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Role of Product of Conception and DNA Profiling in Forensic Cases: A Case Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

At this present time, sexual assault on minors has become a major concern in our country. Social unawareness of victims and perpetrators is an emerging issue in sexual assault cases. It has been observed that most of the cases are being reported in the age group of 17–19 years old. Whenever such cases are reported to law enforcement agencies, the victims are brought to hospitals for the termination of pregnancy. In such cases, the product of conception can play a vital and key role in ascertaining paternity. In this present research work, we considered a case in which three different samples were preserved with different preservatives. The extensive problem was to determine the

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fleshy perceptible as POC because most segments of this material were from mothers. At the present moment, this study concludes that most of the samples provided were not substantive POC. These are part of the placenta and other fleshy material of the mother. Out of three samples, two gave the autosomal STR profile of the victim instead of POC, while one sample was able to conclude the result based on one set of alleles of autosomal STR (Short Tandem Repeat) matching the father. Two of the last samples gave the same STR Y profile to the father.

Keywords: Sexual assault; victim; preservatives; fleshy material; Y profile etc.

1. INTRODUCTION

DNA technology plays a vital role in the judicial system, especially in the identification of victims, sexual assault cases, disputed paternity and maternity cases, etc. In disputed cases, the DNA profile of the foetus/ POC provides the identity of the plaintiff. Moreover, it is very often that minor victims are not aware of the sexual activities [1]. Therefore, by virtue of their hesitation and poor education, they don't divulge their sexual activities / pregnancy. In the follow-up to the above, this present case was studied [2]. This is a case from South Delhi (India) in which a 19-year-old deaf and dump girl reported pain in her stomach. When the girl was hospitalised, she was catechism and acquisition pregnant [3]. The pregnancy was encompassing 45 days; hence, it was terminated, and POC was preserved by using three distinct preservatives i.e., fleshy material in normal saline, fleshy material in alcohol, and fleshy material in absolute alcohol [4]. To understand the language of the victim, a translator was called to translate her statement. Based on the statement of the girl's father, an FIR was registered. All the neighbours of the girl were rounded up, and she identified the perpetrator.

In the aforesaid cases, preservation of the foetus and POC (Product of Conception) is a very important transaction for DNA profiling from exemplifications. The quantification of DNA by implementing real-time PCR (Polymerase Chain Reaction) technology has enabled us to determine the quantity of total human DNA as well as male DNA from exemplars [5]. It assists in the qualitative and quantitative analysis of DNA from the sample. It is very often that the utilisation of improper preservatives degrades the DNA / the inhibit amplification of the DNA profile [6]. Some preservatives, like formalin, work as inhibitors and often result in the absence of DNA in the sample. Proper quantitative analysis of DNA is required to complete the DNA profile; contrarily, split peaks and off-ladder alleles are observed in the profile. Inhibitors in the samples can directly bind with DNA polymerases (Taq polymerase), which don't acquiesce to the sample [7]. These are very sensitive to various diversified inhibitors, as follows, the amplification process is obliterated. An unambiguous utilisation of degraded samples may affect the autosomal STR analysis and result in a partial DNA profile that will lead to a false/ negative result [8]. Therefore, RT-PCR is considered one of the best part antecedents for conventional PCR. DNA technology is a powerful and prevailing technology standard for criminal justice systems. The microsatellites used for human DNA profiling have 4–5 nucleotide repeats.

2. MATERIALS AND METHODS

The samples preserved as POC by a gynecologist were sent to the forensic science laboratory. To diminish any kind of degradation in the samples, all the samples were preserved at -20°C temperatures. To remove any kind of contamination from the samples, tissues containing substantial/ POC were washed with nuclease-free water. Along with it, to remove the preservatives; all samples were washed regularly, 5 to 6 times. The tissues preserved in formalin were unable to generate good quantitative and qualitative analyses; therefore, it is implied to take samples of such tissue material using an automated BTA kit using automated extraction instruments. It is contemplated that the best approach is to isolate the DNA from tissues substantial the foetus/ POC preserved in normal saline.

Subsequently, the isolation and concentration of DNA from each sample were determined using the Quantifiler® Duo Quantification Kit (Applied Biosystems) with the 7500 Real Time PCR machine [9]. All the steps were followed according to the manufacturer's protocols. The real-time PCR precisely enables us to distinguish and measure the DNA sequence from the samples, even if the quantity is very small. Moreover, it also helps to determine the male DNA from the samples if there is a missing Y allele on amelogenin, and a false representation of female DNA in the male DNA can also be

ruled out. DNA segments are amplified and monitored for progress by fluorescent techniques [10]. In this process, the amount of target sequence can be determined by the time how quickly the fluorescent signal reaches the threshold level.

DNA quantification assays combine three 5'-nuclease assays, including a human-specific DNA assay, a human male DNA assay, and an internal PCR control assay. Human Quantification Standards were applied to determine the DNA quantity in each sample. The kit used contains three different types of components, namely Taq Man® (which acts as a probe for the human-specific ribonuclease RNA component H1 (RPPH1) gene), the human male-specific sex-determining region Y (SRY) gene, and an Internal Positive Control (IPC). The duo kit of applied Biosystem is considered best to exclude the misrepresentation of female at the place of male due to the deletion of the Y allele in amelogenin.

2.1 Amplification of DNA

All of us are well aware of the effects of PCR inhibitors; therefore, adequate measures are adopted to avoid them. In the amplification process of DNA, autosomal STRs were amplified by using the AMPF/STR® Identifiler Plus® TM PCR amplification kit (Applied Biosystems) as per the prescribed protocol [11]. Amplified samples, now referred to as amplicons, were loaded onto an ABI 3500 XL genetic analyzer with Gene Mapper IDX 1.4 software for analysis. The generated STR profiles were analyzed for all samples. As a result of this process, degraded samples showing false peak profiles or allelic drop-out in large markers were further processed. The degraded tissues have shown allele dropout samples, which wer amplified by using Minifiler kit [12,13]. It usually targets the big size markers, and accordingly, proper STR profiles were tried to be generated.

3. RESULTS AND DISCUSSION

As per the result of this study, the quantity of DNA (ng/l) was obtained from different samples on a 7500 Real Time PCR machine. The observed percentage of success is given in Table 1.

Table 1. Quantity of DNA (ηg/μl) obtained from different samples

S. No.	POC in normal saline Human /Male (DNA Qty. in ng)	POC in absolute alcohol Human /Male (DNA Qty. in ng)	POC in absolute alcohol Human /Male (DNA Qty. In ng)	Blood sample of accused Human /Male (DNA Qty. In ng)
1.	2.8/---	7.73/.75	29.12/25.8	28.96/31.56

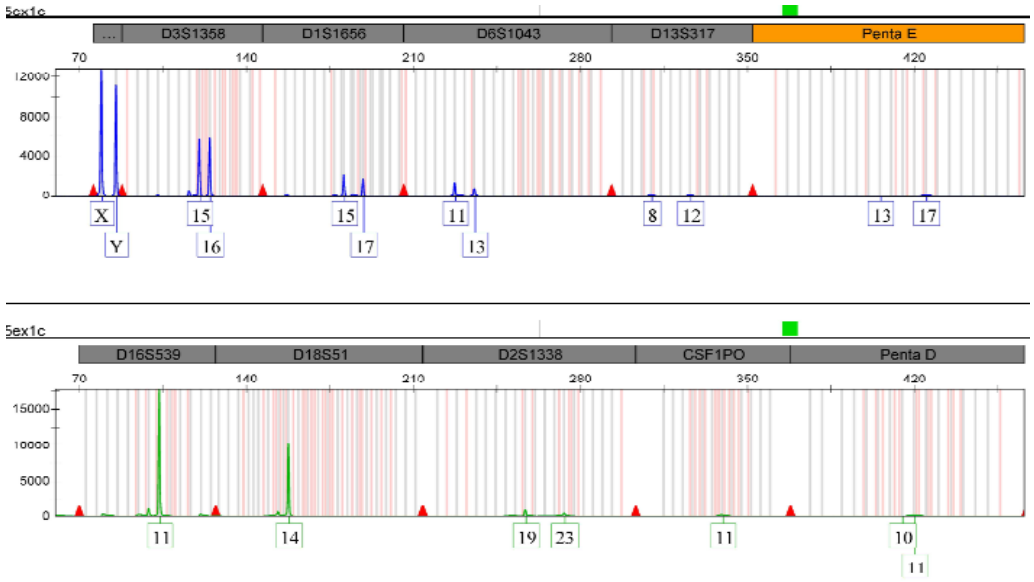

Fig. 1. Third sample showing the DNA profile of POC with poor peaks on big size marker

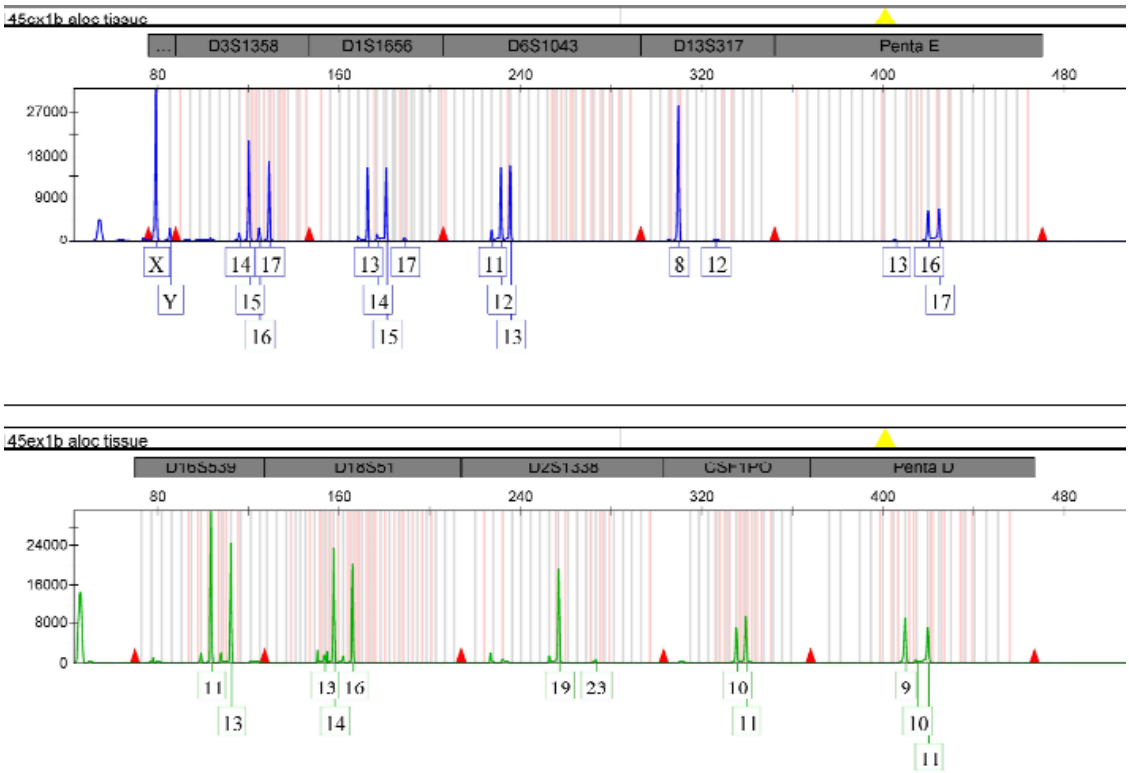

Fig. 2. Second sample which has high amount of female DNA comparative to male DNA and showing the high peaks of mother and low peaks or negligible peaks of POC

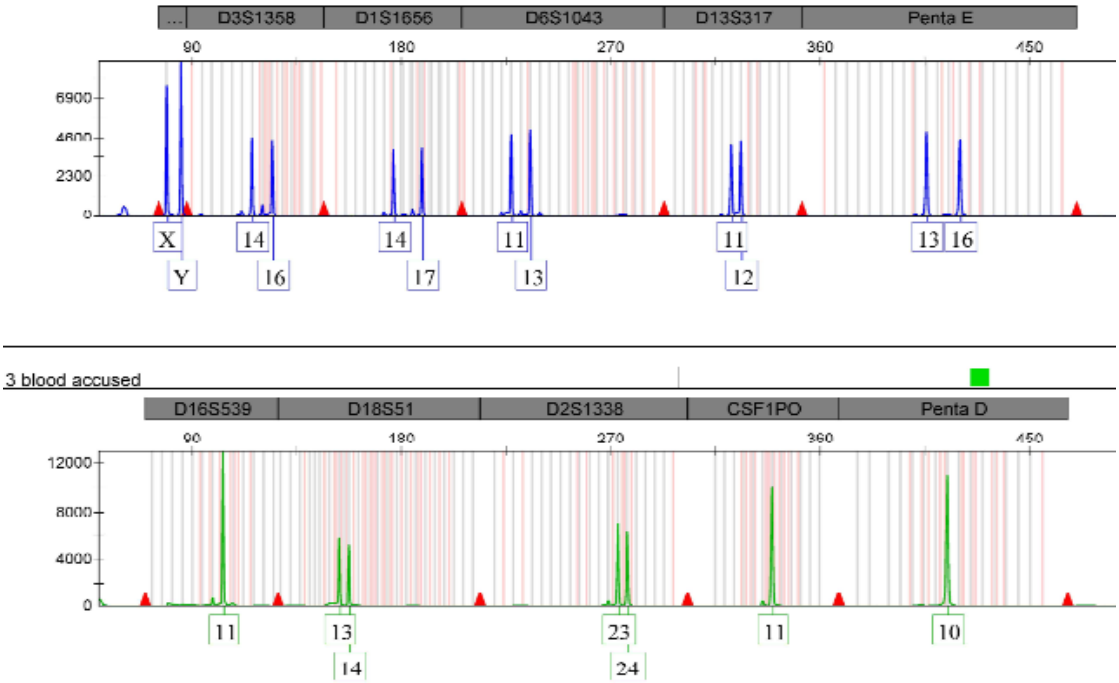

Fig. 3. DNA profile of reference blood of father/accused

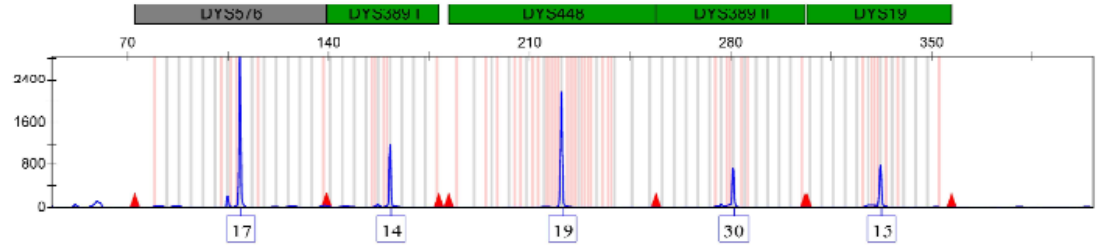

Fig. 4. Alleles of DNA profile of 5 markers of blue dye of Y-STR profile

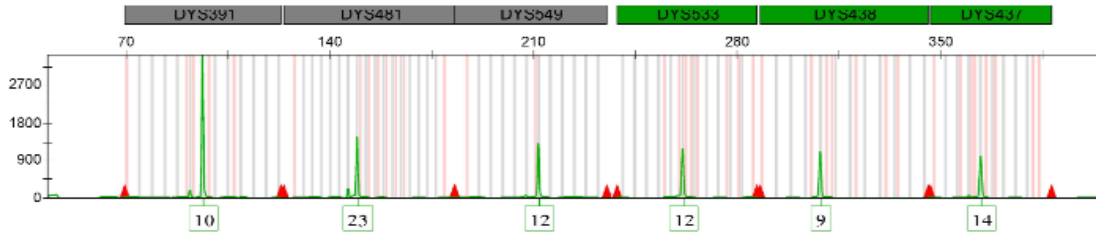

Fig. 5. Alleles of DNA profile of 6 markers of green dye of Y-STR profile

The result indicates that the quantity of DNA from the first sample was isolated, but there was no male DNA in the sample. It couldn't get to know whether the POC is male / female; however, in the absence of male DNA, it is presumed to be female. The quantitative analysis of such samples suggests that the DNA profile may be from the maternal part instep of POC. In the analysis of the second sample, there was a huge difference in human and male DNA of the sample. The generated profile of this sample was given the profile of a female, matching the reference sample of a mother, and giving the profile of a mother due to the high amount of female DNA in the sample but the certainty that it is of a male POC. One very important point was observed in this case that the foetus is male. The quantity of males is shown in the sample, and the Y STR profile can be used if it is not coming in autosomal STR. Analysis of the third sample helped to conclude the case, as the ratio of human and male DNA seemed to be equal, enabling it to determine the male profile in this case. It was also observed that only the first sample gave the female DNA profile in this case. The second sample also gave a female DNA profile in autosomal STR profile, but there was male DNA in the sample. During DNA analysis, low peaks were observed in POC due to the high amount of female DNA in the mother. The extra quantity of human DNA relative to male supersedes the male DNA and gives the female profile only. However, Y STR was able to generate a DNA profile from this sample. The third sample showed an equal quantity of human and male DNA. It showed that the quantity of male DNA is equivalent to that of humans and enables us to generate the male DNA profile, indeed, of the DNA profile of POC. While one set of alleles was found in the DNA profile of the accused or perpetrator.

4. CONCLUSION

Most often, it is observed that improper sampling and preservation will mislead forensic experts or investigating agencies. In this case, if the results had been prepared based on the first sample, it would have misled the matching of the DNA profile. In the second sample, it was showing the variation in the quantity that there is male DNA in the sample, and along with it, it was giving the mother's profile. If POC is female, then it would be difficult to conclude the result on the basis of the above technique. The third DNA profile is of a male and shows the male DNA profile, while one set of alleles was found in the DNA profile of the perpetrator. The Y-STR profile that was obtained in samples two and three was completely matched with the Y-STR profile of the perpetrator. Another important observation of the sexual assault case was that, POC should be preserved in alcohol, which is a good preservative. Various parts of POC, rather than one of less than two months age, should be preserved in alcohol. It facilitates the Y-STR analysis of male DNA samples. If the profile is of a female, it should be compared with the

mother's profile prior to giving the final opinion. In such cases, a fleshy substantial like the placenta can give the mother's profile at the place of POC and help to avoid misinterpretation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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