Analysis of Amelogenin Gene and Short Tandem Repeat(STR) loci LPL, F13B, F13A01, FESFPS, vWA from the Dental Calculus

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Ⅰ. INTRODUCTION

Since restriction fragment length polymorphism(RFLP) was discovered in the mid 1980's, the concept of the deoxyribonucleic acid(DNA) fingerprint was introduced to forensic individual identification of mass disaster and paternity testing [25,26,51].

Individual identification techniques in the 1990's are dominated by the development and rapid establishment of PCR-amplifiable short tandem repeats(STRs) or microsatellites[28]. Typing polymorphic loci at the DNA level has become a routine procedure in the paternity and identity testing fields. This has prompted the investigation of various human tissues as potential sources of genetic evidentiary material. Originally, highly polymorphic variable number of tandem repeats(VNTR) loci were characterized by RFLP analysis. A subgroup of these VNTR loci is the STR loci, which are ideal for use in linkage mapping and DNA fingerprinting. The STR systems are presented according to their chromosomal location, repeat unit, product size, primer sequences and fluorophore tags. The markers - short sequences(2-5bp) that are repeated in a tandem fashion - are abundant, highly polymorphic and uniformly distributed throughout the human genome. Moreover, STR loci, which are generally less than 350 base pairs in length, are amenable to amplification by the polymerase chain reaction(PCR). So this technique is particularly useful in the analysis of highly degraded samples or minute stains, where the initial quantity or quality of DNA is unsuitable other DNA profiling methods. STR loci also have an advantage to detect multiple loci at the same time, it can be lowered the

possibility that some genotype is accorded with other's one accidentally [1,12,38,42,43,59].

Sex determination of forensic DNA samples has been performed by amplification of sex chromosomal genes, such as Y chromosomal repetitive DYZ1 sequence[2,3] in the distal Y chromosome long arm, centromeric α-satellite repeats of X and Y chromosomes, and the X-Y homologous amelogenin gene[36,52].

The amelogenin is the predominant matrix protein in developing enamel and is crucial for proper mineralization. This has been recently found to be shrared by the X and Y chromosomes in humans and animals. The lengths of introns were different between the X and Y sequence[4,10,15,33].

Especially, molecular size of X-Y homologous amelogenin gene is small, therefore it can be detected with a high degree of specificity and sensitivity[56].

Recently, forensic studies using the teeth have been described and applied to individual identification and sex determination[48,49,61,62]. Teeth are good material for DNA analysis, since the dental hard tissue physically encloses the pulp offering an anatomic configuration of great durability[9,23,46,48,59,63,64]. Most DNA analysis of the teeth makes use of pulp. The most characteristic feature of dentin is the closely packed dentinal tubules that traverse its entire thickness and occupied for part or all of their length by odontoblast process. The bodies of the odontoblasts are arranged in a layer on the pulpal surface of the dentin[7,54]. Nuclear DNA in teeth is well preserved. However, when the teeth are used as a forensic material, destruction of the teeth is inevitable. In the aspect of preservation of evidence, it is undesirable. Dental calculus contains gingival crevicular fluid, blood cells and oral exfoliative epithelial cells. So, it can be used for analysis of DNA fingerprinting.

The purpose of this investigation is to evaluate the possibility of individual identification after detection of DNA in the dental calculus. The authors performed AMP-FLPs by PCR from the dental calculus and detected of X-Y homologous amelogenin gene for sex determination and STR loci, Human Lipoprotein lipase gene(LPL), Human factor XIII b subunit gene(F13B), Triplex[Human coagulation factor XIII A subunit gene(F13A01), Human c-fes/fps proto-oncogene(FESFPS), Human von Willebrant factor gene(vWA)] for DNA fingerprinting.

II. MATERIALS AND METHODS

1. Subjects

The 40 supragingival dental calculus were selected from 40 unrelated individuals(male:27, female:13). It was collected without bleeding carefully. The ages ranged from 17 to 65 years. Weight of the samples was 2.0~31.0mg.

The 27 dental calculus samples were rinsed with distilled water(rinsed group) and 13 samples were not(non-rinsed group). Samples were used immediately, or stored at -20℃ .

2. DNA extraction

The dried dental calculus was crushed with a hammer until resulting fragments became a powder. Powder was placed in 1.5ml eppendorf tube and dispersed 200$\mu\ell$ distilled water. The samples were submitted to a conventional DNA extraction method including an overnight incubation at 55℃ in nucleolysis buffer(0.5% SDS, 10mM Tris. Cl, 0.1M EDTA, pH 8.0), and proteinase K. Each sample was spun at 10,000 rpm in a centrifuge for 10 minutes to further purify the DNA and discarded the supernatant. Added ethanol precipitated(5M NaCl and 100%

ethanol), washed in 70% ethanol, dried, overnight incubation at 37℃ for 24 hours, and resuspended in distilled water. The concentration of genomic DNA was determined at 260nm and 280nm with UV visible spectrophotometer. Purity of samples was determined by evaluating the A_{260}/A_{280} ratios.

3. X-Y homologous amelogenin gene detection.

The PCR mixture contained follows. ; 50㎕ reaction containing 8㎕ template DNA, buffer[100mM Tris-HCl(pH 9.0), 500mM KCl, 1.0% Triton] (POSCO), 1.5mM $MgCl_2$, 0.2μM primers according to Sullivan et al.[52](Table 1), 200μM dNTPs and 1.5 unit of Taq DNA polymerase. The PCR was performed under condition of 95℃ for 10 sec, 1 cycle ; 94℃ for 60 sec, 60℃ for 60 sec, 72℃ for 60 sec, 35 cycles; 72℃ for 10 min, 1 cycle in PCR thermocycler(480 model, Perkin Elmer Cetus, NJ, USA). The PCR products(10㎕) were loaded onto 12% natural polyacrylamide gel, 1mm thickness using vertical electrophoresis unit(GIBCO BRL Ins., MD, USA). Electrophoresis was carried out at 80V for 3 hours in TBE buffer(90mM Tris-borate, 2mM EDTA). The gel was subsequently stained with ethidium bromide(0.5㎍/㎖).

4. STR loci LPL, F13B, Triplex(F13A01, FESFPS, vWA) Detection.

The PCR mixture contained follows ; 25㎕ reaction containing 25-50ng template DNA, 10X Taq DNA polymerase buffer[500mM KCl, 100mM Tris-HCl(pH 9.0 at 25℃), 1% Triton® X-100, 15mM $MgCl_2$, 2mM dNTPs, Promega®, WI, USA], 1μM의 LPL locus specific primer, F13B locus specific primer, F13A01 locus specific primer, FESFPS locus specific primer, vWA locus specific primer, 0.01 unit of Taq DNA polymerase/㎕ (Promega®)(Table 1).

The PCR conditions of LPL and F13B locus were 96℃ for 2 min, 1 cycle ; 94℃ for 60 sec, 60℃ for 60 sec, 70℃ for 90 sec, 15 cycles; 90℃ for 60 sec, 60℃ for 60 sec, 70℃ for 90 sec, 25 cycles, 60℃ for 30 min, 1 cycle. The PCR

Table 1. Primer sequences

Locus	Primer pairs
Homologous amelogenin gene	5′-CCCTGGGCTCTGTAAAGAATAGTG-3′ 5′-ATCAGAGCTTAAACTGGGAAGCTG-3′
LPL	ATCTGACCAAGGATAGATATA GZ-15 = CCTGGGTAACTGAGCGAGACTGTGTC
F13B	5′-TGAGGTGGTGTACTACCATA-3′ 5′-GATCATGCCATTGCACTCTA-3′
F13A01	5′-GAGGTTGCACTCCAGCCTTTGCAA-3′ 5′-TTCTGAATCATCCCAGAGCCACA-3′
FESFPS	5′-GGGATTTCCCTATGGATTGG-3′ 5′-ATGCCATGCAGATTAGAAA-3′
vWA	5′-CCCTAGTGGATGATAAGAATAATC-3′ 5′-GGACAGATGATAAATACATAGGATGGATGG-3′

conditions of Triplex(F13A01, FESFPS, vWA) locus were 96℃ for 2 min, 1 cycle ; 94℃ for 60 sec, 60℃ for 60 sec, 70℃ for 90 sec, 15 cycles ; 90℃ for 60 sec, 60℃ for 60 sec, 70℃ for 90 sec, 25 cycles ; 60℃ for 30min, 1 cycle. F13A01, FESFPS, vWA were amplified at the same time. The PCR products(5$\mu\ell$) were loaded onto 4% denaturing polyacrylamide gel(acrylamide : bis-acylamide = 19 : 1) containing 7M urea (Promega®) after mixed 2.5$\mu\ell$ of 2X loading solution (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol FF, Promega®).

Electrophoresis was carried out at 40W for one and a half hour. Bands were visualized by silver staining.

III. RESULTS

1. Detection of X-Y homologous amelogenin gene.

X-Y homologous amelogenin gene was detected for sex determination. Male and female samples have two bands(106bp and 112 bp), one band(106bp), respectively. It was observed 13 samples out of 27 in male, 6 samples out of 13 in female(Table 2). Amelogenin genes in rinsed and non-rinsed group were detected 8 out of 27, 11 out of 13, respectively(Fig. 1, 2, 3).

Table 2. Results of the detection of X-Y homologous amelogenin gene by PCR from the dental calculus

Rinsed dental calculus						Non-rinsed dental calculus		
sample No	Sex	Detection	sample No	Sex	Detection	sample No	Sex	Detection
1	M	-	17	M	-	28	M	+
2	F	-	18	F	-	29	M	+
3	M	-	19	M	-	30	F	+
4	F	-	20	M	+	31	M	+
5	M	-	21	F	+	32	M	+
6	F	+	22	M	+	33	M	-
7	F	+	23	M	+	34	M	-
8	M	-	24	F	-	35	M	+
9	M	-	25	M	-	36	F	+
10	M	+	26	F	-	37	M	+
11	M	+	27	F	-	38	M	+
12	M	-				39	M	+
13	M	-				40	F	+
14	F	-	detected		8	detected		11
15	M	-	non-detected		19	non-detected		2
16	F	-	total sample		27	total sample		13

+ : Band was observed　　- : No specific band was observed

Fig. 1. Results of the detection of X-Y homologous amelogenin gene by PCR from rinsed dental calculus with distilled water.
Lane 1, 9 : size marker Psi 1
Lane 2, 3, : male calculus
Lane 4, 5 : female calculus
Lane 6 : male control.
Lane 7 : female control
Lane 8 : negative control.

2. Detection of STR loci LPL, F13B, Triplex(F13A01, FESFPS, vWA).

The LPL locus was observed 2 samples out of 27 in rinsed group and 5 samples out of 13 in non-rinsed group and 3 alleles were 10, 11, 12 and 3 genotypes were 10-10, 10-11, 10-12, 11-12(Table 3)(Fig. 4). The F13B locus was observed one sample out of 27 in rinsed group and 4 samples out of 13 in non-rinsed group and 2 alleles were 9, 10 and 2 genotypes were 9-10, 10-10(Table 4)(Fig. 5).

The F13A01 locus was observed 3 samples out of 13 in non-rinsed group, and 3 alleles were 3.2, 4, 6 and 3 genotypes were 3.2-3.2, 4-4, 4-6(Table 5). The FESFPS locus was observed only one sample out of 13 in non-rinsed group and 2 alleles were 11, 12 and one genotypes were 11-12(Table 6). The vWA locus was observed one sample out of 27 in rinsed group and one sample out of 13 in non-rinsed group and 3

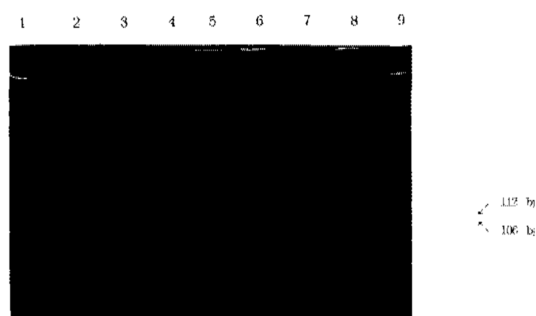

Fig. 2. Results of the detection of X-Y homologous amelogenin gene by PCR from non-rinsed dental calculus with distilled water.
Lane 1, 9 : size marker Psi 1
Lane 2, 5, 6 : female calculus
Lane 3, 4, 7, 8 : male calculus

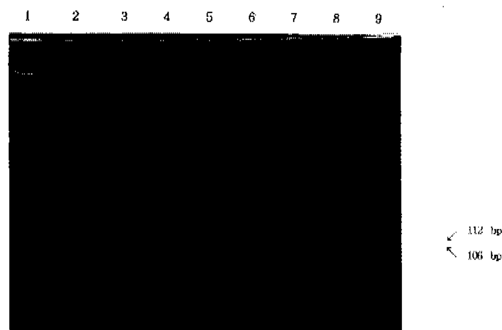

Fig. 3. Results of the detection of X-Y homologous amelogenin gene by PCR from rinsed dental calculus with distilled water.
Lane 1, 9 : size marker Psi 1
Lane 2, 3 : female calculus
Lane 4 : male calculus
Lane 5 : negative control
Lane 6 : male control
Lane 7 : female control
Lane 8 : male calculus

alleles were 14, 16, 17 and 2 genotypes were 14-16, 14-17(Table 7)(Fig. 6, 7).

Table 3. Alleles & genotypes of LPL locus from the dental calculus

allele	Number of alleles observed	genotype	Number observed
7	0	10-10	3
8	0	10-11	1
9	0	10-12	2
10	9	10-13	0
11	2	11-11	0
12	3	11-12	1
13	0	homozygote	3
14	0	heterozygote	4
all	14	total sample	7

Table 4. Alleles & genotypes of F13B locus from the dental calculus

allele	Number of allele observed	genotype	Number observed
6	0	8-9	0
7	0	8-10	0
8	0	9-9	0
9	2	9-10	2
10	8	10-10	3
11	0	homozygote	3
12	0	heterozygote	2
all	10	total sample	5

Table 5. Alleles & genotypes of F13A01 locus from the dental calculus

allele	Number of alleles observed	genotype	Number observed
3.2	2	3.2-3.2	1
4	3	3.2-4	0
5	0	3.2-5	0
6	1	3.2-6	0
7	0	3.2-16	0
8	0	4-4	1
9	0	4-5	0
10	0	4-6	1
11	0	4-16	0
12	0	5-5	0
13	0	5-6	0
14	0	5-16	0
15	0	homozygote	2
16	0	heterozygote	1
all	6	total sample	3

Table 6. Alleles & genotypes of FESFPS locus from the dental calculus

allele	Number of alleles observed	genotype	Number observed
7	0	10-11	0
8	0	10-12	0
9	0	10-13	0
10	0	10-14	0
11	1	11-11	0
12	1	11-12	1
13	0	homozygote	0
14	0	heterozygote	1
all	2	total sample	1

IV. DISCUSSION

Dental calculus is an adherent calcified or calcifying mass that forms on the surface of natural teeth and dental protheses. It consists of inorganic(70% to 90%) and organic components. The inorganic portion consists of calcium

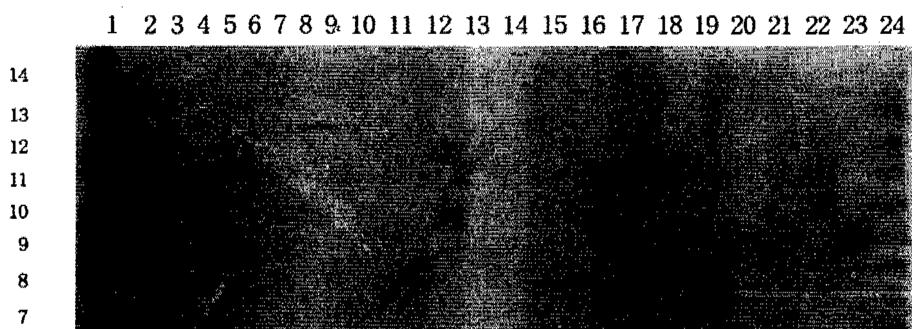

Fig. 4. Bands of LPL locus from the dental calculus

Lane 2-9 : rinsed calculus with distilled water
Lane 10, 11, 14-23 : non-rinsed calculus with distilled water
Lane 12, 13 : blank
Lane 1, 24 : allelic ladder(LPL ladder, Promega®)
Lane 2, 4, 23 : genotype 10-10
Lane 16 : genotype 10-11
Lane 11, 18 : genotype 10-12(faint band)
Lane 22 : genotype 11-12
Lane 3, 5-10, 12-15, 17, 19-21 : band was not observed

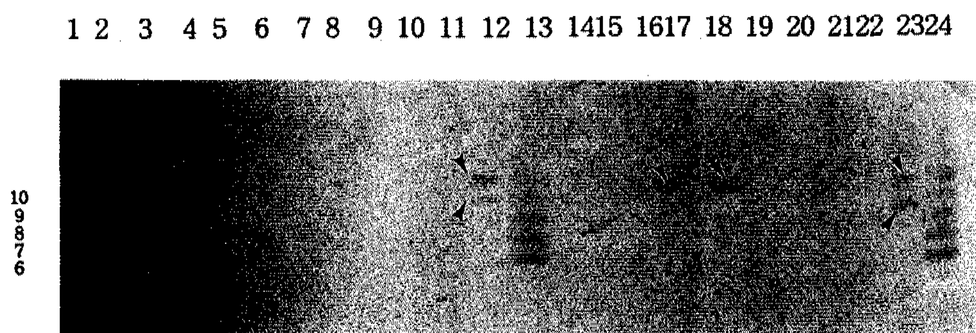

Fig. 5. Bands of F13B locus from the dental calculus.

Lane 1, 12, 24 : allelic ladder(LPL ladder, Promega®)
Lane 2-9 : rinsed calculus, Lane 13 : blank
Lane 10, 11, 14-23 : non-rinsed calculus
Lane 4, 16, 18 : genotype 10-10(faint band)
Lane 11, 23 : genotype 9-10(faint band)
Lane 2, 3, 5-10, 14, 15, 17, 19-22 : band was not observed

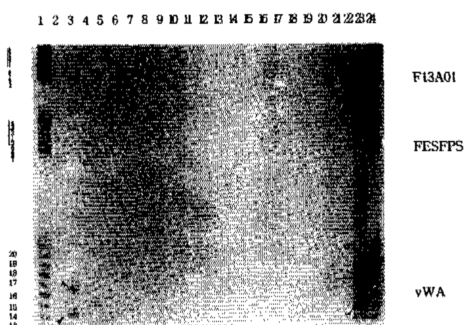

Fig. 6. Bands of Triplex(F13A01, FESFPS, vWA) locus from the dental calculus.
Lane 2-9 : rinsed calculus with distilled water
Lane 10, 11, 14-23 : non-rinsed calculus
Lane 12, 13 : blank
Lane 1, 24 : allelic ladder(Triplex ladder, Promega®)
* F13A01
Lane 16 : genotype 4-4(faint band)
Lane 22 : genotype 4-6(faint band)
Lane 23 : genotype 3.2-3.2(distinct band)
* FESFPS
Lane 23 : genotype 11-12(distinct band)
* vWA
Lane 3 : genotype 14-16(faint band),
Lane 23 : genotype 14-17(distinct band)

phosphate, calcium carbonate, traces of magnesium phosphate and other metals. The organic component of dental calculus consists of mixture of protein-polysaccharide complexes desquamated epithelial cells, leukocyte, and various types of microorganisms[8] .

The organic component in both inside and outside of dental calculus suggests that dental calculus can be a forensic material for DNA typing. Individual identification using the teeth has very significant in forensic odontological investigation. Because a tooth is the hardest tissue of the human body, pulp is surrounded by

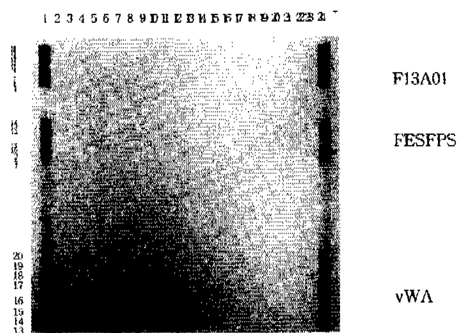

Fig. 7. Bands of Triplex locus from the dental calculus.
Lane 2-11, 14-23 : rinsed calculus with distilled water
Lane 12, 13 : blank
Lane 1, 24 : allelic ladder(Triplex ladder, Promega®).
* F13A01, FESFPS and vWA
Lane 2-11, 14-23 : band was not observed

Table 7. Alleles & genotypes of vWA locus from the dental calculus

allele	Number of alleles observed	genotype	Number observed
11	0	14-14	0
12	0	14-15	0
13	0	14-16	1
14	2	14-17	1
15	0	14-18	0
16	1	14-19	0
17	1	15-16	0
18	0	15-17	0
19	0	15-18	0
20	0	homozygote	0
21	0	heterozygote	2
all	4	total sample	2

enamel, dentin and cementum, so it is well protected. The tooth is distinct anatomic location where DNA is found[46,48,59,63,64]. Although it is possible to analyze the DNA polymorphism from the dental pulp, tooth sample must be destroyed for DNA analysis[60,63]. If dental calculus is attached to tooth, it may be used without destroying the tooth[27].

In this study, the samples were divided rinsed and non-rinsed group, because most of nuclear DNA is distributed in the surface of dental calculus. In actual case, cells of the surface of dental calculus are purified easily. But, it is thought that inside of dental calculus is well preserved.

Sex determination is an important evaluation item in for forensic odontology, archaeology, and anthropology. Sex determination is the first step of individual identification in medico-legal practice and plays an important role in criminal investigation and mass disaster[34]. Various PCR-based methods have been reported ; the X-Y homologous amelogenin gene, DYZ1[4,5].

An advantage of the DYZ1 locus analysis has been that its repetitive nature increases the sensitivity of the assay, reducing the need for template DNA. However, this method lacks an internal control. Therefore, the absence of amplified DYZ1 material could be interpreted as due to either the female sex of the template DNA or amplification failure. Additionally, autosomal homologues of the DYZ1 family are co-amplified resulting in nonspecific amplified fragments in female samples[1,3,16,24,31,47,56, 57], counterparts were detected at the same time.

On the other hand, during development of tooth enamel ameloblst cells within the enamel organ secrete an organic matrix comprising primarily proteins called amelogenins and enamelins(tuft proteins)[10,33] and amelogonenin gene has been recently found to be shared by both X and Y chromosomes in humans and animals[2].

Amelogenins constitute approximately 90% of the enamel matrix protein and are relatively easy to extract from developing teeth[15,55]. Human and animal amelogenin genes have been cloned and sequenced. Transcriptionally active genes are found on both X and Y chromosomes. Amplification of a segment of the amelogenin gene using a pair of primers revealed both Y-and X- specific bands at the same time. The gene has counterparts in both the X and Y chromosomes and a small deletion in the former made it possible to distinguish them[2,11]. Most methods for sex identification detect only Y-specific materials. Thus it was impossible to tell whether the sample was from a female or whether the analysis had ended in failure, when no material was detected[31].

However, X-Y homologous locus is more reliable since co-amplification of distinguishable X- and Y- specific fragments validates the analytical procedure. Using dual amplification technique, it was shown that 0.005 ng(about one molecule) of template DNA in reaction mixture was sufficient for the detection of amplification products[5].

The Y counterpart of the gene has a deletion within one of the introns, making it possible to differentiate between X and Y counterparts based on the difference in size. This difference in size was attributed to that of an intron, and the X-fragment acted as an internal control that validated the analytical procedures[4,5].

Amelogenin genes in rinsed and non-rinsed group were detected 8 out of 27, 11 out of 13, respectively(Table 2). It suggests that most nuclear DNA was washed out during rinsing with distilled water.

Variable number of tandem repeat(VNTR) loci(so called minisatellite) and restriction

fragment length polymorphism(RFLP) analysis are being used as a good identifying test marker, since it contains many alleles, high heterozygosity and high power of discrimination. But VNTR loci have a high molecular weight in compared with STR loci, so it is difficult to detect the VNTR loci from the forensic samples occasionally, especially in degraded samples. RFLP analysis also fails to detect a large fraction of mutations and polymorphisms and has other limitations such as sensitivity, analysis time or the impossibility of typing highly degraded samples. While on the other hand, it is easy to analyze the STR loci(so called microsatellite) from the degraded one[30]. Power of discrimination of STR loci are lower than VNTR loci. Therefore, for increasing the power of discrimination, it is recommanded to examine many of the VNTR loci and STR loci at the same time[38,50].

Although the sensitivity of amplified fragment length polymorphism (AmpFLPs) is suitable for analysing forensic samples, most VNTR loci alleles are too long to be analysed as AmpFLPs ; for example, D1S8(MS32)[53,61,62] on chromosome 1 consists of a tandem array of 29 bp repeat units, the array varying from 0.3 to 20 kb in length. AmpFLPs from degraded DNA samples may fail to obtain target bands[45]. With the advent of PCR, STR marker polymorphisms replaced VNTRs as the markers of choice[19,32,43]. It is estimated that the human genome contains approximately 500,000 STRs(6,000 to 10,000 trimeric and tetrameric repeats)[14,58]. These abundant repeats may be detected using the PCR and polyacrylamide gel electrophoresis. Loci, such as TH01[17,26], CD4[12,13], CSF1PO, D1S53, D21S11, F13A01[39,44], F13B[37], FES/FPS, HPRT[20], HMG14, TRMI[40], CRYG1[21], CYP19[41], vWA[29], LPL[65] have been typed by amplification of DNA by PCR.

Generally forensic samples are mostly corrupted and only a small amount of modified DNA can amplified, the analysis of STR loci by PCR method is indispensable and VNTR loci are not suitable. High ploymorphic STR loci contain 2-5 repetitive base pairs and easy to interpret the type of allele[6]. STR loci have individually different in allele frequency of genotype, heterozygosity and genetic distance, so they have being used for individual identification.

Human Lipoprotein lipase(LPL) gene area is one of STR loci and located on the human chromosome 8q22. The repetitive sequence of the rank is [AAAT]n and molecular size of LPL is 105-133 base pairs. There are 8 kinds of allele 7, 8, 9, 10, 11, 12, 13, 14.[22] Human factor XIII b subunit gene(F13B) is one of STR loci and located on the human chromosome 1q31-q32.1. The repetitive sequence of the rank is [AAAT]n and molecular size of F13B is 169-185 base pairs. There are 5 kinds of allele 6, 7, 8, 9, 10[38]. The F13AO1 locus is one of STR loci and it is [AAAG]n polymorphism and presents within the 5′ untranslated region of the human coagulation factor XIII A subunit gene on chromosome 6p24-p25 and has 3.2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 alleles. These alleles vary in length from 181 through 231 base pairs[39,44]. Human c-fes/fps proto-oncogene(FESFPS) is [AAAT]n polymorphism and locate on the chromosome 15q25-qter and has 7, 8, 9, 10, 11, 12, 13, 14 alleles. Length is from 222 through 250 base pairs. Human von Willebrant factor gene(vWA) is [AGAT]n polymorphism and located on the chromosome 12p12-pter and has 13, 14, 15, 16, 17, 18, 19, 20 alleles. Length is from 139 through 167 base pair[18,29,35].

The LPL locus was observed 2 samples out of 27 in rinsed group and 5 samples out of 13 in non-rinsed group and 3 alleles were 10, 11, 12 and 3 genotypes were 10-10, 10-11, 10-12,

11-12(Table 3). And the F13B locus was observed one sample out of 27 in rinsed group and 4 samples out of 13 in non-rinsed group and 2 alleles were 9, 10 and 2 genotypes were 9-10, 10-10(Table 4).

The F13A01 locus was observed in the 3 non-rinsed samples out of total 40 and observed alleles were 3.2, 4, 6 and observed genotypes were 3.2-3.2, 4-4, 4-6. FESFPS was also detected only one sample in non-rinsed group, vWA was observed one sample out of 27 in rinsed group and one sample out of 13 in non-rinsed group and observed 3 alleles were 14, 16, 17 and observed 2 genotypes were 14-16, 14-17. These 3 loci were detected as triplex at the same time(Table 5, 6, 7).

Authors tried to detect vWA, F13A01, FES/FPS triplex STR amplification system. But only few alleles were observed. In order to obtain approximately even signal intensities for all loci within the triplex, specific primer concentrations must be adjusted. The optimal relative primer concentrations will depend upon the method of product detection. It is essential that any new batch of primer, which has been re-synthesized or newly labelled and purified, is first checked to ensure that it preforms to the required specification within the triplex reaction. The potential requirement to make slight adjustments in relative primer concentrations when employing new batches of primers makes it important to know how variation in primer concentration for one locus will affect the amplification efficiency of the other 2 loci. Although adjusting relative primer concentrations, it failed to detect triplex loci[30].

Singleplex was well detected compared with multiplex. The main reason for using multiplex is to speed the progress. But, multiplex reactions are a compromise of ideal conditions and loss of efficiency of amplication may result[50]. In this study only one sample was detected multiplex. The use of multiplex and singleplex systems is not mutually exclusive. Singleplex reactions may be required to clarify or enhance a difficult result. When analysing poor quality samples the analyst should be familiar with the varied profile morphology that may be expected with forensic samples. With degraded samples or if only limited quantities of DNA of sufficient molecular weight are available the possibility of allelic or locus drop-out must be considered and there may be occasions where it is beneficial to use singleplex tests to assist interpretation.

This study demonstrates the possibility that the dental calculus is useful for sex determination, amplification of singleplex(LPL, F13B), but it is doubtful to detect STR loci triplex(F13A01, FESFPS, vWA).

V. CONCLUSIONS

The organic component in both inside and outside of dental calculus suggests that dental calculus can be a forensic material for DNA typing. Author isolated nuclear DNA from the 40 dental calculus and performed AMP-FLPs by PCR and electrophoresed to detect X-Y homologous amelogenin gene for sex determination and STR locus LPL, F13B, Triplex(F13A01, FESFPS, vWA) gene for DNA fingerprinting. Samples are divided two group(27 rinsed dental calculus group with distilled water and 13 nonrinsed one)

The following results were obtained.

1) Detection of X-Y homologous amelogenin gene for sex determination was possible in rinsed and nonrinsed group, 8 out of 27 and 11 out of 13, respectively.
2) LPL locus in rinsed and nonrinsed group was detected 2 out of 27 and 5 out of 13,

respectively and observed 3 alleles(10, 11, 12) and 4 genotypes(10-10, 10-11, 10-12, 11-12).

3) F13B locus in rinsed and nonrinsed group was detected 1 out of 27 and 4 out of 13, respectively and observed 2 alleles(9, 10) and 2 genotypes(9-10, 10-10).
4) F13A01 locus was observed 3 out of 13 samples, 3 alleles(3.2, 4, 6) and 3 genotypes (3.2-3.2, 4-5, 4-6) in non-rinsed group, but nothing of rinsed group was detected.
5) FESFPS locus was detected 1 out of 27 in nonrinsed group only and observed 2 alleles (11, 12) and 1 genotypes(11-12)
6) vWA locus in rinsed and nonrinsed group was detected 1 out of 27 and 1 out of 13, respectively and observed 3 alleles(14, 16, 17) an 2 genotypes(14-16, 14-17).

From the above results, this study demonstrates the possibility that the dental calculus is useful for sex determination, amplification of singleplex(LPL, F13B), but it is doubtful to detect STR loci triplex(F13A01, FESFPS, vWA) at the same time. It was revealed that dental calculus is useful and applicable to individual identification as molecular biological samples for individual identification.

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국문요약

치석에서 Amelogenin Gene 및 Short Tandem Repeat(STR) 유전좌위 LPL, F13B, Triplex(F13A01, FESFPS, vWA)에 대한 분석

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치석에는 박리상피세포, 백혈구 등이 포함되어 있어 이들의 핵 내에 있는 DNA의 유전자형을 찾아내 개인식별을 할 수 있을 것으로 추정된다. 본 연구에서는 치석만으로 개인식별이 가능한지를 알아보고자 40명으로부터 채취한 치석을 증류수에 세척한 군과 세척하지 않은 군으로 나누어 DNA를 추출하고 중합효소연쇄반응법을 이용하여 증폭절편다형(AMP-FLPs)을 실시한 후 성별검사를 위한 X-Y homologous amelogenin gene과 유전자지문검사를 위한 STR유전좌위 LPL, F13B, Triplex(F13A01, FESFPS, vWA) 등 6개의 유전자를 검색하여 - X-Y homologous amelogenin gene과 LPL, F13B는 각각 증폭하였으며 F13A01, FESFPS, vWA 세 유전자는 동시에 증폭하였음 - 다음과 같은 결과를 얻었다.

1) X-Y homologous amelogenin gene 검색으로 세척군에서 27개의 검체 중 8개, 비세척군에서 13개 중 11개에서 성별검사가 가능하였다.
2) LPL유전자는 세척군, 비세척군에서 각각 27개 검체중 2개, 13개 검체 중 5개가 검색되었으며 3개의 대립유전자(10, 11, 12)와 4개의 유전자형 (10-10, 10-11, 10-12, 11-12)이 나타났다.
3) F13B유전자는 세척군, 비세척군에서 각각 27개 검체 중 1개, 13개 검체 중 4개가 검색되었으며 2개의 대립유전자(9, 10)와 2개의 유전자형(9-10, 10-10)을 관찰하였다.
4) F13A01유전자는 비세척군에서만 13개 검체 중 3개가 검색되었고 3개의 대립유전자(3.2, 4, 6)와 3개의 유전자형(3.2-3.2, 4-5, 4-6)을 관찰하였고, 세척군에서는 나타나지 않았다.
5) FESFPS유전자는 비세척군에서만 13개 검체 중 1개가 검색되었고 유전자 형은 11-12로 나타났다.
6) vWA유전자는 세척군, 비세척군에서 각각 1개씩 검색되었으며, 3개의 대 립유전자형(14, 16, 17)와 2개의 유전자형(14-16, 14-17)을 관찰하였다.

이상의 결과를 종합해 볼 때, 치석은 X-Y homologous amelogenin gene증폭을 통한 성별검사와 단일 STR유전좌위 증폭을 통한 유전자지문형 검사에는 유용하나 복합 STR유전좌위의 검색에는 부적합한 것으로 나타났으며 법의학적시료로 응용이 가능한 것으로 사료된다.

주요어 : 치석, 성별검사, 유전자 지문, X-Y homologous amelogenin gene, STR, LPL, F13B, F13A01, FESFPS, vWA