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DNA extraction and quantification from human saliva deposited on fruits with human bite

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Abstract

Background: Among important aspects of forensic science there stand recovery, preservation and analysis of stains originated by body fluid. DNA Isolated from evidence stains help to exclude an innocent suspect or to identify a perpetrator upon PCR-based typing. This study reports extraction and quantification of DNA from human saliva deposited on fruits. The research work was conducted at Department of Forensic Sciences, University of Health Sciences, Lahore and WTO laboratory of University of Veterinary and Animal Sciences, Lahore.

Methodology: DNA from saliva deposited on bitten fruits. DNA from 55 samples were extracted by Chelax method, Quantifiler® DNA Quantification Kit was used for quantification of the extracted samples and amplification of DNA was done in 7500 Real-Time PCR systems (Applied Biosystems, Foster City, CA). Total 55 samples including controls (positive and negative controls) were collected from bitten fruits by sterile swabs.

Results: All sample swabs with human saliva showed result for quantification. Overall good yield of DNA quantification obtained from all fruits and no sample showed internal inhibition. No sample showed non informative or incomplete quantification which occurs due to LCN (Low copy number) or lesser quantity of DNA extracted from saliva swab.

Conclusion: This study has provided with optimized protocol to isolate DNA from saliva found in very minute quantity on organic surfaces like fruits. Adaptation of this method can play a vital role in establishing new trends in human forensics practiced in Pakistan. Moreover, for future work in human forensics, this study can provide important practical basis.

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Introduction

According to fundamental concept of forensic science, a criminal always brings something at the crime scene and after crime there is always something left behind by him or her. That "some-thing" which is left behind provides certain clues and evidences to forensic investigators. It might be blood, fingerprint, tooth marks, footprints, hair, semen, saliva, fibers, a weapon or less touchable observationally important evidences like type of bruises or wound left on the body of victim [1]. It is highly praised aspect that saliva, which is deposited on skin generates a possibility of extraction and quantification of DNA [2].

DNA profile may not always be complete and it is not strange to get mixed or incomplete profiles. With such small amounts of material the validity of any result is conformed by repeating the analysis on the same DNA extract due to the fact of allele drop in (potential contamination), allele drop out (fails to repeat) and stutters (a small repeat of the the true allele profile peak). Only then is it considered to be a true illustration of the DNA present in Sample [3]. Polymerase chain reaction (PCR) based DNA typing is a significant recent forensic technique for the recognition of origin of biological evidence. In all disciplines of forensic science, this powerful technology is being applied whenever it involves biological evidences [4].

Methods

Locally available and most consumed fruits were bitten by male volunteers. These Fruits were divided in to 5 categories according to time of biting i.e. those were swabbed at 0, 6, 12, 24 and 48 hours respectively for preservation of saliva by sterilized swabs. The neat swabs of volunteers were used as control. A negative control (nc) was also run to evaluate the correct procedure. So, total of 55 swabs were collected.

Saliva was deposited by biting the fruits and it was divided into five categorizes according to time i.e. at 0, 6, 12, 18, 24 and 48 hours. Sterile cotton swab, which was previously impressed in sterile distilled water, was used to recover saliva from the site of bite. In this method, sterile distilled water was used to make sterile cotton swab and cotton tip slightly wet. Over the surface of the "Bitten fruit area", this tip was rolled in circular motions by applying moderate pressure. Upon long axis, rotating the swab made maximum contact with "Bitten fruit area" to collect as much saliva as possible. After completion of this procedure, the swab was air dried completely for 30 minutes.

After saliva collection, the swab samples were labeled before storing at $4^{o}C$ for subsequent DNA extraction and quantification. Isolation of DNA from fabrics of swab was done by using Chelex®100 isolation methodology [5]. Quantifiler® Duo DNA Quantification Kit was used for quantification of the extracted samples. Isolated DNA was amplified in 7500 Real-Time PCR systems (Applied Bio-systems, Foster City, Canada).

Results

Total 55 samples including controls (positive and negative controls) were

collected from fruits bites by sterile swabs. All sample swabs with human saliva showed results for quantification.

DNA quantification result at zero (0 hour)

At zero hour means the bitten fruits were swabbed for saliva as soon as the person bit them. All the fruit swabs showed significant DNA quantities when compared with positive swabs. Positive swabs are the neat swabs taken from the check cells of that person who bites fruits at zero hour. Positive swabs were taken before biting the fruits. The positive swab PS "0" showed DNA quantification 7.5 ng which depicts that the person biting the fruits had cells in saliva which contained DNA. The overall yield of DNA results of fruits were apple (Ap) 15 ng, banana (Ba) 9 ng, apricot (Apr) 15 ng, date (Dt) 15 ng, watermelon (Wm) 12.5 ng, guava (Gu) 10 ng, peach (Pe) 7.5 ng and mango (Mn) 5 ng. Negative control (nc) was established which contained all the regents except DNA sample.

The yield of DNA of negative control was 0 (zero) which indicated that the extraction procedure was totally according to SOP (standard operating procedure) and there was no contamination of DNA at any stage of the procedure. At zero "0" hour maximum amount of DNA was extracted from Apple, Banana, Apricot and Date i.e. 15 ng similarly minimum amount of DNA yielded was from mango i.e. 5 ng. There was no fruit which caused inhibition of DNA.

Overall good yield of DNA quantification obtained from all fruits and no sample showed internal inhibition. No sample showed non informative or incomplete quantification which usually happens due to Low copy number (LCN) or lesser quantity of DNA extracted from saliva swab.

DNA quantification result at six (6) hours

Fruits bites were swabbed for saliva after six hours from biting. All the fruit swabs showed DNA quantity. Positive swabs are the neat swabs taken from the check cells of that person who bit fruits at zero hour. Positive swabs were taken before biting the fruits. The positive swabs PS "6" showed DNA quantity of 27.5 ng which depicts that the person who bit the fruits contained cells in saliva. The overall yield of DNA results of fruits were apple (Ap) 52.5 ng, banana (Ba) 9 ng, apricot (Apr) 10 ng, date (Dt) 2.25 ng, watermelon (Wm) 11.25 ng, guava (Gu) 10 ng, peach (Pe) 7.5 ng and mango (Mn) 15 ng. At six "6" hours maximum amount of DNA was extracted from apple i.e. 52.5 ng similarly minimum amount of DNA yield was from date i.e. 2.25 ng. There was no fruit which caused inhibition of DNA.

DNA quantification result at twelve (12) hours

At twelve hours means the bitten fruits were swabbed for saliva after twelve hours when the person bit them. All the fruit swabs showed DNA quantities. The positive swab means the neat swab which was taken from the check cell of a person who bit fruits at zero hour before person bit the fruits. The positive swab PS "12" showed DNA quantity 10 ng which depicted that the person biting the fruits contained cells in

saliva which had DNA. The overall yield of DNA from fruits were apple (Ap) 50 ng, banana (Ba) 175 ng, apricot (Apr) 82.5 ng, date (Dt) 135 ng, watermelon (Wm) 192.5 ng, guava (Gu) 72.5 ng, peach (Pe) 275 ng and mango (Mn) 15.75 ng. At twelve "12" hours maximum amount of DNA was extracted from peach i.e. 275 ng similarly minimum amount of DNA yield was from mango i.e. 15.75 ng. There was no fruit which caused inhibition of DNA.

DNA quantification result at twenty four (24) hours

At the twenty four (24) hours mean the bitten fruits were swabbed for saliva after twenty four hours when the person bit them. All the fruit swabs showed DNA quantities. The positive swab means the neat swab was taken from the check cell of a person who bit fruits at zero hours before person bit the fruits. The Positive swab PS "24" showed DNA quantity of 57.5 ng which depicts that the person biting the fruits contained cells in saliva which had DNA. The overall yield of DNA results of fruits were apple (Ap) 22.5 ng, banana (Ba) 45 ng, apricot (Apr) 17.5 ng, date (Dt) 13.75 ng, watermelon (Wm) 10.75 ng, guava (Gu) 13.75 ng, peach (Pe) 11.25 ng and mango (Mn) 12.5 ng. At twelve "24" hours maximum amount of DNA was extracted from banana i.e. 45 ng similarly minimum amount of DNA yielded was from watermelon i.e. 10.75 ng. There was no fruit found which caused inhibition of DNA.

DNA quantification result at forty eight (48) hours

At forty eight (48) hours mean the bitten fruits were swabbed for saliva after forty eight hours when the person bit them. All the fruit swabs showed DNA quantities. The positive swab means the neat swab which was taken from the check cell of a person who bit fruits at zero hours before person bit the fruits. The Positive swab PS "48" showed DNA quantity of 11.25 ng which depicts that the person biting the fruits contained cells in saliva which had DNA. The overall yield of DNA results of fruits were apple (Ap) 9.5 ng, banana (Ba) 14.25 ng, apricot (Apr) 10.75 ng, date (Dt) 30 ng, watermelon (Wm) 10 ng, guava (Gu) 9.25 ng, peach (Pe) 9.25 ng and mango (Mn) 11.25 ng. At twelve "48" hours maximum amount of DNA was extracted from date i.e. 30 ng similarly minimum amount of DNA yielded was from guava and peach i.e. 9.25 ng. There was no fruit which caused inhibition of DNA.

Discussion

Forensic genetics has been playing its role in resolving the legal problems such as paternity tests and establishing identity in criminal cases through DNA typing analysis and comparisons. Techniques involved in forensic genetics proceed with the aid of biological evidence, found at crime scenes and case history of individuals. These biological evidences include different body secretions and constituents. Pakistan is lacking in recent advancements in forensic genetics as compared to rest of the world.

Only few departments have been established to adopt updates of forensic science due to lack of research activities.

Present study was designed to validate an efficient methodology for DNA extraction and quantification from different fruits used as salivary substrates in Pakistan. This was pioneer study through which extraction and quantification of DNA was made possible from different fruits. Reasonable quantities of DNA were obtained from different fruit bites that were used as salivary substrates which were used to quantify DNA. Quantities of DNA extracted from different salivary fruits substrate corresponded to the study of DNA extraction from saliva instead of blood [6].

In present study DNA was extracted from salivary substrates using modified Chelex extraction method. Chelax extraction method was found to be very useful especially in Low copy number LCN DNA. The modified chelex extraction method was found to be better in saliva cases as compared to phenol chloroform and chelex method [7]. DNA extraction was followed by Real –time PCR for quantification using Quantifiler^{TM} Quantification kit. This assay was able to detect and quantify even very small amounts of DNA present in the sample. Validation of this quantification technique is comparable to the study in which Quantifiler^{TM} DNA quantification kit was used for Real-time PCR based quantification [8].

As it is evident that DNA quantity recovered from fruit bite surface varies a lot from one fruit to other. Some, as expected, has yielded lower DNA after passing longer periods of time than at 0 hour e.g. apple, but this doesn't stand true when it comes to dates as human DNA quantity from this fruit bite increases in extraction after 48 hours in opposite to extractions after 6, 12 and 24 hours.

DNA extraction and developing its genotypic profile had been in practice in Pakistan from the samples having bulk of living tissue such as blood etc. Present study has established a valid protocol of DNA extraction from salivary substrates and their quantification by Real-time PCR using commercially available kit. Moreover, this investigation can become a strong basis for following research works expected in future in field of human forensics.

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