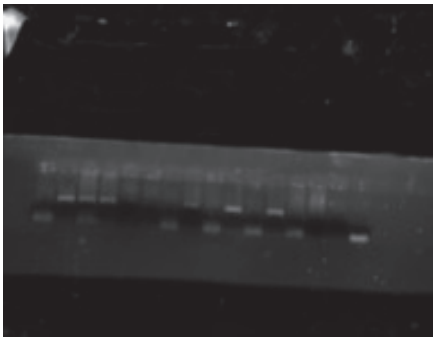

Fig. 3: Gel band of Haemoglobin Genotype AA.

the residue. One hundred µml of Solution 1 (0.5g NaOH, 250ml distilled H_20 at pH 8) was added to the residue, vortexed and the tube sealed firmly with cellotape or paraffin. The tube containing the mixture was gently lowered and left in boiling water on a Bunsen burner or sterilizing unit for 15–20 minutes. Next, 20µml of solution 2 (6.05gm TrisHcl and 250mls of distilled H_2O at pH 7.5) was further added to the solution and centrifuged at 13000 rpm for two minutes. The supernatant containing DNA was carefully pipetted into a new tube and the residue discarded. The extracted DNA was run on PCR, agarose gel electrophoresis and gel imaging to obtain the haemoglobin genotype. The extracted DNA was assessed to be of good quality and quantity by comparison of the intensity of band obtained with bands from confirmed controls.

RESULTS

Table 1 summarises the characteristics of the 10 women who had chorionic villous sampling.

Gestational Age and Weight of Sample

The lowest gestational age was 11 weeks and the highest was 15 weeks. The majority (80%) were within the recommended 11–13 weeks of pregnancy. The minimum wet weight of villi aspirated was 20 mg and the maximum was 45 mg with a mean weight of 25 mg.

Number of Aspirations

Three women (30%) had only one needle aspiration to obtain adequate sample. Five (50%) women had aspiration twice and two (20%) had three aspiration attempts. There was a low negative correlationship between the number of aspirations and the weight of sample obtained ($r = -12$, $p > 0.05$), and also between the gestational age and the number of aspiration attempts ($r = -0.13$, $p > 0.05$).

Table 1: Characteristics of study Women

Subject	Gestational Age(Wks)	Sample Weight (mg)	Number of Aspirations
1	11	25	2
2	15	30	1
3	13	25	2
4	12	25	3
5	11	35	1
6	11	40	2
7	11	35	2
8	13	45	2
9	12	20	1
10	14	40	3

Summary of Results

Of the genotypes, five (50%) were genotype AA, 4 (40%) genotype AS and 1(10%) genotype SS.

Figure 1 shows the prewashed chorionic villi and the photographic documentation of reference standards used to assess the weights of wet chorionic villi samples

Figure 2 shows the DNA bands after gel electrophoresis, ethidium bromide staining and visualisation under translumination. Figure 2 is that os haemoglobin genotype AS and Figure 3 is haemoglobin genotype AA.

DISCUSSION

The extraction of DNA after chorionic villous sampling is crucial in the overall process of prenatal determination of haemoglobin genotype. There are several protocols for DNA extractions each with its strengths and draw-backs. Most of these protocols have been utilised for DNA extraction from other sources apart from the chorionic villi, while their successful application on the villi is scarcely reported. The few reported protocols such as the salting, promega, phenol/chloroform and the glass beads methods, that have been successfully used on the villi are associated with limitations.[4,12,13,16,19] Such limitations include the long time to conduct extraction, multiple steps and reagents, cost of reagents as well as the toxicity associated with some of the reagents.

Chorionic villi are challenging biological samples for DNA isolation. In particular, maternal contamination of the chorionic villi is one vital area of concern because it could lead to inconclusive analysis. It involves washing, cleaning and dissection to remove maternal contaminants and blood clots, prior to isolation of DNA. This was achieved using EDTA and saline, aided by the trypsin which has the ability to degrade various types of proteins especially

histones and ultimately improves yield and purity of extracted DNA. While EDTA dissolves existing clots and prevents new clots formation, trypsin removes heavy protein contaminants. Trypsin also has ability to enhance DNA synthesis through its action on a nuclear component, specifically, DNA polymerase [20]. This effect is very significant because the quantities of chorionic villi aspirated are usually very small compared with other sources of samples for adequate DNA extraction. The quantity of villi sample was made by comparing the sample with an established photographic documentation of varying weights of villi samples as reported in other studies.[18]

The disruption of the cell membrane followed by the nuclear membrane is fundamental steps in DNA extraction. It was achieved through the lysis effect of trypsin, tris and the mechanical effect produced by vortexing the sample. Tris interacts with the lipopolysaccharides component of the cell membrane thus affecting its stability. This action is enhanced by the EDTA. Tris also ensures a stable pH, which is essential during the extraction process. Boiling was achieved using the regular sterilizing unit in place of water bath, which cost more. It degrades DNAses present in the cytoplasm and prevents it from digesting the released DNA from the nucleus. The NaOH precipitates protein that has been denatured by trypsin.

Immediate determination of the quantity and quality of the extracted DNA was not done in this study. Spectrophotometric analysis based on absorbance of DNA solution at 260 and 280 nm is usually used for determination of quantity and quality of extracted DNA. This is however not available in the laboratory because of cost. Instead, it was achieved by comparing the intensity of band obtained after gel electrophoresis and staining with ethidium bromide with the band from a known DNA sample. Similar methods have been reported.[21]

The boiling-based protocol was successfully used for all the sample analysis, with satisfactory outcome. The assurance of quality was based on the bands obtained from the agarose electrophoresis gel and haemoglobin genotype results obtained in all cases. A major benefit of this method is that extraction can be completed within one hour and extracted DNA stored for further use. In addition, the reagents required are minimal, locally available and less expensive.

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