GENETIC VARIATION IN THE DNA PROFILING BETWEEN SMALL POPULATION SAMPLES OF EGYPT AND UINTED ARAB EMIRATES

BY

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ABSTRACT

Short tandem repeats (STRs) are most extensively used for elucidating the genetic variation of human populations because of their abundance and prevalence in the genome. A lot of studies have been reported for autosomal STR allele frequencies for a number of different ethnic groups, however, published data for Arabs are limited. Therefore, it is desirable to establish the population DNA databases for Egypt and United Arab Emirates in general and to determine the extent of genetic variation between the allele frequencies of the two Arabic populations based on the analysis of different STR loci. In this study the Powerplex 1.2 system was used which is able to detect eight different tetrameric STR loci plus the amelogenin locus. DNA extraction was done from blood samples of 200 unrelated Egyptian volunteers and 200 unrelated Emirate volunteers. The coamplification of STRs loci was performed according to the manufacturer's instructions and DNA typing was done in Abu Dhabi by using ABI 310 automated sequencer. Allele frequencies, forensic and paternity parameters were calculated. The two populations were compared by F-statistic estimation. The results showed that from the eight STR loci 60 and 63 different alleles were found in the Egyptian and UAE respectively, their frequencies were similar in the two populations. The data showed that none of the Egyptian population observed a significant deviation from Hardy-Weinberg Equilibrium (HWE). However, two loci in the UAE population displayed significant deviation from HWE. The result of this study confirmed that these STR loci are suitable for forensic and paternity testing in the Egyptian and UAE populations. There was little evidence of genetic differences between the two populations. Therefore one large database for these loci would be sufficient for the two populations. However, more studies for other loci are recommended between large samples of the two countries and also between other Arabic countries to confirm if one large Arabic DNA data could be enough for Arab or would it be necessary to set up one database for each Arabic country.

INTRODUCTION

Several methods of DNA typing have been used in forensic applications including analysis of variable number of tandem repeats (VNTRs), amplified fragment

length polymorphisms (AmpFLPs) and short tandem repeats (STRs). STRs have proven to be the markers of choice in the field of paternity testing and in human identification (Rostedt et al., 1996). There are several advantages to utilizing STRs over the other systems. STR analysis unlike VNTR analysis uses PCR amplification and therefore requires less DNA (Lorente et al., 1994). Alleles of STRs are discrete and can be sized precisely so complex interpretation as used in VNTR systems is not needed, making interpretation of results easier (Gill et al., 1990). In addition STR analysis has lower costs, greater speed and generally amenable to automation (Fregeau and Fourney, 1993).

Moreover, the analysis of STR loci by automated fluorescence has become the method of choice for use in routine forensic investigation and paternity testing (Ziegle et al., 1992).

There are approximately 400 million STR loci in the human genome including di, tri and tetranucleotide loci (Edwards et al., 1991). Tetranucleotide loci are attractive for forensic and paternity analysis because of the low rate of artifacts generated by repeat slippage, which are common features with dinucleotide repeat loci (Beckman and Weber, 1992). There are several multiplexing kits available in which amplification of several loci can be achieved in a single tube reaction, with different dye labels on the primers allowing the identification of alleles at different loci even when they overlap in size. Simultaneous amplification decreases the cost and time of determining genotypes in population studies on several loci. Additionally, less DNA sample is consumed than when analysing each locus independently and fewer reagents are required (Fregeau and Fourney, 1993).

A lot of studies have been reported for autosomal STR allele frequencies for a number of different ethnic groups, however, published data for Arabs are limited. Therefore, it is desirable to establish the population DNA databases for Egypt and United Arab Emirates in general and to determine the extent of genetic variation between the two Arabic populations based on the analysis of different STR loci. In this study the Powerplex 1.2 system was used which is able to detect eight different tetrameric STR loci plus the amelogenin locus.

SUBJECTS & METHODS

Subjects :

Two hundred fresh blood samples from unrelated Egyptian volunteers were collected from Cairo (100 samples) and El-Minia cities (100 samples), and another 200 samples from UAE were collected from two cities, Abu Dhabi (100 samples) and Sharjah (100 samples). The age of

Egyptian and Emirates volunteers ranged from 15 to 65 years, half of them were males and the other half were females. Blood was collected in 5 ml EDTA tubes.

Methods :

Both extraction and Polymerase Chain Reaction (PCR) were done in the department of biochemistry, faculty of medicine, El-Minia university.

Extraction of DNA :

DNA was extracted from blood by phenol-chloroform extraction followed by ethanol precipitation (Sambrook et al., 1989). The concentration of the resuspended genomic DNA was quantitated by resolution on a 0.4% agarose gel electrophoresis. Molecular weight standard samples of known quantity and quality run along side the unknown samples to provide a comparison. The DNA in the gels were visualised under UV light (transilluminator 312 nm UltraViolet, Specronics Corporation, Westbury, NY, USA). Gels were photographed using a Polaroid camera and the amount of DNA could be estimated by comparison with the molecular weight standard.

Polymerase Chain Reaction (PCR) :

The GenePrint™ 1.2 system kit (promega company) which was used to coamplify eight simple tetrameric STR loci. These loci are the following D5S818, D13S317, D7S820, D16S539, VWA, THO1, TPOX and CSF1PO plus a sex determining locus, which is the amelogenin.

The Co-amplification of STR loci was performed according to the manufacturer's instructions. Each sample contained 5.7 µl of distilled water, 0.8 µl Gold STR 10X buffer, 0.8 µl PowerPlex 1.2 10X Primer Pair Mix, 0.2 µl AmpliTag Gold DNA Plymerase and 0.5-2.5 (2-5 ng) diluted DNA was added to each reaction mixture. All tubes were placed in the 840 thermal cycle.

The PCR cycling program carried out as the following: 11 min at 95°C followed by 10 cycles at 96°C for 1 min, then at 94°C for 30 s, then at 60°C hold for 30 s followed by 45 s at 70°C. This was followed by 22 cycles of 90 °C for 30 s, then at 60°C for 30 s, after that at 70°C for 45 s and finally 60°C for 30 min.

DNA Typing :

DNA typing was carried by using the ABI PRISM 310 automated sequencer in criminal investigation center, Abu-Dhabi. There are several different dye sets can be used in different types of experiments. In this study a set of three different fluorescent dyes were used and these dyes are tetramethylrhodamine TMRA (yellow), fluorescein (blue) and carboxy-X-rhodamine (CXR) (red). Loading cocktail was prepared by combining and mixing 1µl of the fluorescent internal lane stan-

dard containing CXR labelled fragments included with the kit with 17 µl of deionised formamide in 0.5 ml tube. 2 µl of PCR product were mixed with 18 µl of the loading cocktail. 2 µl of PowePlex 1.2 allelic ladder mix were also mixed with 18 µl of the loading cocktail. The samples were denatured at 95°C for 3 min and immediately chilled on ice for 3 min. Capillary electrophoresis was carried out by using the automated ABI PRISM 310 genetic analyser.

Data was collected (raw data using the ABI PRISM 310 collection software application, version 1.0.2, with the GeneScan module GS POP4 A). The size of DNA fragments was determined by using 310 GeneScan analysis software application, version 2.0.2 by comparing them to fragments contained in a size standard (Table, 1).

The allele frequencies from the two populations were calculated. The forensic and paternity parameters including matching probability (MP), power of discrimination (PD), typical paternity index (TPI) and probability of exclusion (PE) were calculated for each locus. The standard Chi square test was used to test the data for Hardy -Weinberg Equilibrium (HWE) and for heterozygosity. The FST (co-ancestry coefficient) was used to test the presence of genetic variation between the two populations.

Table (1): The allelic ladder information of loci included in the GenePrint™ 1.2 kit.

STR locus	Fluorescent Label	Size range of allelic ladder components (bp)	Repeat numbers of allelic ladder components	Repeat numbers of allelic not present in ladder
Amelogenin	TMR	209.5 (X) 215.8 (Y)	-	none
D5S818	Fluorescein	112-145	7-15	none
D13S317	Fluorescein	168-200	7-15	none
D7S820	Fluorescein	211-243	6-14	none
D16S539	Fluorescein	261-301	5, 8-15	none
VWA	TMR	126-166	11, 13-21	none
THO1	TMR	176-200	5-11	8.3, 9.3
TPOX	TMR	222-250	6-13	none
CSF1PO	TMR	291-327	6-15	none

Fluorescein = 5 -terminal fluorescein label (blue), TMR = 5 -terminal carboxy-tetramethylrhodamine label (yellow). X= female, Y= male

Statistical Analysis :

The analysis was performed using GDA (Lewis and Zaykin, 1999), TFPGA (Miller, 1998), and PowerStats (Tereba, 1999) software programmes.

RESULTS

The DNA was extraced from 400 individuals from the two populations. The quality and quantity of extracted DNA were estimated by using mini-gel electrophoresis as shown in figure (1). All samples were amplified successfully as a co-amplification of eight tetranucleotide repeats STR loci plus amelogenin locus in a single test tube via PCR technique (Figs. 2a&b).

The allele frequencies for each population was counted from the genotypes observed at eight STR loci and divided by the total number of all alleles for each population. The distributions of observed allelic frequencies for the eight STR loci in the two population samples are shown in table (2).

The forensic and paternity parameters were calculated in both sample populations (Table, 3). Possible departures from Hardy-Weinberg Equilibrium (HWE) were determined and there was agreement between the observed and expected genotype values under HWE for Egyptian population and deviation was observed at two loci only in the UAE population as shown in table (4).

The observed and expected heterozygosity were calculated. The standard deviation and the p value from the chi-square test were calculated. and the results showed that the expected number of heterozygosity was not significantly different from the observed ones (Table, 5).

The F-statistics (F_{ST}) was used to test the presence of variation between the two populations. The overall value of FST over the eight loci was (0.0032). The bootstrapping over the eight STR loci for two populations was applied to generate 95% confidence intervals. The results are shown in table (6).

DISCUSSION

The Arabs in general are believed to be genetically diverse. Major factors that contributed to their diversity include the migrations of Semitic tribes from the Arabian Peninsula, the Islamic expansion in the 7th century AD, the Crusade wars and the recent migration dynamics. These events have resulted in the admixture of the original Arabs with other populations extending from east and south Asia to Europe and Africa (Teebi and Saeed, 2005). Therefore, it is essential to establish if one general DNA database for the Arab would be sufficient or would it be necessary to set up one database for each Arabic country.

This study was performed on 400 fresh blood samples that were randomly selected to compare between the DNA profiling of two Arabic countries, Egypt and UAE. In general, 100 to 200 unrelated individuals (200-400 alleles) are usually recommended to be collected for each DNA system to assess allele frequencies (ISFH, 1992). The coamplification of the nine STR loci was performed using the PowerPlex 1.2 system according to the manufacturer's instructions, except that the PCR was carried out in one third of the recommended total reaction volume containing 2-5 ng template DNA.

All STR loci are simple type except VWA, which is a compound. Simple STR systems are in general, the most suitable loci for comparison results between laboratories (Kimpton et al., 1995). Out of eight STR loci, six of them (TPOX, THO1, VWA, D5S818, D7S820 AND D13S317) plus amelogenin locus have DNA fragment sizes ≤ 250 base pairs which is very important aspect of forensic casework (Gill et al., 1996). A very small sizes of these eight STR loci made them more likely to be successful on old or severely degraded material (Schumm et al., 1993).

From the eight STR loci 60 and 63 different alleles were found in the Egyptian and UAE respectively. The possible number of different combinations of genotypes are 2.07 x 1012, 8.33 x 1011 in the UAE and Egyptian populations respectively. The most common alleles at each of the 8 STR loci were shared between the two populations. The presence of an almost similar allele frequency pattern suggests that these populations might have a common ancestry or probably experienced very high gene flow during the period of their coexistence (Tandon et al., 2002).

The data showed that none of the samples from the Egyptian population observed a significant deviation from HWE. However, two loci (D5S818, THO1) in the UAE population displayed significant deviation from HWE. Although it was not highly significant at D5S818 locus (0.042). Several studies have been reported such as for locus VWA in British population (Drozed et al., 1994) and Russian population (Sajantila et al., 1994) where deviations were observed.

The result of this study confirmed that these STR loci were found suitable for forensic and paternity testing in the Egyptian and UAE populations. The PowerPlex 1.2 system offered improved forensic and paternity parameters over the Quadruplex system (Gill et al., 1995). By using this kit the power of discrimination was reached higher or equivalent to the second generation multiplex (SGM)(Sparkes et al., 1996a, b). However, unlike the SGM system this kit does not contain any of hypervariable or compound loci such as D21S11.

The combined typical paternity index for the eight STR loci in the two populations was higher than the combined paternity index for both CTTv and FFF1 multiplexes in Hispanic American (Lins et al., 1998). The combined probability of exclusion for these loci was higher than other multiplexes as CTTv, GammaSTR and FFFL multiplexes in all American populations (Lins et al., 1998). It was recommended for number of markers to be used in paternity analysis to have a power of exclusion of ≥ 99% (Hammond et al., 1994). However, the English speaking working group (ESWG) reported that the majority of the European laboratories used values in the range of probability of paternity (99.9-99.999%) (Hallenberg and Morling, 2001). Therefor, using the PowePlex 16 system would give better forensic and paternity parameters than other systems (Sprecher et al., 2000).

The interpretation of FST was suggested by Wright (1978) as follows: the range of 0 to 0.05 considered as indicating little genetic differentiation between tested populations. The range 0.05 to 0.15 indicates moderate genetic differentiation. The range 0.15 to 0.25 indicates great genetic differentiation and the values above 0.25 indicate very great genetic differentiation. The value of FST between Egypt and UAE was 0.003 which indicates that there is very little genetic differentiation between these two Arabic populations.

In other reports no significant differences were found between Egyptian and Yemenian Arab populations for many loci (Klintschar et al., 1998a,b,c). Therefore, it could be assumed that the Egyptian database can be used for Arabs of unknown or foreign origin in the absence of population-specific databases without exerting a significant bias on the biostatistical interpretation. However, more studies for other loci are recommended between the two countries and also between other Arabic countries to confirm if one large Arabic DNA data could be enough for Arab populations.

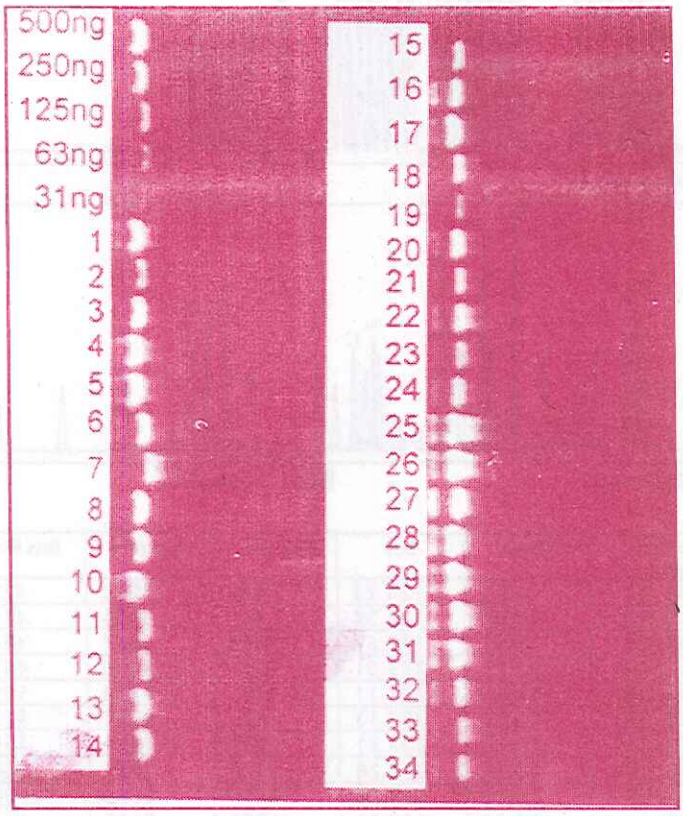
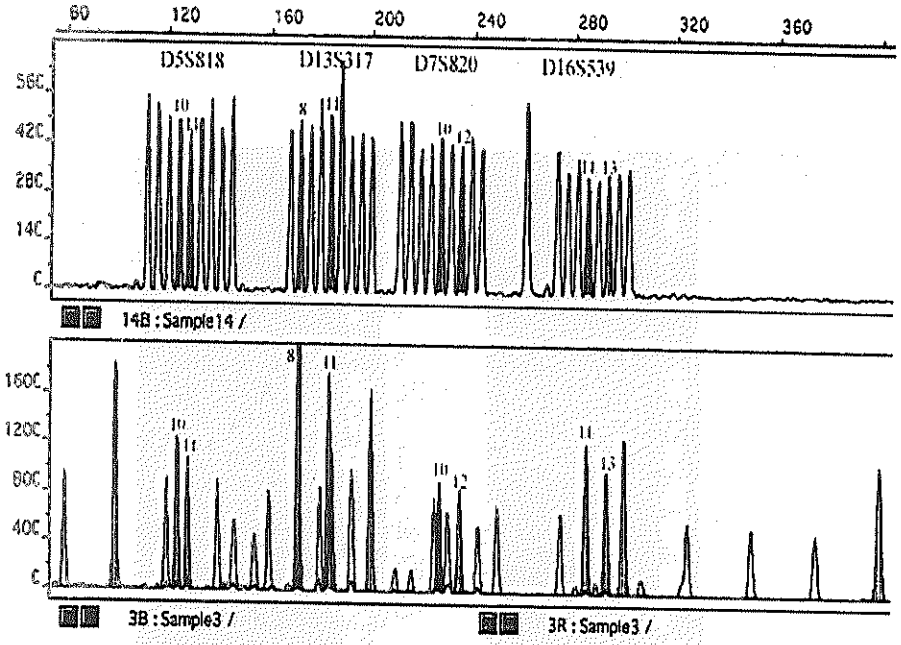
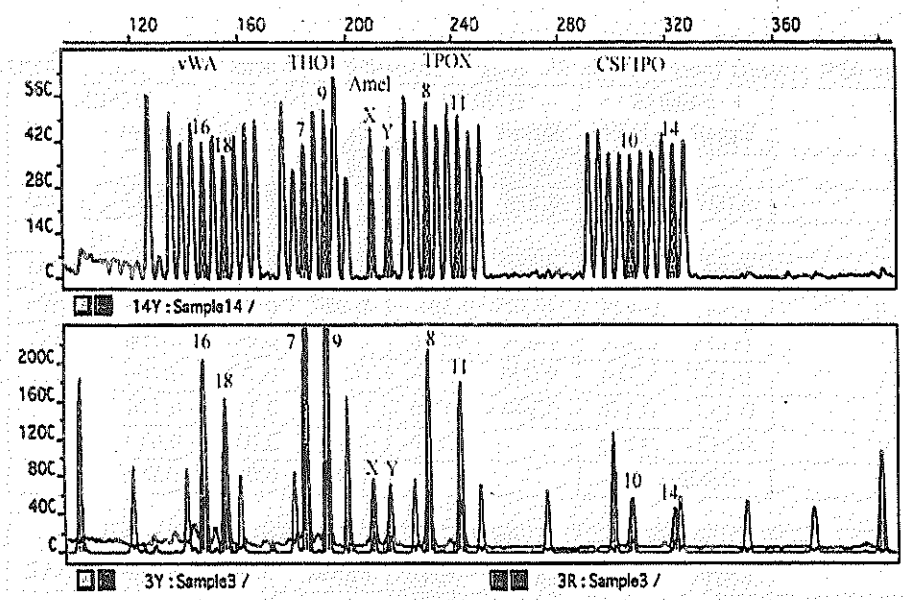


Fig. (1) : Showing the yield of DNA from blood samples. The extracted DNA of unknown quantity was compared to a serial dilution of known quantity of standard DNA (500 ng, 250 ng, 125 ng, 63 ng and 31 ng).



Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
14B, 67	15.15	124.23	488	4060	4132
14B, 68	15.30	128.37	458	3981	4172
14B, 74	16.81	171.67	489	4277	4584
14B, 77	17.23	183.61	511	4570	4697
14B, 86	18.69	227.11	445	4529	5097
14B, 88	18.96	235.13	426	4252	5169
14B, 95	20.57	285.21	339	3484	5609
14B, 97	20.83	293.28	348	3637	5680
3B, 43	14.70	124.18	1258	9793	4009
3B, 44	14.85	128.37	1095	8615	4048
3B, 48	16.30	171.52	2486	23374	4444
3B, 50	16.69	183.42	1794	17893	4552
3B, 52	18.11	227.08	913	8787	4938
3B, 54	18.37	235.18	843	8177	5008
3B, 57	19.92	285.11	1258	12415	5432
3B, 59	20.17	293.01	1004	10111	5499

Fig. (2a) : Electrophoresis profile of a single DNA sample.
The fluorescein labeled loci (D5S818, D13S317, D7S820 and D16S339) are displayed. The allelic ladders for all loci are included. The fluorescent ladder (CXR), 60-400 bases is shown in red and is used as the internal size standard.



Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
14Y, 65	15.94	146.44	419	4219	4345
14Y, 67	16.21	154.48	378	3890	4421
14Y, 73	17.24	184.14	415	4532	4702
14Y, 75	17.52	192.04	520	5626	4776
14Y, 78	18.11	209.47	469	4480	4937
14Y, 79	18.32	215.83	406	3808	4995
14Y, 82	18.80	230.22	543	5554	5125
14Y, 85	19.19	242.20	502	5053	5232
14Y, 92	21.27	307.14	378	4048	5801
14Y, 96	21.78	323.12	412	4685	5938
3Y, 97	15.46	146.32	2062	28747	4214
3Y, 99	15.73	154.40	1650	25240	4288
3Y, 117	16.73	184.41	3303	42064	4561
3Y, 119	16.99	192.29	2887	38574	4632
3Y, 129	17.55	209.52	792	11321	4785
3Y, 131	17.75	215.80	729	10992	4840
3Y, 137	18.21	230.31	2187	27616	4966
3Y, 140	18.59	242.28	1816	22367	5069
3Y, 192	20.60	307.09	588	10025	5617
3Y, 201	21.08	323.03	469	7206	5748

Fig. (2b) : Electrophoresis profile of a single DNA sample.
The TMR labeled loci (VWA, THO1, Amelogenin, TPOX and CSF1PO) are displayed. The allelic ladders for all loci are included. The fluorescent ladder (CXR), 60-400 bases is shown in red and is used as the internal size standard.

Table (2): Allele frequencies for eight loci for Egypt and UAE populations.

Allele	D5S818		D7S820		D13S317		D16S539	
	Egypt	UAE	Egypt	UAE	Egypt	UAE	Egypt	UAE
7			0.021	0.02	0.004	-		
8	0.021	0.023	0.175	0.181	0.157	0.153	0.033	0.052
8.3			-	0.004				
9	0.07	0.039	0.092	0.109	0.054	0.037	0.185	0.162
10	0.141	0.124	0.333	0.301	0.087	0.074	0.069	0.094
11	0.174	0.238	0.279	0.216	0.285	0.279	0.306	0.347
12	0.393	0.384	0.096	0.138	0.289	0.308	0.266	0.183
13	0.182	0.186	0.004	0.026	0.083	0.114	0.125	0.153
14	0.012	0.015	-	0.004	0.037	0.033	0.013	0.008
15	0.008	-			0.004	0.002	0.004	0.002

Allele	VWA		THO1		TPOX		CSF1PO	
	Egypt	UAE	Egypt	UAE	Egypt	UAE	Egypt	UAE
6			0.203	0.284	-	0.011		
7			0.231	0.203	-	0.002	0.011	0.002
8			0.111	0.146	0.508	0.448	0.023	0.004
9			0.322	0.207	0.186	0.118	0.021	0.035
9.3			0.103	0.144				
10			0.029	0.013	0.066	0.111	0.267	0.282
11			-	0.002	0.215	0.275	0.352	0.306
12					0.025	0.035	0.264	0.297
13	0.004	0.002					0.050	0.059
14	0.078	0.037					0.013	0.011
15	0.161	0.106					-	-
16	0.252	0.304					-	0.002
17	0.252	0.322						
18	0.144	0.165						
19	0.083	0.057						
20	0.021	0.008						
21	0.004	-						

Table (3): Forensic and Paternity Parameters for the eight STR loci.

Egypt Pop.	MP	PD	TPI	PE
D5S818	0.093	0.907	1.89	0.486
D7S820	0.096	0.904	1.94	0.496
D13S317	0.087	0.913	2.63	0.618
D16S539	0.084	0.916	2.00	0.510
VWA	0.063	0.937	2.42	0.587
THO1	0.087	0.913	1.89	0.485
TPOX	0.170	0.830	1.32	0.315
CSF1PO	0.120	0.880	1.61	0.412
Combined	0.000000007	0.999999993	188	0.996

UAE Pop.	MP	PD	TPI	PE
D5S818	0.104	0.896	1.66	0.426
D7S820	0.069	0.931	2.08	0.527
D13S317	0.086	0.914	2.34	0.573
D16S539	0.078	0.922	2.16	0.425
VWA	0.095	0.905	1.62	0.415
THO1	0.075	0.925	2.12	0.534
TPOX	0.140	0.860	1.68	0.433
CSF1PO	0.126	0.874	2.06	0.523
Combined	0.000000006	0.999999994	207	0.996

MP= Matching Probability, PD= Power of Discrimination, TPI= Typical Paternity Index and PE= Probability of Exclusion.

Table (4) : *P* value of chi-square .

Egypt Pop.	X^2	Df	*P*
D5S818	34.4	28	0.188
D7S820	21.7	21	0.474
D13S317	41.3	36	0.250
D16S539	20.2	28	0.857
VWA	32.8	36	0.62
THO1	18.5	15	0.238
TPOX	9.7	10	0.466
CSF1PO	20.8	28	0.832

UAE Pop.	X^2	Df	*P*
D5S818	33.4	21	0.042*
D7S820	48.1	36	0.086
D13S317	21.9	28	0.787
D16S539	23.6	28	0.701
VWA	32.3	28	0.261
THO1	35.4	21	0.025*
TPOX	24.2	21	0.285
CSF1PO	48.9	36	0.075

*P ≤0.05 is significant p > 0.05 is insignificant

Table (5) : The observed and expected heterozygosity at eight STR loci in Egypt and UAE.

Locus	Egypt		UAE	
	Observed	Expected±SD	Observed	Expected±SD
D5S818	0.736	0.757±0.039	0.699	0.743±0.029
D7S820	0.742	0.762±0.039	0.760	0.798±0.027
D13S317	0.810	0.791±0.037	0.786	0.783±0.027
D16S539	0.750	0.779±0.038	0.769	0.761±0.027
VWA	0.793	0.811±0.036	0.693	0.792±0.028
THO1	0.736	0.778±0.038	0.764	0.793±0.027
TPOX	0.612	0.656±0.043	0.703	0.696±0.031
CSF1PO	0.670	0.732±0.041	0.756	0.733±0.029

SD= Standard Deviation. No significant differences were detected p > 0.05.

Table (6) : F_{ST} and 95% confidence interval bootstrapping over the eight STR loci in Egypt and UAE.

Compared populations	F_{ST}	Lower bound	Upper bound
Egypt/UAE	0.0031	0.0006	0.0064

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الفرق بين صورة الحمض النووى فى عينة صغيرة من شعبين مصر والإمارات العربية المتحدة

المشتركون فى البحث

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لقد أصبح إستخدام التتابعات الترادفية القصيرة من الوسائل التى يقع عليها الاختيار فى مجال توضيح الفروق الچينية بين الشعوب وذلك لكثرة وجودها فى الجينوم، وقد تم عمل الكثير من الدراسات على تكرار التتابعات الترادفية القصيرة لعدد من التجمعات العرقية المختلفة، ومع ذلك كانت الأبحاث المنشورة فى هذا المجال عن العرب محدودة، ولذلك أردنا فى هذا البحث تكوين قاعدة بيانات لكل من مصر والإمارات العربية المتحدة وتقييم درجة الاختلاف الچينى بين الشعبين معتمدين على تحليل مواقع التتابعات الترادفية القصيرة المختلفة.

وفى هذه الدراسة إستخدمنا نظام "باوربلكس ١٠٢" الذى لديه القدرة على الكشف عن ثمانية مواقع رباعية مختلفة للتتابعات الترادفية القصيرة بالإضافة إلى موقع "أميلوچينين" المسئوول عن تحديد الجنس، تم إستخلاص الحمض النووى من عينات دم ٢٠٠ مصرى لاتربطهم صلة قرابة و ٢٠٠ إماراتى أيضاً لاتربطهم صلة قرابة، تم عمل الإكثار من مواقع التتابعات الترادفية القصيرة تبعاً لتعليمات الشركة المنتجة، وتم تحديد نوع الحمض النووى (ABI 310) باستخدام المسلسل التتابعى الأوتوماتيكى، وقد تم قياس تردد الفرد الچينية، والعوامل التى تستخدم فى مجالات الطب الشرعى وإثبات البنوة، وتم مقارنة نتائج الشعبين باستخدام الطريقة الإحصائية (F).

وقد أظهرت النتائج بعد دراسة الثمانية مواقع للتتابعات الترادفية القصيرة وجود ٦٠ و ٦٣ فردة چينية مختلفة فى كل من المصريين والإماراتيين على التوالى وكان ترددهم متشابه فى الشعبين، ولم تظهر البيانات فى عينات المصريين أى إنحراف ذو دلالة إحصائية عن توازن "هاردى وينبيرج"، ولكن موقعين فى عينات الإماراتيين أظهروا إنحراف ذو دلالة إحصائية عن توازن "هاردى وينبيرج"، وقد أثبتت نتائج البحث أن هذه البيانات مناسبة للاستخدام فى مجالات الطب الشرعى وإثبات النبوة وتوجد دلائل قليلة عن وجود إختلاف بين الشعبين، وعلى ذلك فإن قاعدة بيانات كبيرة يمكن أن تكون كافية للشعبين، ولكن يوصى بإجراء المزيد من الدراسات على عينة شعوب عربية أخرى للتأكد ما إذا كانت قاعدة بيانات واحدة تصلح للعرب جميعاً أم لا.