CASE REPORT

First Paternity Case Resolved by Conventional and DNA Typing Method in Iraq :Acase Report

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ABSTRACT :

In Iraq , DNA Typing Profile has been added to the routine work of disputed paternity cases as a powerful tool of investigation in Forensic in 2007. The old conventional investigation based on blood antigen systems like variable blood groups , HLA Tissue Typing was no more used in such sensitive cases because of the limitation or invariability of loci analyzed.

We report here the first case of paternity test done for a trios (Alleged father , mother and child) rebated by two old and new techniques . The alleged father has been excluded from being a biological father according to the mismatch in ABO, MN blood group &5 genetic markers in DNA profile between the alleged father &son.

KEYWORDS **:** disputed paternity , DNA Fingerprinting , blood relationship testing

INTODUCTION:

Paternity, the state of being a father, can be legally established in several ways. When the parents of a child are married, paternity is commonly presumed .To determine whether a man is the father of a child born out of wedlock, a lawsuit known as a "paternity action" must be brought. In such a suit, paternity may be established if the alleged father admits paternity .[7,9]

Blood-group studies, which commonly employ the ABO system, cannot establish paternity but can conclusively exclude an alleged father from being a candidate. This is the case because a child must inherit his or her blood type from the mother or father; thus, if the child's blood type differs from both the mother's and the alleged father's types, the man could not possibly be a parent of son . A typical population frequency for conventional blood typing might be 1 in 200, for DNA 1 in 5,000,000. This means that only 1 in 5,000,000 people would have the same DNA profile.

Adequate samples for DNA typing can be collected from blood, blood stain & oral swab easily . DNA typing compares strands of genetic material between the child and alleged father.[11] . comparing strands from various locations of the genetic material allows accuracy ratings of 99.9 percent. DNA typing allows an alleged father to be excluded with 100 percent certainty.[5]

CASE REPORT :

A middle age female mother was referred with her husband &son (9 months old) from the court of law in Baghdad. Paternity identification was requested by the mother who convinced that she had been raped forcefully since 18 months past duration time by a foreigner perpetrator, her husband(Who considered during all that time to be the legal and biological father of the child) had no idea about the rape event, he thought the doubt of his wife for disputed paternity as apart of her an un usual psychological personal instability. An appointment was fixed for the trios to attend at early morning at MLI for investigation and as follows :

1) At date of examination : Blood samples for the alleged parents & their son in 3 ml EDTA tube were collected . ABO, Rh , Rh subgroups (E,é,C,ć) and MN system were tested by both slide &tube method using haemagglutination assays .

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2) In early morning at the same date another blood samples of 5 ml were processed to obtain defibrinated blood for Tissue typing Class I , Buffy coat was separated by lymphoprep.Sol. ,washed twice , then adjusted to a final concentration of 2000cell/ µl. The lymphocytes (1 µl)from each trios were added to the previously prepared Terasaki plates with antisera , incubated 30mints at room temperature .Complement (5 µl) was added to each well, incubated again 1 hour at room temperature .Eosin as indicator & formalin as preservative were added .The results were ready to be read in the next after coming 3 days using inverted microscope [10] .

3) Blood stain on FTA cards were collected and preserved for DNA Analysis.
DNA was extracted using chelex method , amplification of DNA samples were performed with AmpFLSTR® Identifiler™ [14] kit .The amplified products were separated and detected using ABI PRISM™ 3130xl Genetic analyzer (Applied Biosystem) . Simultaneous amplification of 16 STR loci (D8S1179,D21S11,D7S820,CSF1PO,D3S1358,THO1,D13S317,D16S539,D2S1338, D19S433,VWA,TPOX,D18S51, D5S818,FGA and AMELOGININ) was completed & analyzed within 3 days .Collection of data from all previous tests was done as follows :

Table 1 : The results of ABO, RH, Rh subgroups (E,é,C,ć) ,MN &HLA –Class I blood markers for putative father, mother and child

Blood Antigen	ABO	Rh	E	é	C	ć	MN	HLA - ABC
Alleged Father	O	+	Neg	+	+	Neg	N	Mismatch
Mother	B	+	Neg	+	+	Neg	M	Match
Child	AB	+	Neg	+	+	Neg	M	HLA - ABC

Table 2 :The results of 15 autosomal genetic markers of CODIS for the trios on the bases of DNA profile,

Genetic Marker	D8 S1179	D21 S11	D7 S820	CSF 1PO	D3 S1358	THO 1	D13 S317	D16 S539	D2 S1338	D19 S433	VWA	TPO X	D18 S51	D5 S818	FGA
Alleged father	14 , 14	28,29	9,10	11 , 12	18,18	8 , 9	11,11	9 , 9	7 , 22	14,16	16,17	10,11	15 , 20	9 , 13	22,24
Mother	11,13	30,32.2	8,10	11,11	16,16	6,7	8,12	9,9	17,22	12,13	15,17	8,11	12,14	11,12	20.2,24
Child	13,14	28,32.2	8,10	11,12	16,18	7,9	8,11	9,14	17,22	12,13	16,17	8,8	14,16	12,12	22,24

In (Table 1), when the alleged father has (O) blood group and mother has (B) the child must be (B or O) according to Mendlian law of inheritance ,the nature of alleles mother must be (Bo or BB) alleles , father are similar (OO) .The husband was excluded according to ABO & MN system as biological Father . HLA Tissue Typing was of mismatch between the alleged father & child which again confirms the conclusion .
In table (2) shown the data at (Red markers & line below) represent 5 different loci between father and child (D16S539 , D19S433 , TPOX , D18S51 and D5S818)declared as exclusion (not-match) as the biological father , when in the mother, no possibly different loci found so she was failed to exclude as a biological mother of child. [3,4]

DISCUSSION :

Disputed paternity investigation is one of the referred cases to the medico- legal institute in Baghdad .As the DNA typing Department was established its work at the end of 2007 in Baghdad[2] . So the decision has been made to run a series of paternity test cases using both Conventional &DNA analysis for some sensitive paternity cases starting from this case to evaluate both in many aspects specially the practical one .

it is important to keep in mind that in STR analysis often examining a similar dozen or more loci, is preferable[7]. and it was achieved in this case.

In such case ,regarding ABO & MN system results ,If we fix the mother as the son ′s biological mother so both tests exclude the father from being the biological real father in absolute way , but in turn they cannot confirm maternity .

The result of HLA Tissue Typing again exclude paternity but it can include maternity to some extent (in case of no another kinship between the mother & son).

In DNA Test , Parentage analysis is based on fact that each allele of a child matches one allele of each parent . The parentage is established on the bases of exclusion ,since there is no possible way to absolutely prove that there are no other man or women in world who could be the father or mother of that particular child [12]

The scientific working group on DNA analysis lists some used recommendations for explain the results. which in turn have been grouped into 3 categories : Exclusion (not-match) , Inconclusive and failure to exclude (match-peak) [14,15] . Accordingly, one can confirm the absolute exclusion of paternity due to 5 mismatch between father & child but one also may confirm maternity relationship with a high rate of certainty because of the full match between mother & her son′ s profiles .

From practical point of view we have found that the result of father exclusion was concluded from both methods but technically with the following differences :

1) Collection of few spots of blood stain on FTA cards from each parents &son for DNA Test is much easer , less invasive than the need to about 7ml of fresh blood for Blood groups &HLA Tissue Typing.

2) The attendance of family must be compulsory in early morning for HLA Test .This is because one should keep the cell alive & the test should be processed at the same day but in DNA test no need for that , one can collect the samples at any time of day work .

3) To complete the standardization , paternity test in conventional histocompatability test should include Class II HLA –Tissue Typing [6] . This test is impractical for daily work because of the need to 20 ml of fresh blood(to obtain B-lymphocyte) ,this is so difficult &invasive in so young children provided that this test needs long time for processing & incubation beyond the time of routine day work in Iraq .In contrast , DNA from minute blood is so enough and fit for paternity as a powerful &safe test if properly performed according to international recommendations standard [13].

4) DNA Test provide simultaneous multiple markers investigation with a high rate of discrimination &polymorphism in one single test while the old one provide the same at the protein level with less polymorphism ,invariability &in multiple variable kinds of recommended manual protocols , tests , kits and instruments.

of coarse ,in contrast to blood antigens systems ,DNA profile provide much information about polymorphisms of each locus & mutational event through generations at the level of 1 or 2 base pair in each[8,14] ,although most of mutation rates are on the order of 1-5 mutation per 1000 allele transfers or generational events and this is very little rate [1] .

CONCLUSION:

We have found from the results & practical point of experience that DNA Test is superior , much easer ,more informative , much practical to test disputed paternity than old conventional one based on blood antigen systems .

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