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Molar pregnancy unveiled by DNA profiling: a rare forensic case study

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Abstract

Background: Forensic DNA analysis is one of the most advanced tools in the criminal investigation. It is used successfully in solving offenses involving rape, paternity disputes, murder or attempt to murder, and dacoity as well as identification of mutilated body remains. DNA profiling is used to determine paternity in sexual offense cases where abortion takes place and the product of conception can be anywhere from 6 to 10 weeks of gestation to 8 months. In the present case, a tissue sample stated as a vesicular mole and blood samples of the mother and suspected father were submitted to the DNA division of our laboratory for paternity analysis.

Results: Genotyping results revealed a single allele at all the tested short tandem repeat (STR) loci. The allele obtained at each locus was common with the suspected father. Such type of genotype was very rare and not observed earlier; therefore, repeated analysis was done and the same genotype was obtained every time. DNA profiling revealed all the alleles in the vesicular mole to be of paternal origin only, devoid of any maternal alleles. After referring to books on gynecology, it was confirmed that the genotype obtained was of hydatidiform mole.

Conclusions: In this POCSO Act case, the product of conception (about 1.5 months old) was termed a vesicular mole, and blood samples of the mother and suspected father were sent for the DNA paternity test. STR profiling of the product of conception sample displayed no maternal tissue contamination and non-inheritance of maternal alleles, showing the case to be of molar pregnancy also called hydatidiform mole, a very rare phenomenon in the forensic scenario. After thorough analysis, the case was reported and it was the first of its kind to be reported in a forensic laboratory in Maharashtra, India.

Keywords: POCSO Act, Vesicular mole/hydatidiform mole, Polymerase chain reaction, STR analysis, Capillary electrophoresis

Background

DNA profiling is helping the judiciary in solving criminal cases such as homicide, rape, burglary, and baby swapping, as well as civil cases involving paternity, maternity or kinship disputes, succession, or inheritance (Jeffreys et al 1985a; Jeffreys et al 1985b). DNA analysis can be done on any biological material containing DNA as the DNA molecule essentially shows no difference from cell to cell, i.e., the DNA in the blood is the same as in the semen, bone marrow, hair, skin, and tissue (Watson

and Crick 1953). DNA profiling is now routinely used in paternity disputes arising from sexual offenses including POCSO act cases.

Protection Of Children from Sexual Offences Act, 2012 (POCSO act) is an extensive law that provides for the protection of children from offenses of sexual assault, sexual harassment, and pornography while protecting the interests of the child at every stage of the judicial process by assimilating child-friendly techniques for reporting, recording of evidence, investigation, and speedy trial of offenses through designated special courts (The Protection of Children from Sexual Offences Act 2012).

Gestational trophoblastic disease (GTD) is the term used to describe the heterogenous group of interrelated

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lesions that arise from the abnormal proliferation of placental trophoblasts (South Asian Edition of Berek and Novak's Gynecology n.d.). GTD begins in the layer of cells called the trophoblast that normally surrounds an embryo (tropho-means nutrition, and -blast means bud or early developmental cell). Early in normal development, the cells of the trophoblast form tiny, finger-like projections known as villi. The villi grow into the lining of the uterus. In time, the trophoblast layer develops into the placenta, the organ that protects and nourishes the growing fetus.

The hydatidiform mole or vesicular mole is a variant of gestational trophoblastic disease which comprises forms of hydatidiform mole, invasive mole, gestational carcinoma, and placental site trophoblastic tumor, according to anatomoclinical criteria (Wells 2007). It is a relatively

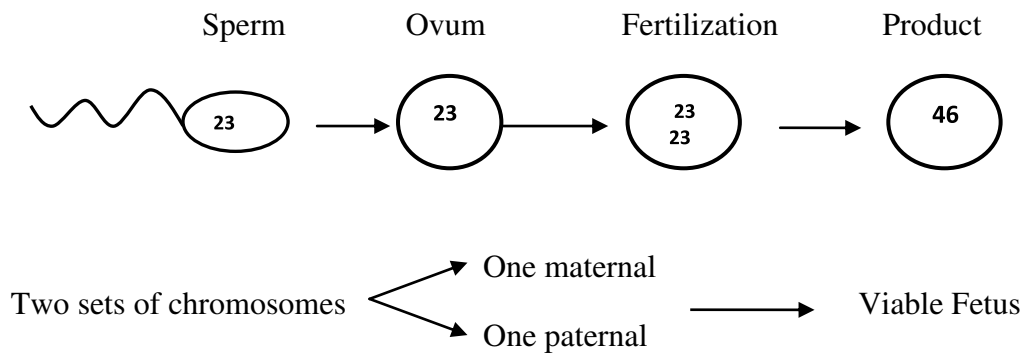
rare condition in which tissue around a fertilized egg that normally would have developed into the placenta instead develops as an abnormal cluster of cells. (This is also called a molar pregnancy.) This grapelike mass forms inside of the uterus after fertilization instead of a normal embryo. A hydatidiform mole triggers a positive pregnancy test and in some cases can become cancerous (Fig. 1).

Hydatidiform mole can be classified into complete or partial. The types are specified based on gross morphology, histopathology, and karyotype.

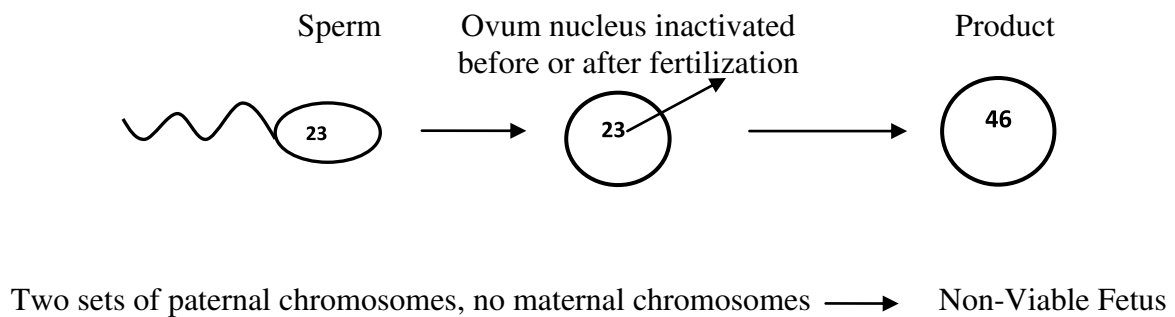
Important features of complete and partial hydatidiform mole are given in Tables 1 and 2 (Howkins and Bourne n.d.):

Schematic presentation of normal pregnancy and molar pregnancy:

Normal pregnancy:



Molar pregnancy: complete hydatidiform mole



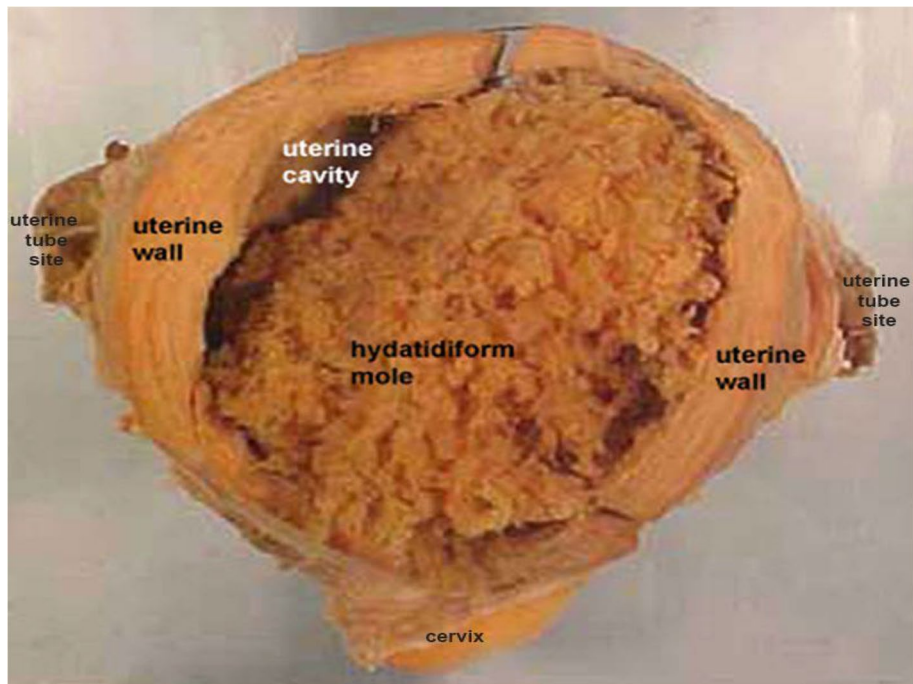


Fig. 1 Image showing hydatidiform mole

Molar pregnancy: partial hydatidiform mole

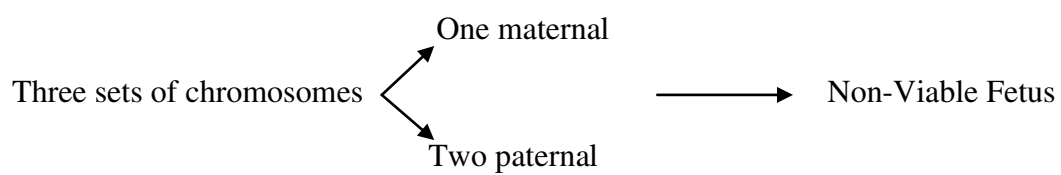
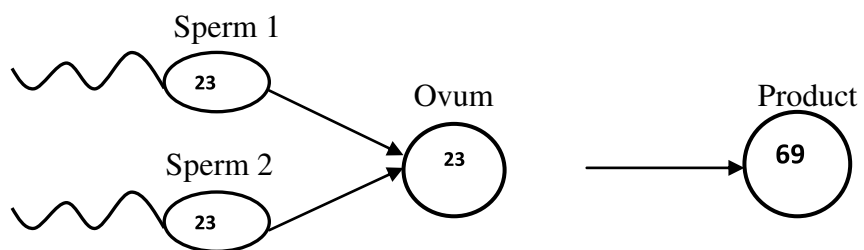


Table 1 Important features of complete and partial hydatidiform mole

S.no.	Features	Complete mole	Partial mole
1	Fetus	Absent	Present, malformed or Intrauterine growth restriction
2	Fetal vessels	Absent	Present
3	Hydropic changes	Diffuse and placenta not present	Focal
4	Trophoblastic hyperplasia	Marked	Mild to moderate
5	β -hCG level	Very high	Comparatively low
6	Karyotype	46 XX and mostly paternally derived	69 XXY
7	Malignant potential	15-20%	Rare

Table 2 Genotypes of blood samples of victim, accused, and vesicular mole

STR loci	Genotype		
	Victim	Vesicular mole	Accused
D8S1179	13, 14	13	10, 13
D21S11	29, 30.2	30	30, 30
D7S820	8, 10	10	10, 10
CSF1PO	10, 13	13	10, 13
D3S1358	16, 18	17	15, 17
THO1	7, 9	8	8, 9
D13S317	8, 10	12	11, 12
D16S539	8, 11	11	11, 11
D2S1338	18, 25	22	22, 22
D19S433	13, 14.2	14	14, 16.2
vWA	16, 16	18	14, 18
TPOX	9, 9	11	10, 11
D18S51	14, 15	12	12, 12
AMELOGENIN	X, X	X	X, Y
D5S818	11, 13	13	13, 13
FGA	22, 22	26	25, 26

It is generally seen that most of the complete hydatidiform moles carry a diploid genome, devoid of maternal chromosomes (Zhang et al 2019; Carey et al 2015). They contain two sets of paternal chromosomes with either 46 XX karyotype (monospermic or homozygous, about 80%) or 46 XY karyotype (dispermic or heterozygous, about 20%). While partial hydatidiform moles are triploid, with one maternal and two paternal sets of chromosomes, having XXX or XXY karyotype (Zheng et al 2014). DNA profiling is a precise method for the confirmation of hydatidiform moles. Short tandem repeats or STRs are DNA sequences that are prevalent in the non-coding region and are repetitive and polymorphic. Since they are genetically stable and the numbers of repeats differ in individuals, they can be utilized to generate a DNA profile or genotype. Therefore, the parental origin of molar pregnancies can be identified through STR genotyping (Carey et al

2015). Several studies exhibit the proof of concept and usefulness of STR genotyping to distinguish between a complete hydatidiform mole and a partial hydatidiform mole (Bell et al 1999; Bifulco et al 2008; Lipata et al 2010).

Case presentation

In the present case, a 17 years old girl was raped by the accused. The victim was one and a half months pregnant and was given a tablet by the accused for abortion. She was then hospitalized following bleeding. Products of conception termed as a vesicular mole, blood samples of the accused, and the victim were sent to the Regional Forensic Science Laboratory, Nagpur for DNA profiling.

Methods

DNA extraction from blood samples and vesicular mole tissue was done using the EZ1[®] DNA Investigator Kit on the EZ1[®] Advanced automated DNA isolation system by Qiagen. Genotyping was performed with an AmpFLSTR[™] Identifiler[™] PCR amplification kit. The reaction consists of an STR multiplex polymerase chain reaction (PCR) assay that amplifies 15 different autosomal STR loci and the sex-determining marker (Amelogenin) in a single reaction. The PCR conditions were 95°C for 11 min followed by 28 cycles of 94° C for 1 min, 59° C for 1 min, and 72° C for 1 min, followed by a final extension at 60° C for 45 min. The amplified products were mixed with size standard, denatured, and then capillary electrophoresis was performed on ABI 3500 Genetic Analyzer. Electrophoresis data collection and analysis were performed using GeneMapper[®] ID-X version 1.5 (Applied Biosystems, Inc) (Budowle and Allen 1988; Gill et al 1995).

Results

Genotyping demonstrates a homozygous allelic pattern in the vesicular mole with exclusive paternal alleles. The vesicular mole showed a lone allele at every locus tested, and the allele was sometimes found to be missing from

the mother's profile. Alleles that are not present in the mother's profile are shown in bold in the table.

Discussion

Forensic Science may be termed as the application of various scientific techniques to analyze evidence found in criminal cases. The word "forensic" is derived from the Latin word "*forenses*" meaning forum. In Rome, a forum was a public place where judicial proceedings and debates were held. Branches of Forensic Science mainly include Forensic Biology, Forensic Chemistry, Forensic Toxicology, Forensic Physics, Forensic Ballistics, and Digital Forensics. The basic principle underlying almost every forensic analysis is Locard's Exchange principle which states that "when two objects come in contact with each other, something is exchanged between them." This forms the basis of the transfer and recovery of all scientific evidence. One of the esteemed discoveries of the twentieth century, DNA profiling has transformed criminal investigation. DNA profiling is a state-of-the-art technique that identifies an individual based on an individual's unique genetic arrangement. The human body is made up of cells and nearly every cell in the human body has the same DNA. It was found that certain regions of DNA contained repeated DNA sequences. DNA regions with short repeat units, usually 2–6 base pairs in length are called short tandem repeats (STR). STRs are extensively used in forensic casework across the world during the past few decades (Hammond et al 1994; Butler 2006). Autosomal STRs are highly discriminating, abundantly present in the human genome, have a low mutation rate, and smaller amplicon size and thus have become very useful markers in human identification, parentage testing, and population genetic studies (Edwards et al 1991). In the instant case, a forcible physical relationship made the victim pregnant. To terminate the pregnancy, the accused gave her a pill which caused her to start bleeding and she was rushed to the hospital. The victim's statement was taken by the police while she was undergoing treatment at the hospital and the accused was arrested. Products of conception termed as a vesicular mole, blood samples of the accused and the victim were sent for DNA profiling. The DNA extracted from the products of conception termed as a vesicular mole, and blood samples of the accused and the victim was typed at 15 STR loci and gender-specific Amelogenin locus using the PCR amplification technique. Results show that there is no maternal tissue contamination and non-inheritance of maternal alleles in the vesicular mole sample. The molecular composition of the vesicular mole indicates replication of only paternal chromosomes leading to probably diandric homozygous diploidy.

Conclusions

Hydatidiform mole or vesicular mole which comes under gestational trophoblastic disease is a phenomenon that is commonly observed in hospitals where the patient is monitored from early pregnancy. Its occurrence in forensic cases is uncommon but DNA profiling using STR analysis helped to identify a hydatidiform mole, thereby resolving the paternity dispute in the POCSO case.

Abbreviations

DNA: Deoxyribo nucleic acid; GTD: Gestational trophoblastic disease; PCR: Polymerase chain reaction; POCSO: Protection Of Children against Sexual Offenses; STR: Short tandem repeats.

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Authors' contributions

AP conducted the extraction, PCR, and DNA typing of the case received in the laboratory for paternity dispute and drafted the manuscript. SD and VM helped in drafting the manuscript. VT reviewed and suggested modifications to the manuscript. AD and MD helped in the laboratory work of the case. The authors read and approved the final manuscript.

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