# ASSESSMENT OF THE ROLE OF BLOOD GROUP SYSTEMS AND THREE DNA LOCI (ALU TPA -25, HUMFES / FPS AND HUMF13A1) IN PATERNITY TESTING IN A SAMPLE OF EGYPTIAN POPULATION

BY

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## ABSTRACT

*A panel of 10 genetic markers has been applied for paternity testing in 51 Egyptian families. The panel included 7 blood group systems (ABO, Rh, MNSs, Duffy, Lewis, Kell, & Kidd), and 3 DNA loci (Alu TPA - 25, HUMFES / FPS, & HUMF13A1). The trio in each family consisted of the mother, the child, and the legal or alleged father. The families were studied as 3 groups of statistical significance: The 1st - 40th family group in which paternity of legal fathers was tested despite the lack of any suspicion of paternity dispute (expected low probability of disputed paternity), the 41st - 51st family group in which paternity of legal fathers was tested due to strong suspicion of paternity dispute (expected higher probability of disputed paternity), and the 1st - 51st family group in which paternity of 10 known foreign men (to represent alleged fathers with 100 % true paternity dispute) was randomly tested in the 51 families of the study. The study included determination of blood groups by the agglutination method, and analysis of DNA loci by agarose gel electrophoresis after DNA extraction and amplification by polymerase chain reaction. Exclusion of paternity was concluded from the knowledge of modes of inheritance of the study markers, and probability of paternity (inclusion of paternity) was calculated from the studied gene frequencies after gene typing of the study population. Results of the study showed that the DNA loci were better than blood group systems in exclusion and inclusion of paternity, though both failed to exclude all the alleged fathers or to give reliable values of probability of paternity. The Lewis, Kell, and Kidd blood groups were nearly of no value in paternity testing whereas the polymorphic DNA loci (HUMFES / FPS and HUMF13A1) provided the best results. Some true disputed fathers were excluded by single markers only, raising the importance of such exclusion which should be considered seriously and cautiously. Its reliability should be scrutinized, and it may be necessary to examine more markers. It has been concluded that the study panel of 10 genetic markers was not adequate in excluding or proving paternity for all test cases, and that the polymorphic markers provide better results in paternity testing. In a certain population, paternity test-*

*ing should rely upon adequate number of the most valuable genetic markers, and regulatory rules regarding reliable paternity exclusion or inclusion parameters are mandatory, as well as strict application of quality control parameters to the concerned laboratories.*

## *INTRODUCTION*

Paternity testing is an important issue both in forensic medicine and in law. It refers to the testing of the inherited genetic markers to determine the presence or absence of a biological relationship between the trio (mother, father, and child). This is most commonly applied to answer the question whether a man is or is not the biological father of a child. The goal of parentage testing is to exclude all falsely accused men or to provide sufficient evidence for inclusion of paternity if a man is not excluded (Harmening et al., 1994). Paternity testing now depends on a constellation of laboratory methods that depend on conventional typing of blood groups, protein polymorphism, and the relatively recent DNA based analysis (Morling and Carracedo, 2002). DNA shows polymorphic regions that include loci of short tandem repeats (STRs). The polymorphism of STRs has proven to be extremely useful in understanding and analysis of genomic diversity, human evolution and in forensic medicine (Mastana and Singh, 2002). Polymerase chain reaction (PCR) has strongly enhanced the usefulness of DNA analysis - based techniques in forensic work, as it allows targeted in vitro amplification of selected short segments of DNA. Potentially suitable STRs loci have repeat units of 3 - 5 base pairs (bp) and allele sizes of about 100 - 300 bp. Some loci have alleles differing by a number of complete repeat units, whereas others display complex polymorphism with some alleles differing in size by only 1 bp (Delghandi et al., 2001).

The role of typing of blood groups and alleles of STR loci of DNA in paternity testing depends largely on the gene or allele distribution in the population (Henry et al., 2001), and this differs appreciably in different populations (Harmening et al., 1994, and Corvelo et al, 2002). The aim of the present work is to assess the role of blood groups and three STR loci of DNA: Alu TPA - 25 (on chromosome 8), HUMFES / FPS (also known as FES, on chromosome 15), and HUMF13A1 (also known as F13A01, on chromosome 6) in paternity testing in a sample of Egyptian population, and to evaluate their reliability for exclusion or inclusion of paternity, on practical grounds.

## *SUBJECTS AND METHODS*

**I- Subjects :**

The study was conducted on 51 families having no history of consanguinity be-

tween parents. Each family consisted of trio: the legal father, the legal mother and a child (as proven in the civil papers). The first 40 families were chosen randomly from the general population with no history of dispute, and the other 11 families (from family 41 to family 51) attended the Genetics Unit of Mansoura University Children Hospital asking for paternity testing. In all the families, the mother–child relationship was not in doubt. In addition, another 10 unrelated men were randomly tested against the legal fathers as if they were alleged fathers. Each of nine men was tested against the legal fathers of five families, and the 10th man was tested against the legal fathers of six families.

**II- Methods:**

**A- Methods of determination of blood groups and DNA loci:**

A peripheral blood sample (10 ml) was collected from each subject by venipuncture on ethylene - diamine - tetracetic acid (EDTA). Each blood sample was divided into two parts:

a) The first part (2 ml) was either used freshly or stored at 4°C for a maximum period of three days for testing the blood group antigens by the agglutination technique (Bethesda, 1993) using appropriate kits. The samples were tested for the major blood group antigens; ABO (A1, A2, B and O) and the Rhesus (Rh) D antigen, as well as the minor blood group antigens which included other Rh antigens (C, c, E and e), MNSs antigens (M, N, S and s), Duffy antigens (Fya & Fyb), Lewis antigen (Lea & Leb), Kell antigen (K & Cellano antigen "k") and Kidd antigens (Jka & Jkb). The tests were done in the Blood Bank of Mansoura University Hospital.

b) The second part (8 ml blood) was either used freshly or stored at -20°C for determining the selected STR loci of DNA. The tests were done in the Genetics Unit of Mansoura University Children Hospital. DNA was extracted by the chemical method (Davis et al., 1986), and DNA solution (1 µg /1 µL) was prepared by the method of Wared et al. (1989). PCR was conducted in HYBAID thermal cycler using 3 groups of primers for amplification of Alu TPA - 25 locus, HUMFES / FPS locus, and HUMF13A1 locus. PCR program of Veerrajul et al. (2001) was applied for amplification of Alu TPA - 25 locus, and PCR program of Frieze and Halldorsson (2001) was applied for the other two loci.

After PCR run, the amplification products were analyzed by agarose gel electrophoresis according to Halos et al. (1999) as follows:

Alu TPA - 25 locus: One or two bands are visible in each lane, indicating that an individual is either homozygous or heterozygous for the Alu insertion on TPA - 25 locus. When Alu insertion on TPA - 25 lo-

cus is present, the 400 - bp fragment is seen, and when absent, 100 - bp fragment is seen (Figure 1).

HUMFES / FPS and HUMF13A1 loci: One or two bands are visible in each lane, indicating that an individual is either homozygous or heterozygous for the STR number (Figure 2). The molecular size of HUMFES / FPS locus ranges from 206 to 238 bp, and it has 9 alleles (from allele 7 to allele 15). The molecular size of HUMF13A1 locus ranges from 179 to 235 bp, and it has 16 alleles (alleles 3, 3.2 and alleles from 4 to 17).

**B- Analysis of data:**

**1) Exclusion of paternity was categorized into 4 classes (Hubbell et al., 1991):**

*I- Direct exclusion:*

**Class Ia:** The child is positive for a genetic marker that is absent in the mother and putative father.

**Class Ib:** The child is negative for genetic markers of a system that are demonstrated in the putative father.

*II- Indirect exclusion:*

**Class IIa:** The child is homozygous for a genetic marker that is absent in the putative father.

**Class IIb:** The child is negative for a genetic marker, whereas the putative father is homozygous for it.

**2) Blood group gene frequency:** It was calculated from the blood group phenotype frequencies in unrelated individuals of the research population sample (51 mothers, 51 legal fathers, and 10 men; total = 112) according to Ibrahim (1987) and Thomson (2003).

**3) DNA allele frequencies:** This was calculated from the phenotype frequencies in the previous population sample according to Thomson (2003).

**4) Inclusion of paternity:** Calculation of paternity index (PI), combined paternity index (CPI) and probability of paternity (PP) (Henry et al., 2001):

PI for non- excluded fathers was calculated from the data of blood groups and DNA allele frequencies. To estimate the PI for a certain man, the frequency (X) with which this man could produce a single sperm with the obligatory paternal gene(s) was compared to the frequency (Y) with which this gene(s) could be expected to occur in a single sperm from a random man of the same population. The **PI** for each genetic system was calculated separately and expressed as **PI = X / Y**. The CPI for a trio was determined by multiplying the individual paternity indices of the tested genetic systems, and then the **PP** was calculated from the Baysian formula for the probability of paternity: **PP = PI / (CPI + 1)** using a value of 0.5 for the prior probability assuming this is a neutral unbiased value.

*Statistical analysis:*

Parametric data, as proved by the Kolmogrov - Smirnov test, were analyzed by calculation of the arithmetic mean, standard deviation, range, and student t - test. Non- parametric data were analyzed by Mann Whitney-U and Fischer's exact tests.

All statistical tests were run on an IBM compatible personal computer by using the Statistical Package for Social Scientists (SPSS) for windows, version 10 (SPPS Inc., Chicago, IL, USA).

## *RESULTS*

*Exclusion of paternity:*

Exclusions made by individual markers are shown in table (1) and figure (3). Numbers and percentages of excluded fathers are shown in table (2) and figure (4). Classification of paternity exclusions according to the number of excluding markers are shown in table (3). The frequencies of classes of exclusion are shown in table (4).

**Inclusion of paternity (expressed as probability of paternity):**

The gene frequencies are shown in table (5). For each family group, the probability of paternity (upper and lower limits, and mean values + standard deviation) calculated from non- excluding systems of blood groups, DNA loci, and combined blood groups and DNA loci, as well as comparisons between non- excluded and excluded fathers are shown in table (6). Comparisons between probability of paternity of non- excluded legal and alleged fathers in the three family groups are shown in table (7).

## *DISCUSSION*

In the present work, a panel of 10 genetic markers was evaluated for parentage testing in 51 families. The markers included the major blood groups (ABO & Rh systems), minor blood groups (MNSs, Duffy, Lewis, Kell, & Kidd), and three DNA loci (Alu TPA - 25, HUMFES / FPS, & HUMF13A1). The families were studied as three groups of statistical significance:

- A group containing 40 families (1$^{st}$ - 40$^{th}$ family): The trio in each family consisted of the mother, the child, and the legal father. The families had no suspicion of paternity dispute so that the probability of disputed paternity may be low in this group.

- A group containing 11 families (41$^{st}$ - 51$^{st}$ family): The trio in each family consisted of the mother, the child, and the legal father. The families of this group had strong suspicion of paternity dispute. The probability of disputed paternity may be higher in this group.

- A group containing all the families (1$^{st}$ - 51$^{st}$ family): The trio in each family con-

sisted of the mother, the child, and a known unrelated man representing the "alleged father". The probability of disputed paternity in this group is 100%.

**Exclusion of paternity:** Analysis of paternity exclusion among the tested families revealed the following:

The group containing the 1st - 40th family (Tables 1, 2): Two legal fathers (5 %) were excluded by the blood group system MNSs, whereas the DNA panel showed no exclusions. In this group where paternity dispute is unlikely, exclusions by a single marker raises a serious question about the reliability of such exclusion, which should be looked at with great caution because of the possibility of false exclusion. Verification of exclusion of those two legal fathers shows that exclusion was indirect (Class IIa) where the children were presumed homozygous for the N or S genes (NN and SS respectively) and the products of such genes were absent in the two fathers. Non- certainty of this exclusion emerges from the inability to detect products of the opposite genes in the children, where the possibilities of weak expression of genes, undetected rare alleles, or the occurrence of rare mutations can not be ruled out. Another finding in favour of the false nature of these two exclusions is the higher mean value of probability of paternity for excluded fathers than for non - excluded fathers of this group (Table 6). For legal issues, the significance of such exclusions and reliability of laboratory tests providing them is critical for any conclusion about the paternity.

The group containing the 41st - 51st family (Tables 1, 2): A total of eight legal fathers (72.73 %) were excluded; four fathers were excluded by the blood group panel (by the ABO, Rh, Duffy, and Kell systems, classes Ia and IIa of exclusion), and other four fathers were excluded by the DNA panel (by the 3 DNA loci, classes Ia and IIa of exclusion). Comparison between exclusions in this group (where paternity dispute is suspected) and the previous group show that the percent of total excluded legal fathers is significantly higher ($P < 0.001$), rising the probability of the presence of true paternity dispute. The possibility that all such exclusions might be false is unacceptable because, if considered false, the percent of exclusion would be comparable to that of the 1st - 40th family group. However, the surprising finding here is that all exclusions were made by single markers only in contradistinction to the 1st - 51st family group in which most exclusions (85.7 %) were made by more than one marker.

The group containing the 1st - 51st family (Tables 1, 2): A total of 49 alleged fathers (96.08%) were excluded: 32 (62.75%) were excluded by blood groups (ABO, Rh, Duffy, and Kidd systems, by all classes of exclusion, mainly class Ia and IIa) as well

as DNA (by the 3 DNA loci, classes Ia and IIa of exclusion), and 17 (33.33%) were excluded by the DNA panel alone (by the three DNA loci, classes Ia and IIa of exclusion). Surprisingly, two alleged fathers could not be excluded by the panel of 10 genetic markers. Taking into consideration the absence of any relationship between the alleged and legal fathers, failure of that panel to exclude two among 51 sure disputed fathers (3.92 % of total) renders it inadequate to exclude all cases of paternity dispute, and warrants the addition of further markers to achieve reliable exclusion. However, if the alleged father is a close relative to the legal father, exclusion may be difficult.

Analysis of exclusions made by individual markers (Table 1) shows that, among the blood groups' panel, the ABO, Rh, MNSs, and Duffy blood group systems were the most efficient in excluding alleged fathers. Lewis and Kell systems were the least valuable (0 % exclusion), and kidd system excluded only 3.92 % of alleged fathers. Among the DNA panel, the HUMFES / FPS & HUMF13A1 loci were more efficient than the Alu TPA - 25 locus. It is observed that the most efficient markers in exclusion are those with polymorphic alleles, a finding which coincides with the theoretical conclusion that the power of exclusion of a genetic marker is proportional to its polymorphism among the population (Ohno et al., 1982). Analysis of exclusions made by the blood group panel versus the DNA panel in the 1st - 51st family group (Table 2) shows that the three DNA loci were more efficient in excluding alleged fathers than the blood groups with a highly significant difference ($P < 0.0001$).

Analysis of exclusions, according to the number of excluding markers, in the 1st - 51st family group (Table 3) shows that 7 of total 49 exclusions (14.3 %) were made by single markers only, whereas 42 exclusions (85.7 %) were made by multiple (more than one) markers. All the exclusions (100 %) in the 41st - 51st family group were made by single markers. Upon comparison between the two groups as regards exclusion by single- marker versus exclusion by multiple- markers, a highly significant difference was detected ($P < 0.001$). It has been shown that most of the exclusions in the 41st - 51st family group were due to true paternity dispute upon comparison with the 1st - 40th family group. Since all the exclusions in the 41st - 51st family group were made by single markers in contrast to the 1st - 51st family group of alleged (who were unrelated to the legal fathers), it can then be concluded that the disputed fathers in the 41st - 51st family group were close relatives to the legal fathers of that group.

As regards classes of exclusion, it has been stated that direct exclusion is more reliable than indirect exclusion because direct exclusion depends on demonstrable markers, whereas indirect exclusion depends on the assumption of homozygosity for a particular gene which can not be determined unequivocally by testing (Polesky, 1996). However, in the present work (Table 4), it has been shown that most exclusions were made by the indirect class IIa (most exclusions by blood groups were by class IIa & class Ia, whereas most exclusions by the DNA loci were class IIa). This leads to the conclusion that any exclusion in parentage resting, even by a single marker and/or by an indirect class of exclusion, should be considered seriously albeit cautiously; its reliability should be verified, and testing for paternity should be extended to involve other genetic markers.

**Inclusion of paternity (probability of paternity):**

Gene frequencies: Gene frequencies were calculated from the studied population sample as a prerequisite for the calculation of probability of paternity (Table 5). Other previous reports or reports done elsewhere about gene frequencies were not used to obtain updated local values derived from the studied population sample. By comparing the obtained values of blood group gene frequencies with values of other studies performed earlier in our locality (Ibrahim et al., 1990) or in other localities (Wagner et al., 1995, Halos et al., 1998, Gamero et al., 2002), variable differences between populations were detected. Indeed, such differences are expected to be present due to such factors as sample size, effect of migration and possible occurrence of mutations (Blumenfeld & Batnaik, 2004).

**Probability of paternity** (Table 6) was calculated for each trio of the studied families using combined blood groups alone, combined DNA loci alone, and combined blood groups and DNA loci. The upper and lower limits, mean, and standard deviation were calculated for the whole group, as well as for the non- excluded and excluded fathers within each group. For excluded fathers, the probability of paternity was calculated from non - excluding markers.

Calculation of probability of paternity for non- excluded and excluded fathers within each group aimed at helping the investigators to suggest whether the exclusions were true or false. The means of probability of paternity derived from blood groups, DNA loci, or both showed higher values for non - excluded than for excluded fathers, except the 1st - 40th family group which had higher mean value for the excluded fathers.

This result is in favour of the assumption that the exclusions in the 1st - 40th family group were most probably false. However, certainty of that assumption is impossible to judge from the used panel of genetic markers because the group of true disputed fathers (1st - 51st family group) showed exclusions by single markers and very high values of probability of paternity for some trios.

Although the mean values of probability of paternity for non- excluded fathers were generally higher than the values for excluded fathers within each family group, yet the upper and lower values showed considerable variation and overlap. The probability of paternity for a sure disputed father (in the 1st - 51st family group) reached a value as high as 99.93 %, while it was as low as 69.2 % for a non- excluded father in the 1st - 40th family group. This renders judgment of paternity inclusion for a tested single trio impossible in many cases "using the study panel of genetic markers".

Comparison between mean values of probability of paternity for non- excluded fathers in the different family groups (Table 7) derived from blood groups, DNA loci, or blood groups and DNA loci showed that the values derived from DNA loci were higher than those derived from blood groups, while the values derived from both blood groups and DNA loci were the highest. The differences were significant in the 1st - 40th family group where all the fathers were mostly true fathers, and in the 41st - 51st family group where a proportion of fathers were not excluded. The superiority of DNA loci to the blood groups coincides with Markowicz et al. (1990) and Wee (1993) who reported that DNA based systems offer higher precision exclusion than protein- based systems, thereby minimizing the chance of falsely including a non - father, as well as giving a more accurate inclusion probability for the identification of true biological fathers.

Although the tested DNA loci yielded better results in paternity testing than blood groups, yet we must keep awareness about some limitations of DNA results. Swarner (1996) mentioned that the genetic systems analyzed by PCR technique were susceptible for inhibition by various factors such as haeme in the blood. Specific primers may be more efficient with one allele than another creating an increased production of the favoured allele. The extreme sensitivity of PCR makes it also susceptible for contamination. Moreover, visual comparison between bands on the gel is limited by a difference of at least 4 base pairs in their sizes. The risk of mutation in DNA is still persistent, and in fact, the rate of mutation in DNA was demonstrated to be greater than that observed with conventional

blood groups (Henry et al., 2001). All these pitfalls may lead to misinterpretation of the results. Many legal authorities consider exclusion at a single locus not enough to establish non - paternity, and reliable exclusion should be based on a minimum of two mismatches (Henry et al., 2001).

**Finally, the following conclusions can be derived from the study:**

- DNA polymorphic short tandem repeat loci provide more conclusive results in exclusion or inclusion of paternity than do blood group systems.

- Some blood group systems proved to be of little value in paternity testing (Lewis, Kell, and Kidd), and should be omitted from such testing in Egyptian population.

- The panel of 10 genetic markers applied in the study is not sufficient to exclude all cases of disputed paternity or to prove paternity (probability of paternity) in case of non- exclusion. Further genetic markers, such as other polymorphic DNA loci and human leucocyte antigens, should be applied to achieve legally reliable results.

- Any exclusion in paternity testing, even by a single marker and / or by an indirect class of exclusion, should be considered seriously albeit cautiously. Its reliability should be verified, and testing for paternity should be extended to involve other genetic markers. Quality control parameters should be strictly applied to the concerned laboratories. Regulatory rules, as regards the reliable number of excluding markers to prove exclusion or reliable values of probability of paternity in case of non- exclusion, are mandatory. Such rules should consider data derived from the concerned population such as the gene frequencies and polymorphism of the selected markers.

- The gene frequencies of any selected panel of genetic markers for paternity testing should be kept updated to obtain the best results.

**Table (1):** Paternity exclusions (number and percentage of excluded fathers) by blood groups and DNA loci of the studied population sample.

| | | Blood groups | | | | | | | DNA loci | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ABO | Rh | MNSs | Duffy | Lewis | Kell | Kidd | Alu TPA-25 | HUMF-ES/FPS | HUMF-13A1 |
| Legal fathers | 1st – 40th family | 0 | 0 | 2 (IIa) (23th,25th) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Percent | 0 % | 0 % | 5 % | 0 % | 0 % | 0 % | 0 % | 0 % | 0 % | 0 % |
| | 41st – 51st family | 1 (Ia) (49th) | 1 (IIa) (48th) | 0 | 1 (IIa) (50th) | 0 | 1 (Ia) (51st) | 0 | 2 (Ia, IIa) (44th, 45th) | 1 (IIa) (41st) | 1 (IIa) (47th) |
| | Percent | 9.09 % | 9.09 % | 0 % | 9.09 % | 0 % | 9.09 % | 0 % | 18.18 % | 9.09 % | 9.09 % |
| Alleged fathers | 1st – 51st family | 15 (Ia)<br>2 (IIa)<br>3 (IIb) | 1 (I)<br>10 (IIa) | 4 (I)<br>1 (Ib)<br>10 (IIa) | 4 (Ia)<br>1 (Ib)<br>2 (IIa)<br>1 (IIb) | 0 | 0 | 1 (IIa)<br>1 (IIb) | 3 (I)<br>5 (IIa) | 1 (I)<br>36 (IIa) | 1 (I)<br>35 (IIa) |
| | | 20 | 11 | 15 | 8 | | | 2 | 8 | 37 | 36 |
| | Percent | 39.21 % | 21.57 % | 29.41 % | 15.68 % | 0 % | 0 % | 3.92 % | 15.68 % | 72.55 % | 70.59 % |

Parentheses: The Latin symbols (I, II) refer to the class of exclusion, and the numerical value to the family number.

**Table (2):** Numbers & percentages of excluded fathers by using blood groups, DNA loci, and combined blood groups & DNA loci.

| Markers | Number & percentage of excluded fathers | | |
|---|---|---|---|
| | Legal fathers | | Alleged fathers* $1^{st} - 51^{st}$ family |
| | $1^{st} - 40^{th}$ family | $41^{st} - 51^{st}$ family | |
| **Blood groups** | 2 (5 %)[1] | 4 (36. 36 %)[1] | 32 (62.75 %) |
| **DNA loci** | 0 (0 %) | 4 (36. 36 %) | 49 (96.08 %) (17 by DNA alone[33.3 %]) |
| **Combined blood groups & DNA loci** | 2 (5 %)[2] | 8 (72.73 %)[2] | 49 (96.08 %) |

( )[1]: significant difference upon comparison against each other (P = 0.015).
( )[2]: significant difference upon comparison against each other (P < 0.001).
*: The alleged fathers in most trios were excluded by more than one marker.

**Table (3):** Classification of paternity exclusions according to the number of excluding markers with percent of total exclusions.

| No. of excluding markers | | Blood groups & DNA loci | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | 1 marker | 2 markers | 3 markers | 4 markers | 5 markers | 6 markers | |
| **Legal fathers** | $1^{st} - 40^{th}$ family | 2 (100%) | | | | | | 2 |
| | $41^{st} - 51^{st}$ family | 8 (100%) | | | | | | 8* |
| **Alleged fathers** | $1^{st} - 51^{st}$ family | 7 (14.3%) | 14 (28.6%) | 16 (32.7%) | 9 (18%) | 2 (4.1%) | 1 (2%) | 49* |

*: significant difference present upon comparison against each other regarding exclusion by more than one marker (P < 0.001).

**Table (4):** Frequencies of classes of paternity exclusions by blood groups and DNA loci of the studied population sample.

| | | Blood groups | | | | DNA loci | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Class Ia | Class Ib | Class IIa | Class IIb | Class Ia | Class Ib | Class IIa | Class IIb | |
| Legal fathers | 1st – 40th family | | | 2 | | | | | | |
| | 41st – 51st family | 2 | | 2 | | 1 | | 3 | | |
| Alleged fathers | 1st – 51st family | 24 | 2 | 25 | 5 | 5 | | 76 | | |
| Total | Class Ia | 26 (41.9%)[1] | | | | 6 (7.06%)[2] | | | | 32 (21.8 %)[3] |
| | Class Ib | | 2 (3.22%)[1] | | | | | | | 2 (1.4 %)[3] |
| | Class IIa | | | 29 (46.8%)[1] | | | | 79 (92.9%)[2] | | 108 (73.5 %)[3] |
| | Class IIb | | | | 5 (8.06%)[1] | | | | | 5 (3.4 %)[3] |

(%)[1] : percent of total exclusions made by blood groups.
(%)[2] : percent of total exclusions made by DNA loci.
(%)[3] : percent of total exclusions made by both blood groups and DNA loci.

**Table (5):** Gene frequencies of blood groups and DNA loci calculated from phenotyping of the studied population sample (n = 112).

| Blood groups | | | | | | DNA loci | | | |
|---|---|---|---|---|---|---|---|---|---|
| Blood group | Gene | Frequency (%) | Blood group | Gene | Frequency (%) | Locus | Allele | Base pair | Frequency (%) |
| ABO | A1 | 28.33 | MNSs | MS | 18.48 | Alu TPA-25 | 1 | 100 | 67.41 |
| | A2 | 4.77 | | Ms | 34.64 | | 2 | 400 | 32.59 |
| | B | 20.71 | | NS | 11.44 | | | | |
| | O | 50.95 | | Ns | 35.44 | | | | |
| Rh | CDE | 9.52 | Duffy | $Fy^a$ | 33.44 | HUMFES/FPS | 11 | 222 | 13.39 |
| | CDe | 21.79 | | $Fy^b$ | 45.08 | | 13 | 230 | 27.68 |
| | Cde | 2.17 | | Fy | 21.46 | | 14 | 234 | 46.43 |
| | cDe | 34.35 | | | | | 15 | 238 | 12.5 |
| | cdE | 0 | Lewis | Le | 78.88 | HUMF13A1 | 3.2 | 181 | 7.14 |
| | cde | 24.28 | | le | 21.12 | | 6 | 191 | 1.78 |
| | CdE | 0 | | | | | 7 | 195 | 30.36 |
| | cDE | 7.89 | Kell | K | 1.79 | | 16 | 231 | 21.43 |
| | | | | k | 98.21 | | 17 | 235 | 39.29 |
| | | | Kidd | $Jk^a$ | 56.25 | | | | |
| | | | | $Jk^b$ | 43.75 | | | | |

**Table (6):** Mean values of probability of paternity of non- excluded and excluded legal and alleged fathers derived from non- excluding markers (blood groups, DNA loci, and combined blood groups & DNA loci) of the studied population sample.

| Marker | | Legal fathers | | | | | | Alleged fathers | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st – 40th family | | | 41st – 51st family | | | 1st – 51st family | | |
| | | No. | Upper & lower limits | Mean ± S.D. | No. | Upper & lower limits | Mean ± S.D. | No. | Upper & lower limits | Mean ± S.D. |
| **Combined blood groups** | Non-excluded | 38 | 69.20 – 98.54 | 89.82 ± 6.18 | 7 | 72.03 – 95.59 | 85.94 ± 8.44 | 19 | 51.86 – 98.63 | 81.82* ± 12.65 |
| | Excluded | 2 | 91.61 – 97.86 | 94.74 ± 4.42 | 4 | 22.59 – 98.04 | 72.80 ± 34.36 | 32 | 28.88 – 94.66 | 68.99 ± 16.01 |
| | Total | 40 | 69.20 – 98.54 | 90.06 ± 6.15 | 11 | 22.59 – 98.04 | 81.19 ± 20.91 | 51 | 28.88 – 98.63 | 73.77 ± 15.99 |
| **Combined DNA loci** | Non-excluded | 40 | 80.18 – 98.78 | 92.07 ± 5.08 | 7 | 82.69 – 99.36 | 96.40* ± 2.33 | 2 | 89.01 – 96.34 | 92.68* ± 5.18 |
| | Excluded | 0 | --- | --- | 4 | 82.96 – 91.70 | 87.49 ± 3.57 | 49 | 00.00 – 95.39 | 62.56 ± 22.49 |
| | Total | 40 | 80.18 – 98.78 | 92.07 ± 5.08 | 11 | 83.58 – 99.96 | 93.16 ± 5.23 | 51 | 00.00 - 96.34 | 63.74 ± 22.82 |
| **Combined blood groups & DNA loci** | Non-excluded | 38 | 95.14 – 99.90 | 99.01 ± 1.13 | 3 | 98.22 – 99.96 | 99.30 ± 0.94 | 2 | 97.55 – 98.35 | 97.95* ± 0.57 |
| | Excluded | 2 | 98.29 – 99.74 | 99.02 ± 1.03 | 8 | 83.58 – 99.92 | 96.29 ± 5.47 | 49 | 37.69 – 99.93 | 83.19 ± 14.48 |
| | Total | 40 | 95.14 – 99.90 | 99.01 ± 1.11 | 11 | 93.14 – 99.36 | 97.11 + 4.81 | 51 | 37.69 – 99.93 | 83.77 ± 14.48 |

No.: Number

*: significantly higher than the mean values of excluded fathers ($P < 0.05$).

**Table (7):** Comparison of the mean values of probability of paternity of non- excluded legal and alleged fathers derived from blood groups, DNA loci, and combined blood groups & DNA loci of the studied population sample.

| Marker | Legal fathers | | | | | | Alleged fathers | | |
|---|---|---|---|---|---|---|---|---|---|
| | $1^{st} - 40^{th}$ family | | | $41^{st} - 51^{st}$ family | | | $1^{st} - 51^{st}$ family | | |
| | No. | Upper & lower limits | Mean ± S.D. | No. | Upper & lower limits | Mean ± S.D. | No. | Upper & lower limits | Mean ± S.D. |
| **Combined blood groups** | 38 | 69.20 – 98.54 | 89.82 ± 6.18 | 7 | 72.03 – 95.59 | 85.94 ± 8.44 | 19 | 51.86 – 98.63 | 81.82 ± 12.65 |
| **Combined DNA loci** | 40 | 80.18 – 98.78 | 92.07 ± 5.08 | 7 | 82.69 – 99.36 | 96.40 (1) ± 2.33 | 2 | 89.01 – 96.34 | 92.68 ± 5.18 |
| **Combined blood groups & DNA loci** | 38 | 95.14 – 99.90 | 99.01 (1,2) ± 1.13 | 3 | 98.22 – 99.96 | 99.30 (1) ± 0.94 | 2 | 97.55 – 98.35 | 97.95 (1) ± 0.57 |

( )(1): significantly higher than the mean value calculated from combined blood groups ($P < 0.05$).
( )(2): significantly higher than the mean value calculated from combined DNA loci ($P < 0.05$).
Comparison between mean values of probability of paternity of the three family groups were insignificant at $P < 0.05$.

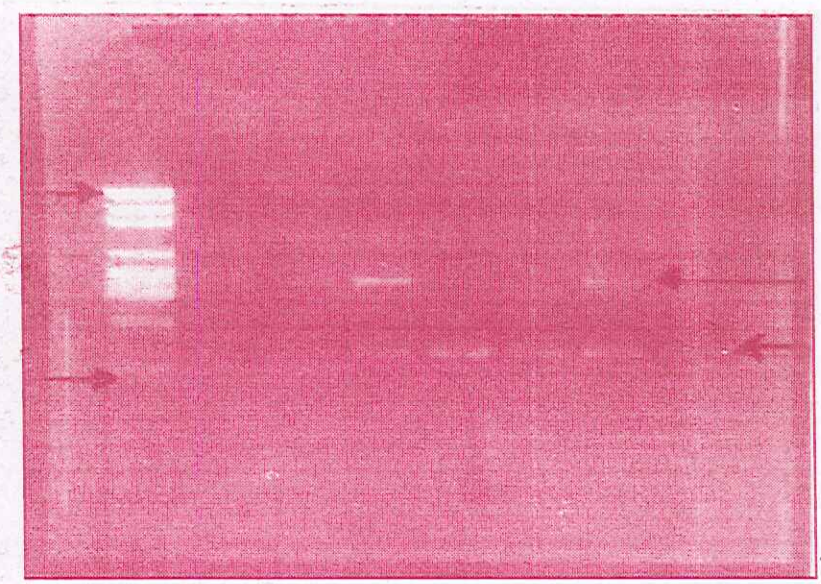

**Fig. (1) :** A photograph of agarose gel electrophoresis of PCR amplification products of the Alu TPA - 25 locus.
**Lane 1:** Bands of the DNA marker 8 (extending from 1116 bp to 110 bp).
**Lane 2:** Allele 1 (100 bp) of the legal father (a).
**Lane 3:** Allele 1 (100 bp) of the mother (a).
**Lane 4:** Allele 1 (100 bp) of the child (a).
**Lane 5:** Alleles 1 & 2 (100 bp & 400 bp) of the child (b).
**Lane 6:** Allele 2 (400 bp) of the legal father (b).
**Lane 7:** Allele 1 (100 bp) of the mother (b).
**Lane 8:** Allele 1 (100 bp) of the alleged father.

These bands provide differentiation between the alleged father (lane 8) and the child (b) (lane 5). The child was heterozygous for Alu TPA - 25 alleles 1 & 2 (100 bp & 400 bp) while the alleged father and the mother (b) were homozygous for allele 1 (100 bp) (Direct exclusion by class Ia).

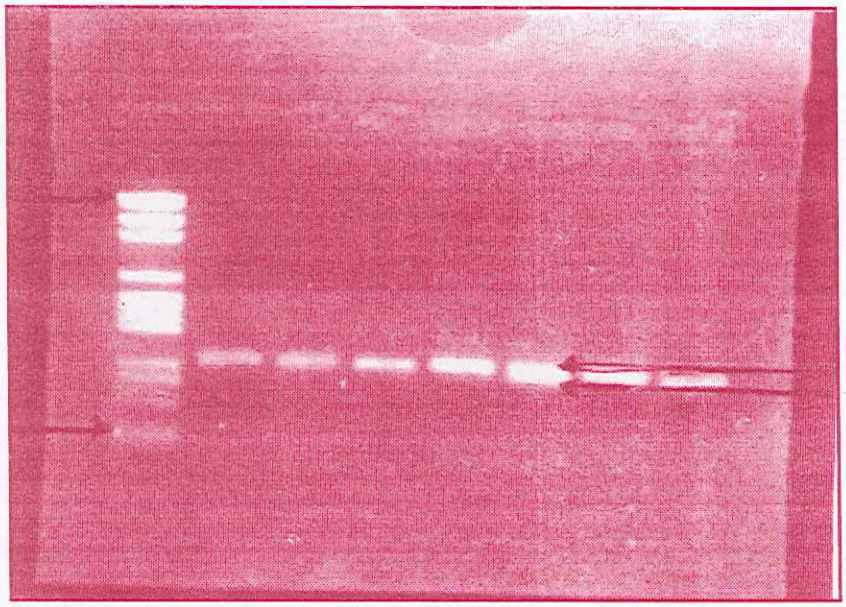

**Fig. (2) :** A photograph of agarose gel electrophoresis of PCR amplification product of STR HUMFES / FPS locus.
**Lane 1:** Bands of the G174A PGEM DNA marker (from 2645 bp to 36 bp).
**Lane 2:** Allele 11 (222 bp) of the legal father (a).
**Lane 3:** Allele 11 (222 bp) of the child (a).
**Lane 4:** Allele 11 (222 bp) of the mother (a).
**Lane 5:** Allele 13 (230 bp) of the alleged father.
**Lane 6:** Alleles 11 & 13 (222 bp & 230 bp) of the child (b).
**Lane 7:** Allele 11 (222 bp) of the mother (b).
**Lane 8:** Allele 11 (222 bp) of the legal father (b).

These bands provide differentiation between the legal father b (lane 8) and the child b (lane 6). The child is heterozygous for HUMFES / FES alleles 11 & 13 (222 bp & 230 bp) while the legal father and the legal mother are homozygous for allele 11 (222 bp) (Direct exclusion by class Ia).

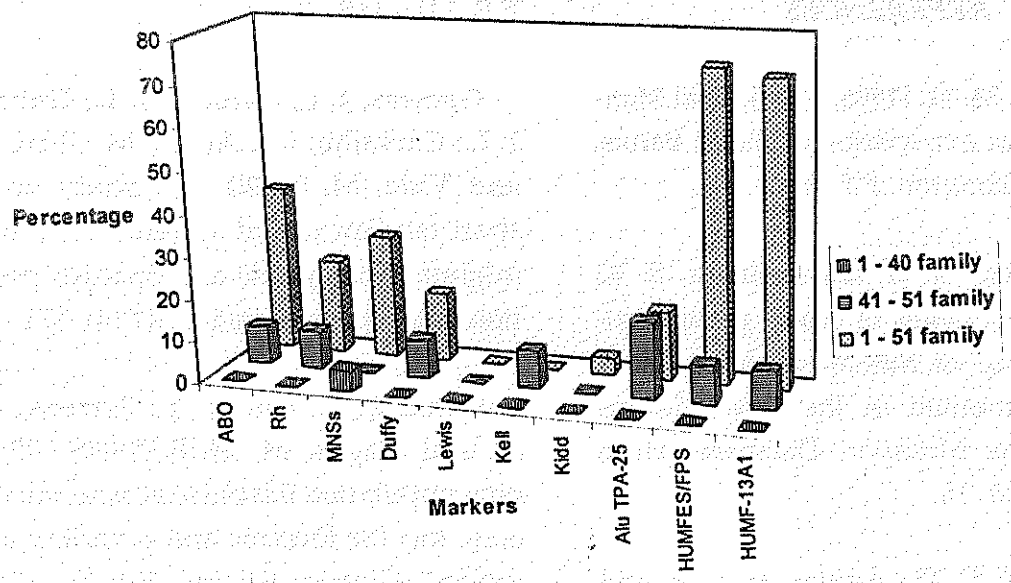


**Fig. (3) :** Percentages of paternity exclusions by blood groups and DNA loci.

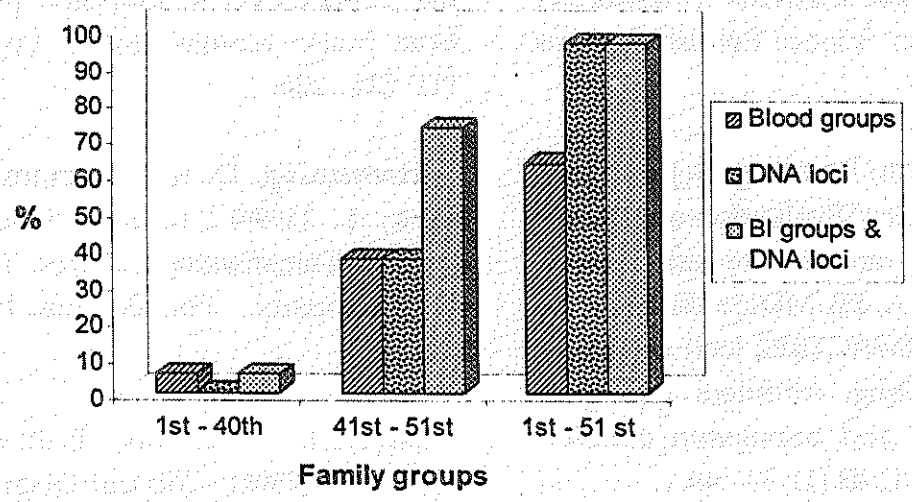


**Fig. (4) :** Percentages of paternity exclusions by using combined blood groups, DNA loci, and combined blood groups & DNA loci.

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# تقييم دور فصائل الدم وثلاث دلالات للحامض النووى فى إختبارات نسب الأبوة فى عينة من المصريين

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من المسلم به أن الاختبارات المعملية لفصائل الدم ودلالات الحامض النووى تلعب الدور الجوهرى فى حل نزاع نسب الأبوة، ولقد أجريت الدراسة الحالية لتقييم دور سبعة فصائل للدم (ABO, Rh, MNSs, Duffy, Lewis, Kell, Kidd) وثلاث دلالات للحامض النووى (Alu TPA - 25, HUMFES / FPS, HUMF13A1) فى عينة من المصريين مكونة من إحدى وخمسين أسرة : أربعون منها ليس لديها أى شك فى أبوة الأب الشرعى للإبن بينما الإحدى عشرة الأخرى محل نزاع حول نسب الأبوة، وتم فحص عينات من دماء كل من الأم والإبن والأب الشرعى بالنسبة لفصائل الدم ودلالات الحامض النووى المختارة، ولمعرفة كفاءة الدلالات الوراثية السابقة فى نفى أو إثبات الأبوة تم فحص دماء عشرة رجال من نفس الوسط لايمتون بصلة قرابة للأسر قيد الفحص باعتبارهم كآباء غير شرعيين حيث تم توزيع نتائج فحصهم عشوائياً بمعدل نتائج فحص رجل غريب واحد لكل خمس أو ست أسر ليمثلوا مجموعة معبرة عن إحتمال ١٠٠٪ لعدم شرعية الأبوة.

وقد تم تحديد فصائل الدم باستخدام إختبار التلزن، وتعيين دلالات الحامض النووى باستخدام تفاعل البلمرة المتسلسل والهجرة الكهربائية على مادة الأجاروز جل، ومن ذلك تم تحديد الطرز المظهرية والجينية للأمهات والأبناء والآباء الشرعيين والرجال الغرباء واستنتاج تكرار الجينات، بالإضافة إلى نفى الأبوة أو إستنتاج إحتمال إثباتها بالنسبة للآباء الشرعيين والرجال الغرباء بتطبيق القواعد الوراثية المعروفة لهذه الدلالات.

وقد أظهرت نتائج الدراسة أن دلالات الحامض النووى كانت أكثر كفاءة من فصائل الدم فى نفى الأبوة أو إستنتاج إحتمال إثباتها برغم أن كلتا المجموعتين لم تنجح فى نفى أبوة جميع الرجال الغرباء أو فى إعطاء قيم يعول عليها فى إحتمال إثبات الأبوة لمن لم يكن نفى أبوته سواء بالنسبة للآباء الشرعيين أو الرجال الغرباء، وكانت فصائل الدم (Lewis, Kell, Kidd) هى الأقل فائدة فى إختبارات الأبوة بينما كانت دلالات الحامض النووى (HUMFES / FPS, HUMF13A1) الأكثر فائدة. وقد لوحظ أن بعض الرجال الغرباء تم نفى أبوتهم بواسطة

عامل وراثى واحد فقط من بين الدلالات محل الدراسة مما يرفع من أهمية هذا النفى فى الحالات العملية ويستدعى تمحيص مدى التعويل عليه بالإضافة إلى ضرورة فحص عوامل وراثية إضافية.

يستنتج من هذه الدراسة أن مجموعة فصائل الدم ودلالات الحامض النووى التى تم إختيارها غير كافية لنفى الأبوة أو إستنتاج إحتمال إثباتها بطريقة مؤكدة فى جميع الحالات، كما أن الدلالات الوراثية المتعددة الچينات هى الأفضل فى إختبارات الأبوة. بناء على ذلك، فإنه فى مجموعة معينة من الناس، يجب أن تعتمد إختبارات الأبوة على عدد كافى من أكثر الدلالات الوراثية فائدة، كما يجب وضع قواعد منظمة لمقاييس نفى أو إثبات الأبوة، بالإضافة إلى إتباع معايير الجودة بصورة ملزمة بالنسبة للمعامل التى تجرى إختبارات تحديد نسب الأبوة.