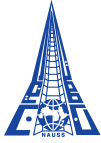|	
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Statistical Calculations in Case of Brother-Sister Incest

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التحليل الإحصائي في حالة زنا المحارم (الأخ - الأخت)

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Case Report

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Abstract

Incest is a sexual activity that happens between close family members that are not allowed to get married due to customs or law. In a case of incest between a brother and his married sister, a paternity test and a statistical analysis were performed at the laboratories of the Paternity and Kinship Division of the Medico-legal Directorate Baghdad, Iraq.

Blood samples were taken from the concerned subjects in this case (newborn baby, mother, alleged father, and the husband) and placed on FTA cards. DNA extraction was done using Chelex®, then the amplification of extracted DNA was carried out using an AmpFlSTR® Identifiler kit. PCR products were run with a 3130xl Genetic Analyzer, and the data were analyzed with Gene-Mapper ID® Analysis Software V.3.2 software.

The analysis of DNA profiles using 15 loci as well as the statistical analysis for calculating the paternity index confirmed the allegation of the brother-sister incest, since the baby inherited all the obligate alleles from the alleged father (suspected brother). With a 99.9998% probability of paternity, these results showed that even in the case of brother-sister incest, paternity could be proved using 15 DNA loci with a high rate of certainty.

Keywords: *Forensic Science, Paternity, Incest, DNA Profile, Combined Paternity Index, Probability of Paternity.*

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المستخلص

زنا المحارم هو نشاط جنسي يحدث بين أفراد الأسرة المقربين الذين لا يُسمح لهم بالزواج بسبب العادات أو القانون.

في حالة زنا المحارم بين أخ وأخته المتزوجة، تم إجراء اختبار للأبوة وتحليل إحصائي في مختبرات قسم الأبوة والقرابة في المديرية الطبية ببغداد في العراق. أخذت عينات دم من الأشخاص المعنيين في هذه الحالة (المولود الجديد، الأم، الأب المزعوم والزوج) وضعت على بطاقات FTA، تم استخلاص الحمض النووي باستخدام محلول الكيليكس ®Chelex، ثم تم تكثير الحمض النووي المستخلص باستخدام طقم التكثير AmpFlSTR® Identifiler. تم فصل نواتج التكثير (PCR products) باستخدام جهاز التحليل الوراثي فئة ٣١٣٠ إكس إل، Genetic Analyzer 3130xl، وتم تحليل البيانات الناتجة عن الفحص باستخدام برنامج Gene-Mapper ID® Analysis Software V.3.2.

أكد تحليل ملفات العلامات الوراثية للحمض النووي لخمس عشرة موقعاً وراثياً وكذلك التحليل الإحصائي لحساب مؤشر الأبوة على ادعاء حصول سفاح الأخ بأخته، حيث ورث الطفل جميع أليلات الإلزامية من الأب المزعوم (شقيق مشتبه به). مع قوة احتمال إحصائي تساوي ٩٩,٩٩٩٨ ٪ لأبوة الأب المزعوم، كما أظهرت هذه النتائج أنه حتى في حالة سفاح المحارم من الأخت، يمكن إثبات الأبوة باستخدام ١٥ موقع وراثي للحمض النووي DNA مع معدل عال من اليقين.

الكلمات المفتاحية: علوم الأدلة الجنائية، الأبوة، زنا المحارم، ملف العلامات الوراثية للحمض النووي، مؤشر الأبوة المشترك، احتمال الأبوة.

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1. Introduction

Incest is any sexual activity that happens between close family members that are not allowed to get married due to customs or law. In most populations (recent and past), incest is a taboo and is prohibited in most religions. Usually, people who commit incest are severely punished. Incest is also defined as any sexual contact between any close related individuals, so their marriage is also considered as illegal. In cases of abuse, the older family member is usually the one who abuses a child or adolescent [1,2]. Many experts consider incest a distinct damaging form of sexual abuse, because it was done by a trusted and dependable person closely related to the victim. Also, in the majority of cases, it is very difficult to find support and victims are forced to keep silent as a result of fearing family break up, which can affect other family members. People who have been severely abused by adults in their childhood often suffer from many psychological and mental disorders such as low self-esteem, difficulties in social relations, and sexual dysfunction [3].

Since 1984, DNA-based identity testing has been the method of choice and has been widely used for many purposes (criminal cases, social security, and immigration). During the 1990s, DNA testing was commercialized by private companies, making it available to consumers on a fee-for-service basis. Parentage testing (particularly paternity testing) is one of the first applications of DNA identity testing that helped to identify the paternity of people for the first time in human history with reasonable confidence. Implicitly, the test has implications and consequences for family relations [4]. DNA parentage tests involve the use of biological materials for two or more subjects to confirm or exclude paternity. A comparison of non-coding DNA re-

gions (band patterns or peaks) is often used, then the probability of paternity is calculated for ascertaining the claim of paternity [5]. A probability of ≥ 99.9% can regularly be obtained and indicates that the tested man is the biological father of the child [6]. Since a child's maternity is usually intuitive, most tests are requested for paternity. Routine biological samples include bloodstains, mouth swabs or finger pricks in the licensed laboratory context. Other samples obtained outside the laboratory are taken from drinking cups or hair follicles [5,7].

2. Case Report

In a family with 5 children, two brothers and 3 sisters, one of the sisters accused one of her brothers (the oldest one) of raping her and that he was the father of her baby. She claimed that he raped her before she got married; the marriage was a way to hide the brother's crime. After the baby's birth, she dumped it and told her husband about the rape. The husband reported the baby missing, and when the police found it he filed a case against the brother. The police then ran a complete criminal investigation and blood samples were taken from the mother, her baby, the alleged father (the oldest brother), and the husband for DNA analysis.

3. Methodology

DNA was extracted from the bloodstain of approximately 3 mm² in diameter and dried on FTA filter-paper using the Chelex method (7). PCR amplification was performed with an AmpFlSTR® Identifiler PCR amplification kit (Applied Biosystems, USA) using thermocycler® PCR Systems 9700 (Applied Biosystems, UAS). PCR products were seperated on a 3130xl Genetic Analyzer®

(Applied Biosystems, USA) according to the manufacturers innstructs. The data were analyzed with Gene-Mapper ID® Analysis Software V.3.2 software (Applied Biosystems, USA).

4. Results and calculation

Parentage analysis is based on the fact that each allele of a baby's profile matches one allele of each parent. The parentage is established based on exclusion, since there is no possible way to prove that there is no other man or woman in the world who could be the father or mother of that particular baby. The results of the DNA profile for all partners, in this case, are listed in Table 1. The husband was excluded to be the biological father of the baby in five loci (D21S11, D3S1358, D16S539, D2S1338, and D2S1338) as is shown (underlined) in Table 1.

Table 1- *The results of DNA profiles of the alleged father, husband, mother, and the baby.*

Loci	Baby		Mother		Husband		The brother/ Alleged father		Paternity Index
D8S1179	13	10	15	10	13	11	13	10	1/2Pj**
D21S11	29	27	31.2	27	33.2	30	31.2	29	1/2Pj
D7S820	12	9	12	8	12	12	12	9	1/2Pj
CSF1PO	13	10	13	10	10	10	10	10	1/(Pi+Pj)
D3S1358	16	16	17	16	18	17	16	15	1/2Pi*
THO1	9.3	7	9.3	7	7	6	9.3	8	1/2(Pi+Pj)
D13S317	11	8	12	8	11	8	11	8	1/2Pj
D16S539	12	9	11	9	11	10	12	9	1/2Pj
D2S1338	23	23	23	18	17	17	23	21	1/2Pi
D19S433	16	14	15.2	14	14	13	16	14	1/2Pj
VWA	18	16	19	18	17	16	18	16	1/2Pj
TPOX	9	8	8	8	10	8	9	8	1/2Pj
D18S51	20	16	20	14	19	16	16	15	1/2Pj
D2S1338	12	12	12	8	10	10	13	12	1/2Pi
FGA	20	19	20	19	21	20	21	20	1/2(Pi+Pj)
Amelogenin	Y	X	X	X	Y	X	Y	X	--

* i: alleles inherited from the mother, **j: alleles inherited from the father

Statistical analysis is necessary and required to provide a better understanding of the results and increases the power of discrimination of the obtained DNA profiles. Paternity index (PI) is the most commonly used test for this purpose. Paternity PI is defined as "the ratio of two conditional probabilities where the numerator assumes paternity and the denominator assumes a random man of a similar ethnic background was the father" [8].

The PI is generally represented in the formula of X/Y, where X is the probability of the alleged father (AF) to transmit the obligate allele while Y is the probability of any other man of the same race to transmit the allele. The value of X is assigned as 1 when the AF is homozygous for the locus of interest while 0.5 is assigned when the AF is heterozygous. The PI is calculated for each locus (Table 2) then Combined Paternity Index (CPI) for the entire set of genetic loci is obtained by multiplying the individual PI values for each locus of the DNA profile. The minimum accepted standard value for inclusion of paternity is a CPI of 100 or greater [9]. In this case, it is (5354041.628). The PI was calculated for each locus, as mentioned in 4 and 10.

Table 2- *Equations used for Paternity Index calculation*

D8 :	$1/2P_j =$	$1/2 * P\ 13 =$	$1/(2 * 0.2925) = 1.709$
D21 :	$1/2P_j =$	$1/2 * P\ 29 =$	$1/(2 * 0.255) = 1.961$
D7 :	$1/2P_j =$	$1/2 * P\ 9 =$	$1/(2 * 0.09) = 5.556$
CSF :	$1/\ P_i + P_j =$	$1/(P10+P13) =$	$1/(0.2975 + 0.45) = 1.338$
D3 :	$1/2P_i =$	$1/2 * P\ 16 =$	$1/(2 * 0.2825) = 1.770$
THO1	$1/2(P_i+P_j) =$	$1/2 * (P\ 7 + P9.3) =$	$1/2 * (1.775 + 0.1) = 0.267$
D13 :	$1/2P_j =$	$1/2 * P\ 11 =$	$1/(2 * 0.0125) = 40$
D16 :	$1/2P_j =$	$1/2 * P\ 12 =$	$1/(2 * 0.215) = 2.326$
D2 :	$1/2P_i =$	$1/2 * P\ 23 =$	$1/(2 * 0.115) = 4.348$
D19 :	$1/2P_j =$	$1/2 * P\ 16 =$	$1/(2 * 0.0675) = 7.407$
VWA :	$1/2P_j =$	$1/2 * P\ 16 =$	$1/(2 * 0.2725) = 1.834$
TPOX :	$1/2P_j =$	$1/2 * P\ 9 =$	$1/(2 * 0.105) = 4.762$
D18 :	$1/2P_j =$	$1/2 * P\ 16 =$	$1/(2 * 0.1075) = 4.651$
D5 :	$1/2P_i =$	$1/2 * P\ 12 =$	$1/(2 * 0.3825) = 1.307$
FGA :	$1/2(P_i+P_j) =$	$1/2\ (P\ 19 + P20) =$	$1/2\ (0.07 + 0.105) = 2.857$

* *i: alleles inherited from the mother, j: alleles inherited from the father*

Then the Combined Paternity Index (CPI) was calculated as follows:

PI D8 * PI D21 * PI D7 * PI CSF * PI D3 * PI THO1 * PI D13 * PI D16 * PI D2 * D19 * PI vWA * PI TPOX * PI D18 * PI D5 * PI FGA

CPI= 1.709* 1.961 * 5.556 * 1.338* 1.77* 0.267* 40* 2.326* 4.348 * 7.407* 1.834* 4.762 * 4.651* 1.307 * 2.857 =

5354041.628 times more likely that the brother is the biological father of the baby, rather than any other man that is not related to the tested man.

Probability of paternity= likelihood ratio*non genetic evidence

(likelihood ratio*non genetic evidence)+ (1- non genetic evidence)

= 5354041.628 *0.1 = 0.999998

(5354041.628*0.1)+ (1-0.1)

The probability of paternity is 99.9998%

5. Discussion

Incest is a taboo and prohibited in most religions, and people who commit incest are usually severely punished [1]. The legal definition of incest includes the nature of the interpersonal relationship as well as the type of sexual activity, which varies by country, and even within individual states or provinces in a country. In Iraq, anyone commits incest is punished with death according to the Revolutionary Command Council (defunct) No. 448 on 11 / 4 / 1978, as stated in the resolution:

First: Punished by death

1 - Any person who commits adultery with a female of his relatives to the third degree without her consent, if she had reached fifteen years of age, and the act led to her death or to make her pregnant or remove her virginity.

2 - Any person who commits adultery with a female of his relatives to the third degree without her consent and she was younger than fifteen years old.

3 - Any person who commits adultery with a female of his relatives to the third degree with her consent and she had not reached fifteen years of age, and the act led to her death or to make her pregnant or remove her virginity.

Second: The guilty shall be punished with life imprisonment if the act of intercourse or sodomy is committed (male or female), by mutual consent and they were eighteen years old and they were third-degree relatives.

Parentage testing is primarily aimed to exclude the topmost number of individuals who could be considered as biological fathers of the baby in question. This is achieved by recognizing the obligate allele in the baby and determining whether the alleged father carries this allele or not [5]. The obligate allele is known as the allele which a baby obligatorily inherits from the biological father at a certain locus. When the tested man (alleged father) is not excluded, then the probability of each obligate allele is calculated. Since progenitors are closely related, then the child's alleles inherited from the mother are likely to be included in the father's DNA profile [11].

The paternity index (PI) was created for a single genetic marker or locus (chromosome site or DNA sequence site of interest) and is associated with statistical strength or weight of that locus in favor or against ratios given the phenotypes of the participants tested and an inheritance scenario. The paternity index is a calculated value used to measure the strength of a particular match for a child-parent dispute. First, it is calculated for a single genetic marker or locus, then the product of multiplying the indexes for all the tested loci creates the Combined Paternity Index (CPI). The CPI value must be at least 100 for the alleged father to be considered as the child's father (to be "not excluded" as the biological father). A CPI value of 10,000 is referred to as

a probability of 99.9900%, and so on [9,12]. For this case, the CPI was 5354041.628, with a 99.9998% probability of paternity. This analysis of results showed that the baby had inherited all of the obligate alleles from the alleged father (the brother), so he was not excluded as the biological father of the baby in question. These are in accordance with previous studies concerning the use of STR analysis and paternity index in solving incest cases [13,14,15].

In conclusion, the use of 15 loci and Amelogenin for paternity testing plus the statistical calculations revealed that even in a case of brother-sister incest, paternity could be proven with a high degree of certainty.

Conflict of Interests
None.

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