Article published online: 2020-04-19

Original Article

Quality and Quantity of Extracted Deoxyribonucleic Acid (DNA) from Preserved Soft Tissues of Putrefied Unidentifiable Human Corpse

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ABSTRACT

Context: The appropriate collection and preservation of soft tissues from putrefied unidentifiable human corpse for the purpose of identification using DNA profiling technique is critically important especially in developing countries like India having different levels of health-care set ups with largely varying facilities and varying climatic conditions.

Aims: The present study was carried out, mainly focusing on quality and quantity of extracted DNA from the soft tissues of putrefied unidentifiable human corpse stored upto 4 weeks at 4°C and at −80°C for DNA analysis.

Materials and Methods: The present study was conducted on 16 different putrefied unidentifiable human corpses after getting approval from institutional ethical committee. Around 2 g of four different tissues (brain, kidney, heart and muscle) were collected and preserved for one month followed by DNA extraction using the organic method, the quality and quantity of high molecular weight-DNA was estimated using the spectrophotometer and gel electrophoresis. Further, the amplification polymerase chain reaction (PCR) was also performed (AmpFLSTR® Indentifiler™ PCR Amplification kit for multiple loci, of Applied Biosystems, Lab India) and was checked using continuous PAGE.

Results: The yield of DNA was significantly higher at −80°C for all the four tissues collected and was best for brain followed by heart, kidney and worst for muscles in all cases.

Conclusions: It is suggested that the brain tissue preserved at −80°C is the best among soft issues for DNA extraction. Refrigeration or deep freezing facility should be available at all the centers.

Key words: DNA-profiling, putrefaction, soft tissues.

INTRODUCTION

The Identity of a human corpse can be best established by using DNA-profiling technique. DNA, as known, can be extracted from all the body tissues, but it is of real concern to decide the best tissue for DNA extraction and analysis. Blood, long bones, teeth are the common source to establish the identity of individuals using DNA-profiling technique.[1-4] Body tissues collected for DNA profiling are recommended to be preserved at –80°C until the time analysis is carried out.

Access this article online

Quick Response Code:

Website: www.jlponline.org

DOI: 10.4103/0974-2727.129088

Due to varying climatic conditions, dealing with putrefied human corpse is not infrequent in forensic practice in India. However, the stages of putrefaction may vary. It may be difficult to identify human corpse by just external features in case they are in an advanced stage of putrefaction. This situation is further added by (1) mutilation of the skeletal remains in situations like run over situations of road traffic accidents and in train accidents and (2) due to contamination of mutilated skeletal remains (3) in situations of mass disaster in which human corpse may be exposed to extreme thermal, physical or chemical insult.[5] Putrefaction and inappropriate preservation leads to degradation of genomic DNA. Therefore, the issue of appropriate tissue collection and preservation is

critical, particularly in view of the fact that the majority of medico-legal autopsies are conducted by the person lacking adequate knowledge and also done out at the places, which lack required equipment and infrastructure. Advance facilities of the deep freezer (−80°C) are available at few selected tertiary care centers only.

The present study is focused on deciding the best soft tissue among the four (Brain, Muscle, Kidney and heart preserved at 4°C and −80°C for DNA analysis in case of unidentifiable putrefied human corpse and to compare the DNA yield at these two temperatures.

MATERIALS AND METHODS

After getting approval from the Institutional Ethical committee, present prospective study was conducted from January 2010 to December 2011 on 16 putrefied unidentifiable human corpses with post-mortem interval up to 14 days, which came for autopsy at the Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, New Delhi.

Tissues from heart (part of the right ventricle), brain, kidney (from the upper pole) and muscle (biceps) of all the putrefied unidentifiable human corpse under study were collected and stored in sterilized plastic containers at 4°C and −80°C without any preservative and then subjected for DNA analysis after one month of storage. DNA was extracted from all the samples by phenol chloroform extraction method (organic method).[6] DNA quality and quantity was measured using ultraviolet spectrophotometer at 260 nm and 280 nm wave length.

The presence of high molecular weight DNA was also checked by agarose gel electrophoresis (0.8%). Extracted DNA was amplified using polymerase chain reaction (PCR) and the amplification was verified by continuous polyacrylamide gel electrophoresis (12%) to ascertain the presence of amplifiable DNA (using AmpFLSTR® Identifiler™ PCR Amplification kit (Applied Biosystems, Lab India).

Mean value of DNA yield was calculated for individual organs from all samples at different temperatures. *P* value was calculated individually for all means using *t*-test. Further, one-way ANOVA analysis was applied separately for all the samples at −80°C and 4°C and *P* value was used to compare the significance of difference in DNA yield of different organs at both these temperatures (−80°C and 4°C).

RESULTS

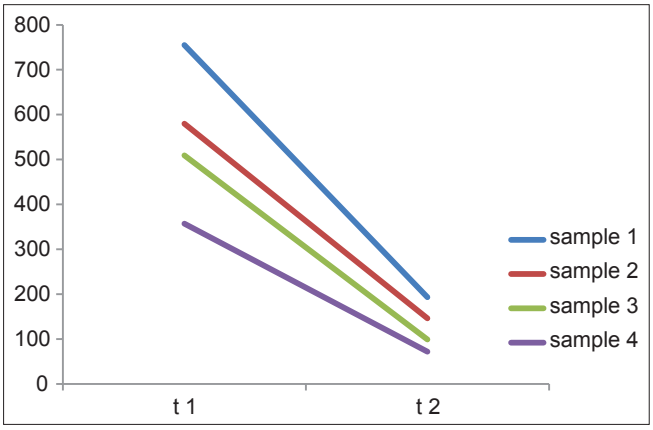
The yield of DNA from tissue samples (brain, heart, kidney and muscle) of all the sixteen cases at different temperature (4°C and −80°C) were obtained and then mean was calculated**.** Among the two temperatures 4°C and −80°C, the mean of DNA quantity obtained was highest for brain followed by heart, kidney and muscle respectively in decreasing order. The mean yield of all the four organs was compared at both temperatures and yield at −80°C was found to be significantly higher than at 4°C (*P* value 0.0001) [Table 1]. This difference in DNA yield at −80°C and 4°C is shown graphically below in graph [Graph 1].

At −80°C DNA yield was found highest in the brain with a significant difference from kidney ($P = 0.005$) and muscle ($P = 0.0001$), but the difference was not significant in relation to heart ($P = 0.090$). DNA yield was high in the heart at −80°C with a significant difference from muscle ($P = 0.014$). There was no significant difference in yield of DNA from the heart and kidney ($P = 1.000$). Furthermore, there was no significant difference in yield of DNA from kidney and muscle ($P = 0.200$).

Table 1: Mean yield of DNA for all the organs at 4°C and −80°C along with the *P* value

Sample (DNA)	Mean±SD (at −80°C) ngm/µltr	Mean±SD (at 4°C) ngm/µltr	*P*
Muscle	357.63±107.36	72.13±33.87	0.0001
Kidney	509.94±209.48	99.31±37.79	0.0001
Heart	580.63±221.36	146.06±54.14	0.0001
Brain	755.94±228.43	193.69±49.87	0.0001

*Calculated using one way ANOVA, #Calculated using *t*-test,
DNA: Deoxyribonucleic acid, SD: Standard deviation, ANOVA: Analysis of variance



Graph 1: Decrease in deoxyribonucleic acid yield with change in temperature from -80°C to 4°C, sample 1 = brain, sample 2 = heart, sample 3 = kidney, sample 4 = muscle

At 4°C DNA yield was found highest in brain with a significant difference from heart ($P = 0.023$), kidney ($P = 0.0001$) and muscle ($P = 0.0001$). DNA yield was high in heart at 4°C with a significant difference from kidney ($P = 0.027$) and muscle ($P = 0.0001$). There was no significant difference in yield of DNA from kidney and muscle ($P = 0.543$).

The quality and quantity of extracted DNA checked on agarose gel for all the samples showed more amount of high molecular weight (HMW)-DNA in Brain tissue at both −80°C and 4°C and the least amount of HMW-DNA was shown in muscle samples. Figure 1 and amplicon was first run on continuous PAGE (12%), Figure 2 and then subjected to genotyping.

All the samples from 16 bodies showed amplification [Figure 3].

DISCUSSION

Selection of tissue for DNA analysis is very important to obtain appropriate quality and quantity of DNA for further investigation. Furthermore, DNA analysis facility is available only in few laboratories in our country and hence samples need to be stored and transported appropriately until analysis.

In the present study, four organ tissues (Brain, Heart, Kidney and Muscle) were collected from each of 16 decomposed bodies to find out the suitable storage temperature (4°C and −80°C) used for DNA analysis. Collected samples were preserved for one month at specific temperatures without using any preservative.

Hoff-Olsen *et al*.[7] has recommended silica gel extraction procedure for putrefied human tissue, but in this study phenol chloroform extraction method (organic extraction) was used following the protocol of Sambrook *et al*.[6] as per the availability in our lab and good results was observed with the stored sample also.

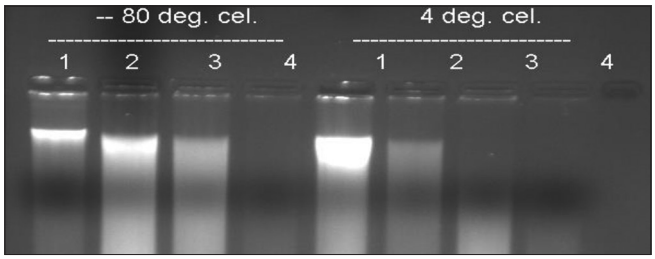


Figure 1: Extracted deoxyribonucleic acid on agarose gel electrophoresis. 1 = brain, 2 = heart, 3 = kidney, 4 = muscle

It was observed that preservation of tissues at −80°C has given good DNA yield than tissues preserved at 4°C without the use of any preservative. Raina *et al*.[8] have observed that preservation at −80°C temperature and normal saline as a preservative is best combination for storage of forensic samples until processed further.

Our results are showed that the yield of DNA was significantly higher from the tissues when stored at −80°C then 4°C for all the four tissues collected. This suggests that preservation at −80°C is better for DNA analysis. Huckenbeck and Bonte[9] have recommended freeze drying as a suitable method for preserving tissue samples for DNA profiling. Bomjen *et al*.[1] also observed that higher amount of genomic DNA can be recovered from blood samples stored at temperature 4°C or below.

On comparing the yield of HMW-DNA from different tissues, brain tissue was found to be more suitable for DNA analysis in case of putrefied bodies than heart, kidney and muscle for both the temperatures. Ludes *et al*.[10] have also reported the extraction of HMW-DNA from brain cortex regardless of postmortem age and that tissues such as blood and kidney were unsuitable for DNA fingerprinting because of rapid degradation of the DNA after a period of one week. However, Bär and Hummel[11] and Bar *et al*.[12] have found good DNA stability in brain cortex and psoas muscle over a 3 weeks postmortem period. In the same study, kidney reported to be showing the good DNA stability up to 5 days (at room temperature) of post-mortem, but later to this rapid degradation was observed. Rerkamnuaychoke *et al*.[13] could isolate STR allele of DNA from decomposed muscle, but it was degraded and could not yield amplified products of large size STR alleles.

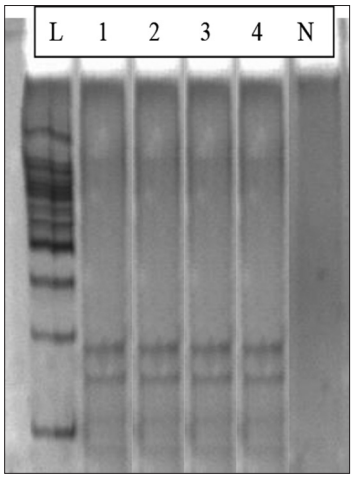


Figure 2: 12% PAGE (continuous) (Lane L = uncut ladder, Lane 1 = brain, Lane 2 = heart, Lane 3 = kidney and Lane 4 = muscle, Lane N = negative control)

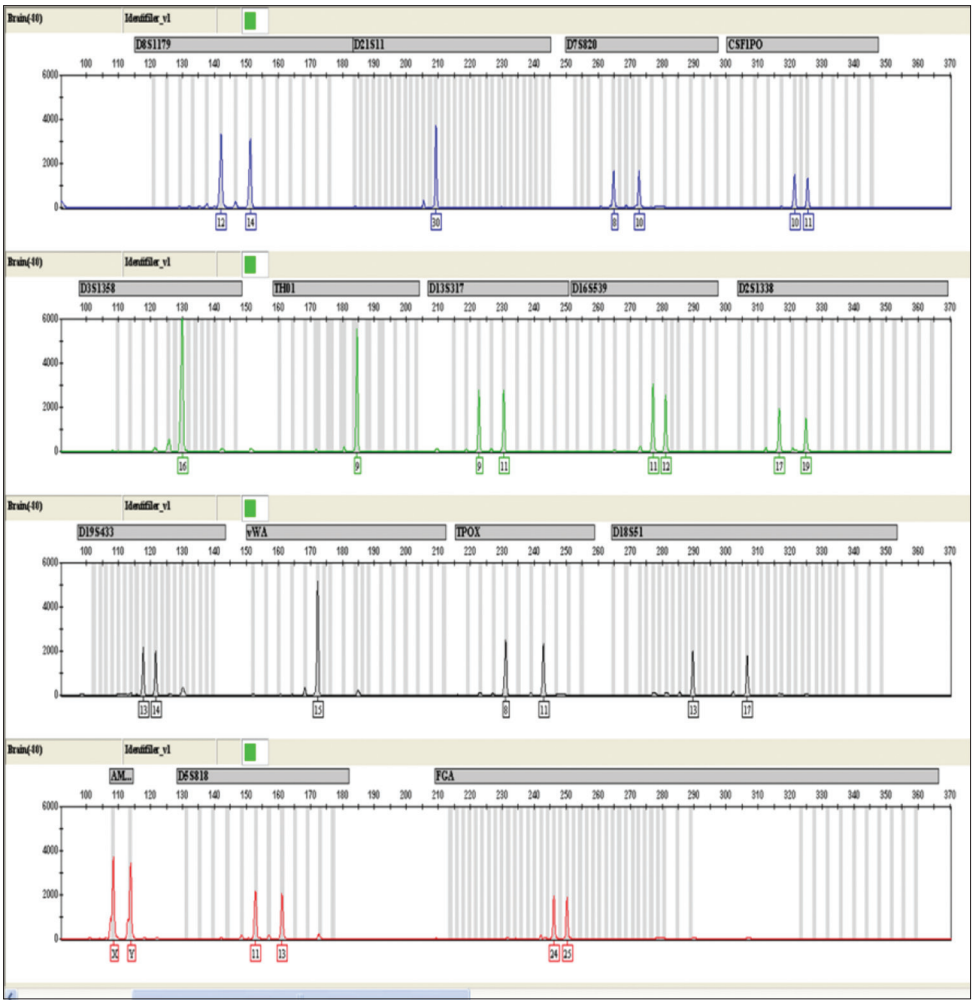


Figure 3: Electropherogram of brain tissue stored at −80°C showing amplification at all 16 loci in the form of sharp allele peaks

Haglund *et al*.[14] were able to isolate DNA from a tissue sample of decomposed body after 2 weeks. Piasecka-Pazik *et al*.[15] successfully extracted DNA from blood and decomposed tissues, particularly from the heart and kidney. DNA extraction from the liver was poor. Extraction of good quality DNA from brain tissue could be due to brain's location within the skull, preserving it from early environmental bacterial contamination.[12]

CONCLUSION

Our study suggested that quality and quantity of DNA extracted from tissues of putrefied unidentifiable human corpse was best seen in brain followed by heart and kidney preserved at −80°C and 4°C. Muscles were found to be least useful for DNA extraction. However, further work is required in this field taking other tissues such as cartilage, blood vessels, Achilles tendon and nails as other studies [16-19] have shown the usefulness of these tissues in DNA extraction in cases of putrefied unidentifiable bodies. This study recommends preservation of brain at both −80°C and 4°C (preferably −80°C) for DNA extraction in case of decomposed unidentifiable mutilated and contaminated dead bodies. We recommend refrigeration or deep freezing facility to be available at all the centers where medico-legal work is carried out starting from district hospitals to higher centers.

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How to cite this article: Pooniya S, Lalwani S, Raina A, Millo T, Dogra TD. Quality and quantity of extracted deoxyribonucleic acid (DNA) from preserved soft tissues of putrefied unidentifiable human corpse. J Lab Physicians 2014;6:31-5.

Source of Support: Nil. **Conflict of Interest:** None Declared.

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