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Comparing Genotyping of 8 Regions of Short Tandem Repeats (STR) in the Human Genome using DHPLC and Capillary Electrophoresis in People Referring to Forensic Medicine

Research Article

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Abstract

Introduction: STRs are short components of 2-7 pairs which spread through human genome and are used for identity detection. According to researches, loci of STRs having four repetitions (tetra-nucleotide) play a more important role in the system of identity detection. Today, various methods are used for studying loci of STRs and introduction of genetic analyzer machines utilizing capillary electrophoresis with high output power led to a great revolution in this context. Owing to considerable costs of the above method, the aim of the present paper is to use a prevalent and accessible method using multiplex PCR and DHPLC methods which can be used for initial screening due to high accuracy and speed of the machine as well as low cost of the method. In addition, in this study, determination of the number of alleles as well as statistical indicators of identity detection for clients of the forensics of the Semnan Province was conducted using two above methods.
Materials and Methods: In this work, 8 loci of STR including 6 loci from the set of 13 loci of FBI (CSF1PO, VWA, D18S51, TPOX, Amelogenin, FGA) and loci SE33 of the international German database (GCL) and an important mini-STR introduced recently are selected based on size and allele abundance. Primers of the above STRs are prepared from GenBank and the quad-STR multiplex system was designed. Then, 20 blood samples were taken from clients of the forensics of Semnan Province in a random manner. First, adjusted quad multiplexes of the intended loci were prepared and PCR results were studied on 3% Agarose gel. Then, the PCR product was analyzed using DHPLC machine. At the same time, blood samples were sent to the forensics of Mashhad for determination of the genetic profile by capillary electrophoresis machine as golden standard.
Results: Multiplex PCR technique was optimized by means of the concentration of primer, temperature and concentration of magnesium. In results, the efficiency of DHPLC was improved using temperature and concentration gradient. A non-denaturing method in 50-60ºC temperature range was used by a 2ºC increase per injection was used for optimization of the conditions. Then, according to the size and genomes of loci, genotype was determined. Results were compared to capillary electrophoresis and from six comparable loci, in four loci; TOPX, Amelogenin, CSF1PO and D18S5, chi-square statistical analysis results were not statistically significant ($p < 0.05$) and in two loci FGA and VWA, significant results were obtained.
Conclusion: Results revealed that DHPLC method can be used as a screening method for preparation of genetic profiles of STRs, TOPX, Amelogenin, CSF1PO and D18S51 in identity detection of people.

Keywords: STR; PCR Multiplex; DHPLC; Capillary Electrophoresis; Identity Detection.

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Introduction

Forensic DNA testing is very important in order to solve some criminal cases and rigor and accuracy of DNA testing are vital [1]. According to the previous researches, the short sequence repeats (STR) are short 2 to 7 bp components scattered throughout the human genome. Today, several STR loci have been identified to determine the genetic profile of individuals, which are used in legal genetic laboratories to identify their identities [2]. These sequences are highly polymorphic and can easily be amplified by PCR method [3]. The small size of these fragments (200bp) makes it possible to analyze them from very small amounts of DNA and the degraded sample. Therefore, we can use the investigation in the above areas as a sensitive and accurate method for selection in the identification of persons [4]. The analysis time of STR Typing and the cost of conducting the test are important and substantial considerations [1]. By investigating the recent developments, whether in the field of promoting conventional executive methods and in the manufacturing new equipment, new and previous methods in different areas and stages of this process should be inevitably compared. Today, a great change in the legal DNA testing has been done on a large scale by manufacturing Genetic Analyzer devices using capillary electrophoresis with high output power. But expensive prices of these devices and its needed laboratory kits Led to further studies on the use of other methods, one of which is DHPLC.

Aim of the Research/Research Questions

The aim of this study was to evaluate the accuracy of STR Typing results using DHPLC method. For this purpose, PCR products are analyzed using DHPLC method in this study.

With respect to the high accuracy and speed of this device and the cheaper price, this method and can be raised as a primary screening method for expensive genetic analyzer devices.

Research Material and Method

Sample Collection

In this study, venous blood samples were randomly taken from 20 unrelated subjects. The blood sample taken from the subjects was divided into two parts, 3 ml of blood in a sterile tube containing EDTA to setup DHPLC method and 5 punches of 1.2 mm of blood were kept on FTA paper of each sample in order to prepare the genetic profile using Electrophoresis Capillary as the gold standard at 20°C.

DNA Extraction and Quantification

DNA extraction was carried out using PrimePrep™ Genomic DNA isolation Kit of GeNet Biocompany. And the concentration of DNA extracted sample was determined by a UV spectrophotometer (Ratio of A260/A280 which is the estimate of DNA purity is 1.7-2).

Locus Selection and Primer Design

Sequences of the eight STR loci (the names and some of their properties are presented in Table 1) were searched and received from STR Base database. According to characteristics such as the polymorphism amount and allelic spectrum in various populations and novelty and based on size and location as well as the allele size range that these markers created after PCR, These loci were divided into two groups of four loci in the multiplex so that there was no overlap in the size of product of any two primers (Figure 1). Primers used in this study are received from A. Odriozola et al.,'s study. Then all the primers were reviewed and approved using Primer-BLAST in terms of the necessary characteristics and mentioned requirements, particularly lack of formation of dimer between different primers in each multiplex and also in terms of specific performance in NCBI Web site.

Table 1. STR Locus Name and Some Characteristics of the STR Loci Under Examination in this Study.

Locus	Genbank accession	Allele Range	PCR Fragment (bp)	Primer Sequence (5–3)	TM
CSF1PO	X14720	6-15	75–111	F: ACTGCCTTCATAGATAGAAGAT R: GCCCTGTTCTAAGTACTTCCT	56.6 59.4
TPOX	M68651	6-16	61–101	F: CTTAGGGAACCCTCACTG R: GCAGCGTTTATTTGCCCAA	64.89 65.99
FGA	M64982	16-51/2	151–293	F: CTCACAGATTAAACTGTAACCA R: TTGTCTGTAATTGCCAGC	58.20 57.96
VWA	M25858	11-24	121–173	F: TCAGTATGTGACTTGGATTGA R: GTAGGTTAGATAGAGATAGGACAGA	55.5 62.5
D18S51	X91254	7-27	213–293	F: GTCTCAGCTACTTGCAGG R: GGAGATGTCTTACAATAACAGTTG	56.11 60.51
SE33	V00481	10-34	450–523	F: AATCTGGGCGACAAGAGTGA R: ACATCTCCCCTACCGCTATA	58.4 58.4
PentaD	AP001752	30-45	400–429	F: GAAGGTCGAAGCTGAAGT R: ATTAGAATTCTTTAATCTG-GACACAAG	57.33 60.75
Amelo-genin	M55418 M55419	X, Y	121,127	F: CCCTGGGCTCTGTAAAGA R: AGGCTTGAGGCCAACCAT	56.1 56.1

Figure 1. Designing Two Sets of Quadriplex PCR Based on Fragments' Size, the Scale of BP. There is no Overlap between the Size of the Product any Two Primers.

PCR Amplification, Electrophoresis and Data Analysis

PCR was performed using a thermal cycler (BioRad -Germany) in DNase and RNase-free vials of 0.2 ml. PCR reactions were performed in several stages. To verify the function, the received primers were first entered in Monoplex PCR reactions and were evaluated in terms of product development. To perform the Monoplex PCR test, PCR vial containing 50 ng of DNA from an individual, PCR 1X buffer, and 1.5 Mm of $MgCl_2$ (for FGA from $MgCl_2$ 2 Mm) were used. Besides, 0.6 mM of each of the primers and 200 mM of dNTP (From Taz- Germany) and a Taq polymerase unit (Native Fermentas, Germany) were used. Eight loci of this study were classified into two groups of four: CSF1PO, VWA, D18S51, PentaD and TPOX, Amelogenin, FGA, SE33 with the changes in temperature conditions, magnesium ion, primer concentration and setting-up Hot Start Multiplex PCR of four markers. The schedule was as follows:

The initial denaturation temperature (95°C for 3 minutes), 35 cycles of denaturation (94°C for 30 seconds), annealing (64°C for 45 seconds), elongation (72°C for 45 seconds) and the final elongation step phase (72°C for 10 min).

PCR product on agarose gel electrophoresis (2.5 %) and products size and the results of Genotypes were determined by the genetic analyzer.

dHPLC Analysis

Non-Denaturing method was used to separate fragments of PCR products. Before injecting the sample into the column, first Buffer A (5% triethylammonium acetate and 95% water) passed through the column for 15 minutes at a speed of 75ml / min until the column was ready for the injection. 10µl of the PCR product of the studied sample was poured in the micro-tubes and was centrifuged a few seconds so that possible fine particles deposit. The related sample was slowly injected into the device and buffers passed the column with an above-mentioned gradient. The column was washed based on the following conditions between the two injections to avoid possible overlap between samples.

1- 5Ml Buffer B (5 % triethylammonium acetate, 25% acetonitrile, and 70% water).
2- 3-5Ml 40-20% acetic acid at a temperature of 60 ° C to column.
3 - Again 5ml buffer B.

Also, the sample injection syringe was washed several times, respectively with 40-20% acetic acid and buffer B and was ready for the next sample injection [5].

The device's harvest percentage from the buffers A and B was determined 65% and 35%, respectively during its total run time. Samples' absorption rate was read at a wavelength of 260 nm after being eluted the column and the pertinent peak curve as well as its staying time in the column was read on the Wave software.

dHPLC Instrumentation

(DHPLC), using a DNAsep Cartridge (Transgenomic® WAVE® System 4500, Glasgow, UK).

Capillary Electrophoresis

Two microliters of each amplified product were analyzed, mixed with 9 µl of Hi-Di™ Formamide (Applied Biosystems®, Foster City, CA, USA) and 0.5µl of GeneScan™ 500 LIZ® Size Standard (Applied Biosystems®, Foster City, CA, USA). Following denaturation of the amplified products (6 min at 95°C), they were cooled (4 min at 4°C) and separated in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems®, Foster City, CA, USA). The results of the electrophoresis were analyzed using version 4.0 Genmapper software (Applied Biosystems®, Foster City, CA, USA).

Statistical Analysis

Comparison of two methods of DHPLC and CE was performed using Chi-square test and other parametric and non-parametric tests.

Research Results/Discussion

To examine the functionality of primers, in the step of primers design, a set of factors such as the melting point, four pairs of primers associated with a QuadruPlex, the size range provided by the alleles after PCR and electrophoresis, and the effectiveness of banding 4 pairs of primers associated with the same quadruPlex were evaluated. Due to these properties, 2 quadruplex systems consisting of eight STR markers: (CSF1PO, VWA, D18S51, PentaD and TPOX, Amelogenin, FGA, SE33) were designed. Then, the markers in a QuadruPlex system were set up. In the following, according to the protocols obtained for Monoplex systems, a protocol was presented for the relevant QuadruPlex protocol systems and it was finally brought to the optimization step with many changes.

Interpretation of Multiplex systems

The results showed that at a temperature of 62°-66° and *1.5 mM* **magnesium ion** concentration, all STR loci studied are detectable as Monoplex PCR. Besides, Multiplex PCR primers results also showed that when the concentration of primers and temperature are measured against a fixed concentration of magnesium,

CSF1PO, and D18S51 loci bands are weaker than desired.

Using common buffer and fixed Magnesium conditions against primer concentration and temperature changes, when Taq polymerase is added to the test tubes at a temperature of 94° C, Multiplex PCR bands are visible desirably (Figures 2 and 3).

DHPLC Results

DHPLC method was used to identify STR loci. As expected, the temperature applied to the column and gradient of buffer concentrations was effective in the work efficiency. Temperature range of 50°C to 60°C with an increase of two centigrades were applied per injection for optimizing conditions and the optimum temperature of 56°C was selected after multiple injections and investigating the resulting peaks. Amplified fragments were eluted from the column based on the band size. Optimized conditions for calculating the size the PCR fragments in DHPLC method (Table 2).

Images 4 and 5 show Quadriplex 1 and 2 , in which it is compared with the standard sample of DHPLC device and its size is mentioned.

Figure 2. QuadruPlex image (1*1) taken from the sample under study. This image has a size marker (Fermentas) with product number 0321 SM on the right side of the gel and the band size is specified at the top of each. Bands are in the expected size range. This gel is Agarose (2.5%).

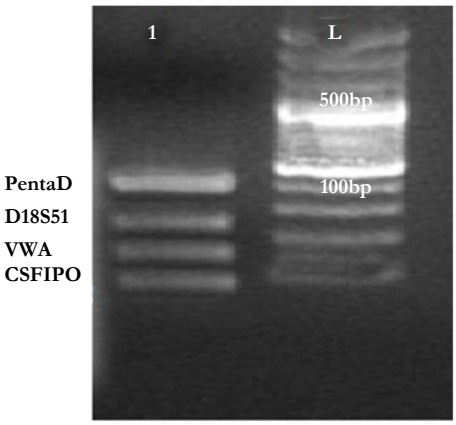


Figure 3. QuadruPlex Image (2*1) in a sample under study.
This image has a size marker (Fermentas) with product number 0371 SM at the right side of the gel, that the size of the bands is identified at the top of each. Bands are within the expected size range. The mentioned gel is agarose (2.5%).

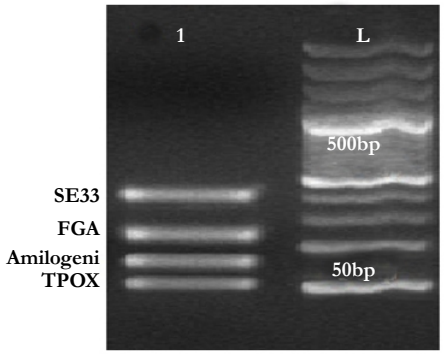


Table 2. Optimized Conditions for Calculating the Size the PCR Fragments in DHPLC Method.

Optimization Steps	Optimized Conditions
Mode	Nondenaturing
Volume of PCR Product	5-10µl
Temperature	55°C
Loading Duration	0.1 min
Loading Drop	5%
Gradient Slope	2% Buffer B /min
Gradient Duration	4 min
Clean Duration	0.5 min
Equilibration Duration	1 min
Flow Rate	0.9 mL/min
Run Time	20 min

Figure 4. DHPLC chromatogram of Quadriplex 1 in one sample. The chromatogram obtained from the injection Quadriplex PCR sample of a chromatogram Quadriplex 1 sample, including four CSF1PO, VWA, D18S51, PentaD loci are specified in the image and compared with the standard and the size of each locus was determined.

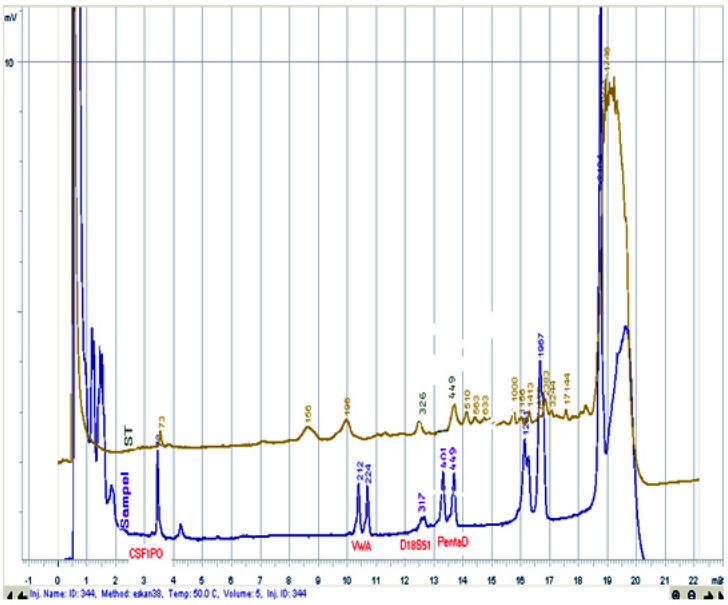
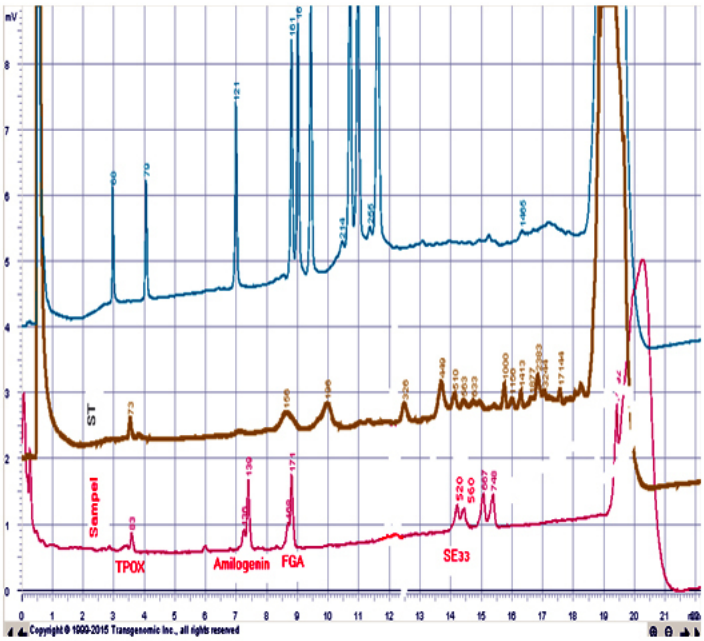


Figure 5. DHPLC chromatogram of Quadriplex 1 in one sample.



Loci are in the expected range. As it is clear, in this sample chromatogram of D18S51, CSF1PO and VWA , PentaD loci are homoduplex and heteroduplex, respectively.

Column temperature of 56°C.
Concentration buffer A = 0.1 M TEAA and buffer of concentration B = 0.1 M TEAA + % 25 Acetonitrile.
Working runtime of 20 minutes at a wavelength of 260 nm.

The chromatogram obtained from the injection of Quadriplex 2 PCR sample, including four *TPOX*, *Amelogenin*, *FGA*, *SE33* loci were specified in the image and were compared with the standard and the size of each locus was determined. Loci are in the expected range. As it is clear, the chromatogram of *TPOX and Amelogenin, FGAa* and *PentaD* loci is homoduplex and heteroduplex, respectively.

Column temperature of 56°C.
Concentration buffer A = 0.1 M TEAA and buffer of concentration B = 0.1 M TEAA + % 25 Acetonitrile.
Working runtime of 20 minutes at a wavelength of 260 nm.

The number of alleles per locus is calculated using the exact size of fragments pieces obtained in the desired locus as follows:

The number of primer nucleotides of any locus is summed up and the obtained number is subtracted from total fragment size reported by the DHPLC device. The obtained number is later

divided by 4 since all the loci have four nucleotides, except PentaD, which had 5 nucleotides, thus it is divided by 5 and the resulting number is considered as individual's alleles number.

Capillary electrophoresis method was used for genotyping of STR loci. Using Genetic Analyzer ABI 3130, capillary electrophoresis was carried out. At the end, chromatogram analysis was performed using the Gene Mapper software (Figure 6).

There was a significant difference between VWA and FGA loci in the results of the above-mentioned methods and no significant difference was observed in other loci. It should be noted that two loci of PentaD and SE33, as was explained in detail in Chapter One, have recently been introduced and have been included in some specialized PCR kits of Promega Co. and applied Biosystems; therefore, they are not analyzed in the capillary electrophoresis system to determine the genetic profile since the Identifier PCR kits, in which and these two loci don't exist.

Considering the outdated nature of some loci evaluated in these kits and the lack of new PCR kits companies, the same 13 loci introduced in 2003 by the FBI are used in forensic identification system of in Iran and new loci are not analyzed. According to the above-mentioned discussion, two loci of PentaD and SE33 were analyzed in this study.

Discussion and Conclusion

As mentioned earlier, DHPLC is a useful tool to separate STR fragments amplified by PCR so that PCR products are separated from each in the chromatography Column and each person's DNA information is identified. According to above discussion on the research needs listed in chapter one, the present study aims to investigate some common STR in the identification process using a new, quick and easy method can be raised as a method of choice for DNA testing in the forensic field. Therefore, in this study, a manual multiplex PCR method was initially set up that consists of 7 loci STR and one amelogenin locus, by using which 8 loci with the size of less than 550 bp can be amplified using PCR in two groups of four loci. This multiplex set covers 6 cases of loci in CODIS system and a Mini STRs as well as SE33 locus that is recently introduced by Germany National Database (GCL) that are included in FBI's total 13 STR standard and can be used as an appropriate system to differentiate persons from each other. The main method of identification by STR loci is capillary electrophoresis method. But one of the problems of this method is the need to very specific materials such as fluorescent primers and expensive multiplex PCR kits so that the cost of doing it is

Figure 6. Genetic Profile Image, shows One Subject under Study in STR loci (CSF1PO, vWA, D18S51, PentaD) with CE Method. Standard size or liz2500 and kit Ladder and Polymer (POP-4) capillary y36 column.

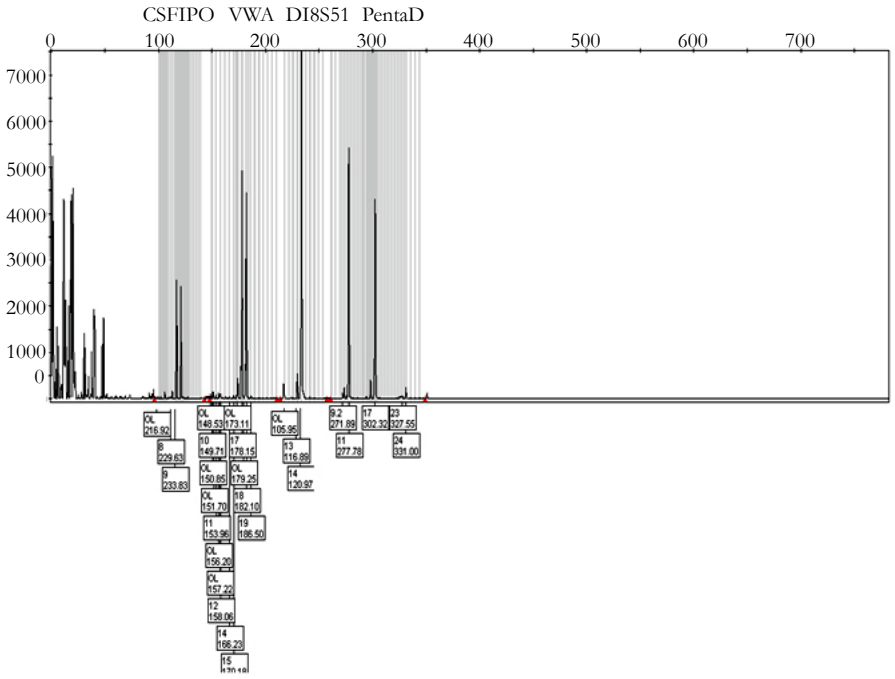


Table 3. Comparison of DHPLC and CE methods in the analysis of studied loci.

Result	CSF1P0		VWA		D18S51		PentaD		TPOX		Amelogenin		FGA		SE33	
parameter	+*	-**	+*	-**	+*	-**	+*	-**	+*	-**	+*	-**	+*	-**	+*	-**
CE	95	5	95	5	95	5	----------		95	5	95	5	95	5	----------	
DHPLC	90	10	50	50	75	25			80	20	90	10	55	45		
P	0.548		0.001		0.077				0.151		0.548		0.003			
X^2	0.360		10.157		3.137				2.0157		0.360		8.533			

* Separation of alleles on DHPLC and CE

** Lack of separation of alleles on DHPLC and CE

very expensive to analyze a person's DNA [6].

DHPLC method

It is necessary to consider several factors in order to optimize DHPLC in method the analysis of STR loci and features and manner of setting up DHPLC devices will be discussed below:

Device mode

Two methods can be used for DNA analysis based on their size according to field surveys and study on DHPLC device Manual [7, 8].

1- Nondenaturing
2- Fully denaturing

In this study, Nondenaturing method was used to improve the sensitivity of PCR products at a temperature of 50°C to calculate the size of the PCR products for the analysis of STR loci. In this regard, in a study the multiplex analyses using DHPLC device, Pental et al., showed that Nondenaturing method was more accurate and easier compared with Fully denaturing [7]. In a study on comparing and sequencing DNA fragments analysis using DHPLC by examining the mode DHPLC's mode, the Nondenaturing method was introduced by Ellis et al., as the best method to separate DNA fragments based on their size [8]. Therefore, the results of the present study were consistent with the results of the above studies and STR loci were clearly distinguished from each other using Nondenaturing method.

Device Column

In this study, the DNAse column, which consists of poly(styrene-divinyl benzene) particles and was in agreement with the method used in Herbert's study, was used to separate STR fragments [9]. In a study, Herbert Oberacher et al., studied some methods to separate oligonucleotides and Xterra column were used in the separation of the oligonucleotide. Since these columns are made from a hybrid combination of small size and large particles, they enhance the separation of the oligonucleotide and thus, was introduced as a powerful method for the high-quality separation of the oligonucleotide. Also, the performance of these columns was compared with that of the capillary electrophoresis device. At the end, polystyrene-benzene columns were considered as the best columns for the isolation of DNA fragments and oligonucleotides and were later used in DHPLC to for STR analysis [3].

PCR product size

According to the manufacturer's recommendations and for DNA fragment analysis, the size of PCR product fragments was considered between 50 to 1000 BP, like A. Odriozola et al.,'s study. Loci with variable sizes of 60Bp to 500bp were selected. Also, according to A.Odriozola et al.,'s study and to prevent overlap in STR loci, loci were selected according to their size and were placed in two quadriplex categories [6]. In a study, A. Odriozola who studied 49bp to 279bp 15 STR as an (I-DNA) set using DHPLC method and alleles range along with the repeated sequences of 15 STR was determined, DHPLC was considered as a technology with high advantages , high speed and low cost of which were among advantages introduced this device as the primary screening for expensive devices [6].

In general, since some parts of the project in A.Odriozola et al's study, including PCR and primer design and PCR product sizes were consistent with the present study, similar results were obtained on the PCR product and DHPLC chromatograms [10]. Also, the results of a study conducted in South Korea showed that the shorter the amplified fragment length, the faster the elusion occurs and the shorter its retention time is at the same temperature.

The results of this study are fully consistent with our findings so that smaller loci were eluted faster than larger loci in the same temperature in this study [11].

DHPLC Temperature

The standard temperature for each of the four monoplex loci was selected based on the temperature proposed by Stanford University Website and several others were high and low temperatures were also used for each fragment in addition to the temperature predicted by the above website until the best practical temperature was obtained. In this study, in a review study on methods used in DHPLC system, XIO et al., stated that the use of nonspecific temperatures in identifying STR loci leads to a lack of identification of heteroduplex from the homoduplex loci and removal of a chromatogram of some loci with low size. They also concluded that the temperature shown on the device oven does not usually match with the actual temperature required. Temperature measurement probes show a deviation of 2 degrees from the optimum temperature [12]. The results of this study were fully consistent with results of the present study so that based on temperatures predicted by the Stanford University site, the appropriate temperature of 56°C was selected in the present study after applying 2°C changes and investigating its chromatograms. In a study on column's temperature changes, Jones AC et al., concluded that when the column temperature increases samples are eluted faster [13], which was consistent with results of the present study. Indeed, the results showed when the column temperature was 50°C, TPOX locus fragment was identified during by the detector five minutes after the injection, while the same sample was eluted from the chromatography column during about four minutes when the column temperature was 58.5°C.

Gradient Buffer

According to a study by GR Taylor who used DNAsep column to separate STR Loci and noted that the device column was a non-polar stationary alkylating phase and the buffer is liquid and polarized phase acetonitrile. Since the charge of DNA molecule is negative because of the presence of phosphate groups in its structure, connecting the stationary phase (column) and DNA requires organic cations, which bind DNA molecules to the column due to their positive charge and on the other hand, because of the presence of non-polar fragments, they are linked with the stationary phase. The most common organic cation compound was introduced TEAA, which is bonded with the DNA molecules because of its positive charge and is linked with the stationary phase with because of its triethyl fragment so that DNA molecules have a weak relationship with acetonitrile and thus small fragments of DNA pass through the column more

quickly than larger fragment as it passes. Acetonitrile and TEAA buffers raised in this study were used in the DHPLC devices and based on the results, such as what was stated earlier, loci were eluted from the device column in the order of big to small in size [14].

In a study, Shi et al., discussed on the percentage of the intended gradient buffers and it was that determined in the case of the inappropriateness of concentration of device buffers, loci with small size has been removed and cannot be detected while passing through the device column. Concentration gradients used in this study were selected according to the study by Shi et al., and DHPLC results and four chromatograms are indicative of the proper conditions of temperature and buffer gradient [15].

Analysis of Chromatogram Obtained from DHPLC

WAVER Maker software was also used in this study to investigate the size of the resulting chromatograms, which is consistent with the results obtained in Joseph. M et al.,'s study.

Joseph. M conducted a study on the separation and purification of one STR locus using HPLC method in 2005 and reached the conclusion that determining the identified genotype requires for more separation of besides PCR method so that STR heterozygous alleles is distinguished from non-specific components of hetero-duplex produced in PCR. In this study, the researchers could purify hetero-duplex changes in STR using WAVER System in HPLC and used for the sequencing of PCR products. Also, DHPLC was considered as a powerful tool in DNA Profiling studies [16].

Furthermore, in a study, A. Odriozola et al.,'s who focused on the SETUP of PCR, HPLC methods in their research, the annealing temperature was investigated several times in order to conduct Multiplex PCR, thus, it was thus confirmed that as the annealing temperature increases, height of the peaks gradually decreases in DHPLC and causes false negative results by creating a heterozygous imbalance in STR alleles. Also, it was proved that an increase in the number of PCR cycles led to increasing in peak heights, but it had no effect on heterozygosity and since the size of studied STR fragments was mostly small, 30 cycles were used for having better PCR amplification [6].

Comparison of DHPLC and CE Methods

DHPLC in the analysis of CSF1PO, D18S51, TPOX, Amelogenin Loci was more successful compared to CE according to the results of the reports on studying loci using DHPLC and CE methods and by comparing their results using chi-square analysis, as is shown in Table 2, and acceptable answers were obtained by optimizing multiplex PCR and DHPLC conditions in the above Loci.

Despite achieving individuals' genotypes in VWA and FGA loci using DHPLC, this method was not more acceptable compared with CE based on Chi-square analysis, which is due to the PCR, gene expression and the methodology conditions in this study conditions. However, it should also be noted that although poor results were obtained in investigating two above loci in the discussion of methodology, the same small positive percentage has legal value compared to other loci in order to calculate statistical indicators while importing the percent of positive results in PowerState Software, which shows the importance of these two loci in discussing the legal indices. Also, PentaD and SE33 loci, which are not available in the PCR commercial kits used in forensics Iran were also examined using DHPLC method in this study. Moreover, the allele frequency and legal parameters obtained from the analysis of these two loci were in conformity with international standards and they can be introduced two good markers for identification Iran.

DHPLC technique has an advantage over CE and that is the low cost of DNA analysis so that the cost of doing DNA tests performed in this study using DHPLC was 12 times less than CE method.

Therefore, this technique is used as a new method to identify STR loci in the genetic identification of various genes.

This method was used for the first time in this study to identify genetic identity in STR loci in our country study due to its high efficiency.

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