EXTRACTION OF DNA FROM HUMAN SALIVA AND BLOOD AND GENETIC ANALYSIS OF STR MARKERS-A COMPARATIVE STUDY.

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Summary
As a diagnostic fluid, saliva offers many advantages over serum especially in forensic science as it is good source for DNA retrieval which could be used for individualization. The objective of this study was to evaluate the use of salivary DNA for amplification of Short tandem repeats (STRs) and its comparison with same STRs in blood DNA. Blood and Saliva samples were collected from 20 patients reporting in Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pardesh, after informed consent and approval from Ethics Committee. Extraction of DNA was done through Phenol Chloroform method both in blood and saliva. PCR was used for amplification of 2 Short tandem repeats (STR), Amelogenin (AMG) and von Willebrand factor (vWF) which were then matched with blood. On amplification of AMG in blood all 20 samples (100%) amplified, whereas in case of saliva, out of 20 samples 17 samples (85%) amplified. Out of these 17 samples 16 samples (94.1%) of salivary DNA matched with amplified samples of blood DNA. For the vWF gene all 10 samples (50%) which amplified, matched positively with blood DNA. Based on the obtained results, it may be concluded that amplification of AMG from salivary DNA samples have a high potential and is not inferior to blood.

Keywords: Saliva, Short tandem repeats, Amelogenin, von Willebrand factor.
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Introduction
Human identification is one of the major fields of study and research in forensic science because it deals with the human body and aims at establishing human identity. The revolution caused in by Watson and Crick (1953), who discovered the double-helix structure of DNA, was the basis for the development of techniques that allow characterizing each person’s individuality based on the DNA sequence. Several biological materials may be employed for isolation of DNA and accomplishment of laboratory tests for human identification, including bone tissue, hair bulb, biopsy sample, saliva, blood and other body tissues. It is possible to obtain DNA from virtually all human body tissues, only with variations in the quantity and quality of the DNA extracted from each tissue (Ricardo et al., 2007).

The use of saliva for diagnostic purposes is increasing in popularity. Saliva offers an alternative to serum as a biologic fluid that can be analyzed for diagnostic purposes and has a number of advantages. Whole saliva can be collected non-invasively, and by individuals with limited training. No special equipment is needed for collection of the fluid. Collection of the fluid is associated with fewer problems as compared with the collection of blood. Further, analysis of saliva may provide a cost-effective approach for the screening of large populations. Saliva has a huge potential to be used in forensic science as it is good source for DNA retrieval which could be used for individualization (Eliaz and Ira, 2002; Streckfus et al., 2002).

Short tandem repeat (STR) are DNA markers, also called microsatellites or simple sequence repeats (SSRs) (Comey et al., 1994). STR in DNA is a class of polymorphism that occurs when a pattern of two or more nucleotides are repeated and the repeated sequences are directly adjacent to each other. The pattern can range in length from 2 to 10 base pairs (bp) for
example (CATG)n in a genomic region and is typically in the non-coding intron region, making it junk DNA. It is possible to evaluate DNA from samples with a significant grade of degradation (Anzai-Kanto et al., 2005).

In this study we selected two STRs. One was Amelogenin (AMG) and the second was von willebrand factor (vWF). Amelogenin (AMG) is a sex determining gene which is located on the X and the Y chromosome (Chang et al., 2003). Von willebrand factor (vWF) is located at the tip of the short arm of chromosome 12 and is very useful for application in forensic analysis especially the paternity determination (Comey et al., 1994; Zaverio et al., 1993).

The aim of this study was to evaluate the DNA extracted from saliva and its use for amplification of STRs and its comparison with STRs in DNA obtained from blood.

**Materials and Method**

The sample selected for this study consisted of blood and saliva collected from 20 different subjects reporting in Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pardesh, after informed consent and approval from Ethics Committee. Blood was collected using sterile syringes and were stored in EDTA. Saliva was collected by spitting method by asking the subjects to spit into a sterile test tube. This was later on transferred to a sterile vial at -20°C using a sterile pipette.

**DNA extraction:** DNA extraction in blood and saliva was done by salting out method using phenol-chloroform as described by Comey et al., 1994.

**Selection criteria for AMG and vWF:** Selection of AMG STR marker was done on basis of study conducted by Sullivan et al., 1993. Selection of vWF STR marker was based on the global survey carried out by Perez-Lezaun et al., 1997, in which 20 STR loci were used on samples belonging to 16 worldwide populations which were spreaded over five continents and were analyzed (Table 1). AMG and vWF were then amplified using PCR under suitable conditions (Table 2).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Locus</th>
<th>Repeat Motif</th>
<th>Chromosomal position</th>
<th>Gene</th>
<th>Primer sequences</th>
<th>Size range (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AMG</td>
<td>NA (Butler)</td>
<td>Xp22.1-.3 Yp11.2</td>
<td>Amelogenin</td>
<td>5'-CCCTGGGCTCTGTAAAGAATAGTG-3' 5'-ATCAGAGCTTAAACTGGGAAGCTG-3'</td>
<td>785-953 sullivan</td>
</tr>
<tr>
<td>2</td>
<td>vWF1</td>
<td>TCTA</td>
<td>12p13.3-p13.2</td>
<td>Unknown</td>
<td>5’ CCCTAGTGGATGATAAGAATAAC 3’ 5’ GGACAGATGATAATACATAGGATGGATG 3’</td>
<td>99-134</td>
</tr>
</tbody>
</table>

**Table 1:** Showing details of AMG and vWF markers.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>STR marker</th>
<th>Denaturation Temp</th>
<th>Time</th>
<th>Annealing Temp</th>
<th>Time</th>
<th>Extension Temp</th>
<th>Time</th>
<th>Type of PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AMG</td>
<td>94°C</td>
<td>45 sec</td>
<td>62°C</td>
<td>45 sec</td>
<td>72°C</td>
<td>45 sec</td>
<td>Single</td>
</tr>
<tr>
<td>2</td>
<td>vWF-1</td>
<td>94°C</td>
<td>30 sec</td>
<td>57°C</td>
<td>45 sec</td>
<td>72°C</td>
<td>1 min</td>
<td>Single</td>
</tr>
</tbody>
</table>

**Table 2:** Showing PCR conditions for amplification of AMG and vWF.
Results

Mean Quantity of DNA obtained in blood was 140.65 ± 45.1 µg/ml and in saliva was 48.5 ± 8.1 µg/ml. Quality of blood DNA obtained as assessed by ratio of OD 260 / 280 was found to fall within the range of 1.6-2 whereas it was lower for saliva. Eleven out of twenty samples of salivary DNA were found to fall within this range whereas the remaining 9 samples had lower ratio which may be due to contamination with phenol or protein.

On amplification of AMG in blood all 20 samples (100%) amplified, whereas in case of saliva, out of 20 samples 17 samples (85%) amplified. Amplified samples show two bands in males [heterozygous, XY] and single band in case of females [homozygous, XX] (Fig 1). Out of 17 amplified saliva samples 16 samples (94.1%) matched with amplified samples of blood DNA. On overall matching of saliva and blood DNA samples, 4.2% (3/20) saliva samples did not amplify, 94.1% (16/17) amplified saliva samples matched blood samples, and 5.9% (1/17) amplified saliva samples did not match to amplified blood samples thus giving an overall positive matching of 80% (16/20).

On amplification of vWF in blood, except one sample all the samples got amplified but only half of the samples of saliva amplified. Out of 20 blood DNA samples 19 samples (95%) amplified and in salivary DNA samples, only 10 samples (50%) amplified. 10 samples of saliva which got amplified, all salivary DNA samples matched positively with the corresponding blood DNA samples giving 100% result. On overall matching of saliva and blood DNA samples, 50% success rate was found.

Discussion

DNA can be successfully isolated from salivary samples in optimal quantity for purpose of amplification of STR using polymerase chain reaction. DNA fingerprinting or DNA typing (profiling) was first described in 1985 by an English geneticist named Alec Jeffrey. He found that certain regions of DNA contained sequences that were repeated over and over again next to each other and the number of
repeated sections present in a sample could differ from individual to individual (Comey et al., 1994).

This is a study performed on Forensic Dentistry that used salivary DNA analysis for human identification, highlighting the importance of molecular biology and the importance of saliva over serum in cases of forensic investigation. The purpose of this study was to match the DNA obtained from salivary sample to blood DNA sample using two STR markers Amelogenin (AMG) and von willebrand factor (vWF). Our study was in accordance with the study conducted by Comey et al., 1994 and Holt et al., 2002.

Amelogenin gene is located on the X chromosome (Xp22.1 -22.3) and Y chromosome (Yp11.2) (Chang et al., 2003). As it is located on the sex chromosomes it is suitable for sex determination. It has been widely used for gender identification as it has the ability to designate whether a sample originated is from a male or a female source (Atsushi et al.). It is useful in sexual assault cases, where distinguishing between the victim and the perpetrator’s evidence is important.

Our results showed that on amplification of blood & salivary DNA samples with STR, AMG we found that all 20 samples of blood DNA amplified and 17 samples (85%) of salivary DNA amplified the STR for AMG. Out of total 20 salivary DNA samples 16 samples (80%) matched with the amplified blood samples. Hence, this STR could be amplified in majority of cases (85%) and in large number of samples (80%) sex could be correctly identified. As we achieved high success rate by using this STR in salivary samples, it shows that saliva is not inferior to blood.

vWF has been shown to exhibit high allelic variability, high average observed Heterozygosity, high Polymorphism information content (PIC), and high power of exclusion (Suraksha, 2009). In past it was used for forensic purposes by various groups (Evelyn, 2005; Dawid, 1999). It has also been used for individual identification, phylogenetic reconstruction, and paternity identification cases ((Suraksha, 2005).

In this study we tested the possibility of amplifying vWF in salivary DNA samples and its efficacy in matching with blood samples. We found that though vWF can be amplified for salivary DNA isolates the results were not consistent. Out of 20 samples of blood and saliva, 19 samples (95%) of blood DNA and 10 samples (50%) of salivary DNA amplified the STR. There may be couple of reasons for this finding, most important among them may be the lower purity of DNA obtained from saliva. Other reasons may be small quantity of DNA obtained from saliva (Atsushi et al.) or presence of certain unknown PCR enzyme inhibitors in saliva sample that may be a hindrance for amplification of more sensitive STRs (Dawid, 1999).

Even though the amplification of vWF was not consistent but all samples which were successfully amplified gave correct matching with blood samples. Hence it may be concluded that even though amplification of STR vWF is a technique sensitive procedure, requiring strict control, it is an efficient marker for matching and individualization.

Conclusion

Based on the obtained results, it may be concluded that amplification of AMG from salivary DNA samples have a high potential and is not inferior to blood to be used for forensic individualization process. Even though amplification of STR vWF is a technique sensitive procedure, requiring strict control, it is an efficient marker for matching and individualization. Hence, studies on molecular biology applied to human identification further enhance DNA extraction with less material available and under increasingly adverse conditions. Hence, Saliva has a huge potential to be used in forensic science as it is good source for DNA retrieval which could be used for individualization.
References


