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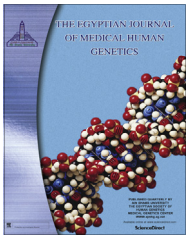

ORIGINAL ARTICLE

Determination of DNA profiling of siwak and toothbrush samples used in Kingdom of Saudi Arabia

Nagy Alfadaly [a,*], Ahmed Kassab [b], Fahd Al Hedaithy [b]

[a] Forensic Medicine, College of Medicine, Qassim University, Qassim, Saudi Arabia
[b] Department of Forensic Biology, College of Forensic Sciences, Naif University, AlRiadh, Saudi Arabia

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Abstract *Background:* DNA profiling is an integral part of forensic work. Enough and good samples for DNA quantification and profiling are mandatory.

Aim of the study: To quantify and profile DNA from siwak and toothbrushes and study the effect of time on this process.

Methodology: The present study included DNA profiling from siwak and effect of time on extracted DNA. Then; obtained data were compared to those extracted from toothbrushes. For this purpose, 25 uncovered siwaks, 4 covered siwaks, and 25 toothbrushes were used. Samples were divided into 8 groups; first group (4 months; 4 volunteers); second group (3 months, 5 volunteers); third group (2 months, 4 volunteers); fourth group (1 month, 5 volunteers); fifth group (1 week, 3 volunteers); sixth group (the same day, 4 volunteers and covered siwak was added); seventh group (reference samples) and group 8 (positive and negative control samples). Extraction of DNA was done using Promega kit and then PCR was used for amplification and DNA profiling was done.

Results: Considerable quantity of human genomic DNA was successfully extracted from siwaks. There was no proof of the existence of any substance in siwak or its components that may interfere with amplification of DNA by PCR or interfere with obtaining DNA profiles. Siwak proved to be a good and reliable source of human genomic DNA that is enough for DNA analysis. There has been no effect of time on DNA analysis and DNA profiling in this study (within the targeted period which is 4 months).

Conclusion: Siwak contains enough quantity of DNA, and retained good DNA profiling; and when compared to toothbrushes, siwak is a reasonable source of DNA profiling when found at the scene of crime. In addition, time of storage up to 4 months had no or little effects on results.

* Corresponding author at: Forensic Medicine, College of Medicine, Qassim University, Buraida, Saudi Arabia. Tel.: +966 543474172.
E-mail address: nmbayoumy@yahoo.co.uk (N. Alfadaly).

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1. Introduction

DNA profiling, since its introduction in 1985 [1,2], has been frequently employed by the forensic community to assist in the identification of individuals and has become an indispensable tool in criminal investigation, disaster victim identification and parentage analysis. The prevailing technology calls for amplifying the DNA for genotyping with the polymerase chain reaction (PCR), targeting relevant segments in the short tandem repeat (STR) loci, in the size ranges of 100–480 bp. These loci, which are well distributed throughout the human genome, are highly polymorphic and capable of generating DNA typing results from very little material through multiplex amplification using PCR [3].

However, the DNA samples in forensic casework are often far from pristine (pure samples) which poses a range of impediments to a forensic scientist's profiling attempts. The amplification of compromised DNA samples, for example those exposed to harsh environmental conditions, skeletal remains of missing persons or human remains from mass disasters, can result in partial or no genetic profile. This loss of signal could be due to the presence of PCR inhibitors co-extracted with the forensic evidence, or a degraded DNA template due to bacterial, biochemical or oxidative processes [4]. There are several methods developed to overcome these problems [3].

Toothbrushes are an often selected source of reference DNA sample in victim identification cases [5].

Although profiling DNA from toothbrushes is routinely performed, there have been reports of failure to generate results in some cases in Thailand. One cause could be attributed to the moisture in bathrooms together with the tropical temperature and humidity, which promotes growth of bacteria and thus accelerates DNA degradation [6].

Furthermore, although the common practice of using all of the bristles (stiff hairs of toothbrush) improves the chance of collecting enough intact genetic material, it may also increase the concentration of PCR inhibitors found in toothpaste residue, which compromises the quality of the resulting DNA profile [7].

"Siwak" is an Arabic word meaning "tooth-cleaning stick," and *Salvadora persica* siwak has a wide geographic distribution. Traditionally, in Middle Eastern countries, many cultures use chewing sticks of Arak for medicinal purposes especially, for oral cleanliness care. It was used by Muslims for cleaning of teeth and highly recommended to be used by Muslims during the whole day [8].

In the present study we intended to use both toothbrushes and Siwak for DNA profiling.

2. Aim of the study

The present study was designed to investigate the possibility of extracting human genomic DNA from used siwaks and toothbrushes. The effect of storage time on genotyping, quantitation of genomic DNA, verification of PCR inhibitor existence were also investigated.

3. Methodology

The present study was done at Genetic Tests Division at criminal lab department; Department of criminal evidence; Hael District; Kingdom of Saudi Arabia.

The present study included DNA profiling from siwak and effect of time on extracted DNA. Then; obtained data were compared to those extracted from toothbrushes. For this purpose, 25 uncovered siwaks (unpacked), 4 covered siwaks (tightly packed or placed inside a covering envelope), and 25 toothbrushes were used.

Then, the study samples were divided into eight groups:

- *First group:* included 4 siwaks, and 4 toothbrushes that were assigned for 4 volunteers (one siwak and one toothbrush for each volunteer) before sampling by 4 months.
- *Second group:* included 5 siwaks and 5 toothbrushes assigned for 5 volunteers (each one siwak and one brush) before sampling by 3 months.
- *Third group:* included 4 siwaks and 4 toothbrushes for 4 volunteers as previous; before sampling by 2 months.
- *Fourth group:* included 5 siwaks and 5 toothbrushes assigned for 5 volunteers (each one siwak and one toothbrush) before sampling by one month.
- *Fifth group:* included 3 siwaks and 3 toothbrushes assigned for 3 volunteers (each one siwak and one toothbrush) before sampling by one week.
- *Sixth group:* included 4 uncovered siwaks, 4 covered siwaks and 4 toothbrushes assigned for 4 volunteers (each volunteer used one uncovered siwak, one covered siwak and one toothbrush) at the day of sampling.
- *Seventh group (reference samples):* 5 mouth swab samples that were taken by cotton-tipped swabs and their DNA content was extracted and used for comparison as reference ones.
- *Eighth group (positive and negative control samples):* the negative control samples were unused siwaks and unused toothbrushes, in addition to one positive control sample with known DNA profiling (negative control samples are taken from unused siwaks and unused toothbrushes, to check if the process of DNA collection is strict and there was no DNA contamination, while positive control sample was known, standard DNA sample, supplied with the Amp FlSTR®Kit, used as a routine positive control in the lab, its DNA profile, was already known). Control samples were extracted the same as other samples by Promega kit as described in methodology.

4. DNA extraction by Promega kit

Each sample was prepared separately as the following: siwak head or toothbrush were handled by sterilized forceps; 0.5 cm from siwak head and ¼ of the anterior part of toothbrush were cut by a new sterilized scalpel; each sample was transferred to a sterilized plastic tube (1.5 ml); then 320 µl of lysis buffer was added, vortexed and incubated at 71 °C for 1 h.

Samples were then transferred to a plastic basket tube (1.5 ml) and centrifuged at 14,000/min for two minutes; the supernatant was obtained in the tube, covered well and vortexed at high speed for 3 s; 7 µl of resin was added for each sample. Samples were reshaken by high speed vortex for 3 s; left for 10 min at room temperature, then vortexed for 3 s every two minutes.

Samples were then vortexed at the maximum speed for 3 s; rapidly transferred to magnetic stand; all fluid was removed

from each tube without touching resin at the sidewall of the tube; 100 µl of lysis buffer was added and samples were then vortexed at the maximum speed for 3 s and returned back to the magnetic stand.

All lysis buffer was removed; wash buffer was added; the samples were then vortexed for 3 s on the highest speed and returned back to the magnetic stand; washing process was repeated for additional two times; then samples were allowed to stand on the magnetic stand, left uncovered for 5 min for drying.

50 ml of elution buffer was added, the samples were then covered, vortexed for 3 s at the maximum speed, and incubated at 66 °C for 5 min. Finally, samples were then vortexed at the highest speed and returned back to the magnetic stand, transferred to a new tube (1.5 ml) and the samples then were ready for DNA quantification.

DNA quantification was done using optical 96 well reaction plate (MicroAMP™; Applied biosystem company) as described by the manufacturer; using Quantifiler™ Human DNA Quantification kit. DNA quantity was measured by Sequence detection system 7000. DNA quantity was amplified by Amp FlSTR®Kit Amplification Identifiler® PCR.

Finally DNA profiling was done using Genetic Analyzer 3130XL, and Gene Mapper software was used to read samples.

5. Results and discussion

Table 1 presents DNA quantity (ng/µl) obtained from each sample in siwak and toothbrushes and the results of DNA profiling (either complete, partial or cannot be profiled).

Table 2 presented the total amount of DNA (ng/µl) extracted from siwak and toothbrushes; and showed statistically, non-significant variance between as regards to DNA extracted from either siwak or toothbrushes. However, there was a significant increase of DNA quantity extracted from siwak samples as a whole (18.96 ± 16.15) when compared to total quantity extracted from toothbrush samples as a whole (1.76 ± 1.07), ($p < 0.001\%$). These results mean that, siwak is an important source for DNA profiling in criminal and civil cases (as siwak samples returned quantity 10.77 times as toothbrush). Thus, when found in crime scene, siwak represents an important source for DNA profiling.

In addition, no PCR inhibitors were found in siwak samples, and thus, no interference with DNA profiling (except one sample that returned partial DNA profiling). Finally, there was no difference between covered and uncovered siwaks as regards DNA quantity. Higher quantity of DNA material extracted from siwak can be attributed to a larger volume of pure saliva presented in siwak compared to a small amount mixed with toothpaste in toothbrushes.

Saliva is a good source for DNA profiling, this was indicated by authors in forensic reports [9]. Their findings demonstrated that genomic DNA of reasonable quantity can be obtained from 2 ml of saliva, with average yield of 35 µg. This is comparable, perhaps somewhat less, than that typically obtained from a same volume of blood (20–80 µg). Nevertheless, this quantity is higher than that reported using mouthwash (16–30 µg) or from buccal cells scrapped using cytobrushes or swabs (1–2 µg per swab or cytobrush) [10].

Going with results of the present study Ng et al. [9] added that, besides a reasonable DNA yield, their study showed that the various saliva storage conditions failed to negatively affect DNA quality as shown by results from real time PCR experiments. Furthermore, there was no obvious indication that prolonged storage following preservation of saliva, diminished genotyping fidelity.

Table 1 Data obtained from studied groups.

	DNA (uncovered siwak)	DNA (toothbrush)	DNA (Covered siwak)	DNA profiling siwak	DNA profiling toothbrush
Group I	9.96	0.576		Complete	Partial
Group I	2.93	0.303		Complete	None
Group I	30.12	0.844		Complete	Complete
Group I	0.558	2.02		Partial	Complete
Group 2	78.18	1.78		Complete	Complete
Group 2	8.05	2.01		Complete	None
Group 2	16.53	2.18		Complete	Complete
Group 2	7.95	1.27		Complete	Complete
Group 2	5.8	3.92		Complete	Complete
Group 3	15.13	1.69		Complete	None
Group 3	40.64	2.06		Complete	None
Group 3	19.1	2.63		Complete	None
Group 3	22.83	4.39		Complete	Complete
Group 4	23.8	1.92		Complete	Partial
Group 4	35.77	1.05		Complete	None
Group 4	9.01	1.62		Complete	Complete
Group 4	9.57	0.915		Complete	Complete
Group 4	24.04	3.24		Complete	Complete
Group 5	10	2.15		Complete	Complete
Group 5	8.07	0.558		Complete	None
Group 5	22.86	0.486		Complete	Complete
Group 6	16.96	2.63	9.71	Complete	Complete
Group 6	4.66	2.46	12.02	Complete	None
Group 6	27.33	1.14	14.64	Complete	Partial
Group 6	24.28	0.264	33.4	Complete	Complete

Table 2 Comparison between different groups as regards total DNA quantity extracted from siwak and toothbrush.

		Mean	S.D.	Minimum	Maximum	*F*	*p*
DNA siwak	Group I	10.89	13.42	0.56	30.12	0.38	0.85 (NS)
	Group 2	23.30	30.95	5.80	78.18		
	Group 3	24.42	11.25	15.13	40.64		
	Group 4	20.43	11.27	9.01	35.77		
	Group 5	13.64	8.03	8.07	22.86		
	Group 6	18.30	10.08	4.66	27.33		
	Total	18.96	16.15	0.56	78.18		
DNA Brush	Group I	0.93	0.75	0.30	2.02	1.76	0.16 (NS)
	Group 2	2.23	1.00	1.27	3.92		
	Group 3	2.69	1.19	1.69	4.39		
	Group 4	1.75	0.92	0.92	3.24		
	Group 5	1.06	0.94	0.49	2.15		
	Group 6	1.62	1.12	0.26	2.63		
	Total	1.76	1.07	0.26	4.39		
Paired comparison	Total samples ($t = 5.30$, $p < 0.001$), NS means non significant						

Both Tables 3 and 4 present DNA profiled from siwak in all groups (Table 3) and from toothbrush in all groups (Table 4) and reveal that, there was no significant difference between groups as regards DNA profiling. These results indicated that, the time has no or low effect on DNA quantity and sequenced characters. However, DNA extracted from siwak showed complete DNA character profiling in 24 samples (96.0%); while DNA extracted from toothbrush showed complete sequencing in 14 cases (56.0%); partial in 3 samples (12.0%) and 8 samples (32.0%) returned negative results. These indicated the effectiveness of siwak samples in identification. These results are comparable to Vandewoestyne et al. [11], who reported that, Short Tandem Repeats (STR) profiling of cellular and extracellular DNA showed the same pattern for every donor. A previous study that examined different STR loci also showed the same profile patterns in cellular and extracellular DNA derived from saliva. Therefore, identification of individuals using extracellular DNA from saliva is possible.

Going with results of the present study, other studies: Walsh et al. [12] and Kamodyova et al. [13] reported that, saliva and saliva-stained material have proven to be good and valuable sources of DNA for genotyping in certain forensic settings.

The inability of samples of toothbrushes to return complete DNA profiling was not ascertained to time as the samples obtained just before analysis were unable to return complete profile. This can be explained by the effect of toothpaste itself, or humidity of bathroom that lead to multiplication of bacteria that interfere with DNA extraction and profiling irrespective of toothpaste use [6]. In addition, presence of PCR inhibitors on toothpaste itself may be a factor that leads to destruction of DNA material [14].

Table 3 DNA profiling from siwak.

	Siwak				Statistics	
	Complete		Partial			
	n	%	*n*	%	X^2	*p*
Group I	3	75.0	1	25.0	5.46	0.36 (NS)
Group 2	5	100.0	0	0.0		
Group 3	4	100.0	0	0.0		
Group 4	5	100.0	0	0.0		
Group 5	3	100.0	0	0.0		
Group 6	4	100.0	0	0.0		
Total	24	96.0	1	4.0		

Table 4 DNA profiling from toothbrush.

	Toothbrush						Statistics	
	None		Complete		Partial			
	n	%	*n*	%	*n*	%	*n*	%
Group I	1	25.0	2	50.0	1	25.0	7.04	0.72 (NS)
Group 2	1	20.0	4	80.0	0	0		
Group 3	3	75.0	1	25.0	0	0		
Group 4	1	20.0	3	60.0	1	20.0		
Group 5	1	33.3	2	66.7	0	0		
Group 6	1	25.0	2	50.0	1	25.0		
Total	8	32.0	14	56.0	3	12.0		

This postulation was supported by another study from Brazil, where 3 groups of buccal mucosa cells were used; the first exposed to lysis solution without preservation for a period of time; the second preserved in room temperature; the third was preserved at 3 °C. Results revealed no significant difference as regards quantity of DNA extracted from each group. However, there was a significant difference between groups as regards quality [15].

In addition, decreased quantity of DNA in toothbrushes may be due to decreased number of cells on toothbrush due to frequent and massive washing. This explanation was in agreement with the study of Yamamoto et al. [16]. Furthermore, some reports [17] indicated that, toothpaste contains PCR inhibitors that lead to destruction of DNA and decrease DNA profiling.

Finally, it can be said that, siwak contains enough quantity of DNA, and returned good DNA profiling; and when compared to toothbrushes, siwak is a reasonable source of DNA profiling when found in the scene of crime. In addition, time of storage up to 4 months had no or little effects on results. However, it is needed to design future studies to estimate the

longest period with capability of DNA quantification and profiling.

6. Conclusion

Siwak contains enough quantity of DNA that can successfully result in good DNA profiling. When compared with toothbrushes, siwak is a reasonable source of DNA profiling that can help in forensic identification when found at the scene of crime. In addition, siwak can be used as a source of identification even after months of its use as the time of storage up to 4 months was found to have no or little effects on results.

Conflict of interest

There is no conflict of interest.

References

[1] Gill P, Jeffreys AJ, Werrett DJ. Forensic application of DNA 'fingerprints'. Nature 1985;318:577–9.
[2] Jeffreys AJ, Wilson V, Thein SL. Individual-specific 'fingerprints' of human DNA. Nature 1985;316:76–9.
[3] Ip SCY, Lin S-W, Li C, Lai K-M. Forensic DNA typing strategy of degraded DNA on discarded cigarette ends using the AmpFℓSTR® Identifiler®, Identifiler® Plus and MiniFiler™ PCR Amplification Kits. Sci Justice 2014;54:311–5.
[4] Bar W, Kratzer A, Machler M, Schmid W. Postmortem stability of DNA. Forensic Sci Int 1988;39:59–70.
[5] Jobim MR, Gamio F, Ewald G, Jobim M, Jobim LF. Human identification using DNA purified from residues in used toothbrushes. Int Congr Ser 2004;1261:491–3.
[6] Butler J. Forensic DNA typing: biology, technology and genetics of STR markers. 2nd ed. San Diego: Academic Press; 2005. p. 38.
[7] Bandhaya A, Panvisavas N. Optimization of DNA recovery from toothbrushes. Forensic Sci Int Genet Suppl Ser 2008;1:9–10.
[8] Ibrahima MM, AL Sahlia AA, Alaraidha IA, et al. Assessment of antioxidant activities in roots of siwak (*Salvadora persica*) plants grown at two different locations in Saudi Arabia. Saudi J Biol Sci 2015;22(2):168–75.
[9] Ng DPK, Koh D, Choob S, Chia K-S. Saliva as a viable alternative source of human genomic DNA in genetic epidemiology. Clin Chim Acta 2006;367:81–5.
[10] Steinberg K, Gallagher M. Assessing genotypes in human genome epidemiological studies. In: Khoury MJ, Little J, Burke W, editors. Human genome epidemiology: a scientific foundation for using genetic information to improve health and prevent disease. Oxford University Press; 2004. p. 79–91.
[11] Vandewoestyne M, Van Hoofstat D, Franssen A, et al. Presence and potential of cell free DNA in different types of forensic samples. Forensic Sci Int Genet 2013;7:316–20.
[12] Walsh DJ, Corey AC, Cotton RW, et al. Isolation of deoxyribonucleic acid (DNA) from saliva and forensic science samples containing saliva. J Forensic Sci 1992;37:387–95.
[13] Kamodyova N, Durdiaková J, Celec P, et al. Prevalence and persistence of male DNA identified in mixed saliva samples after intense kissing. Forensic Sci Int Genet 2013;7:124–8.
[14] Achirapa B, Nathinee P. Optimization of DNA recovery from toothbrushes. Forensic Sci Int Genet Suppl Ser 2008;1:9–10.
[15] Nedel F, André Dde A, de Oliveira IO, Tarquinio B. Demarco Buccal cells submitted to three different storage conditions before DNA extraction. J Appl Oral Sci 2009 March–April;17(2):113–5.
[16] Yamamoto Y, Hara M, Takada A, Kido A, Miyazaki T, Saito K. STR loci analysis of buccal cavity cells captured by laser microdissection. Int Congr Ser 2006;1288:666–8.
[17] Alaeddini R, Walsh SJ, Abbas A. Forensic implications of genetic analyses from degraded DNA—a review. Forensic Sci Int Genet 2010;4(3):148–57.