Project Report

On

Comparative Analysis of DNA from Fingernail (Exogenous DNA) Sample stored in different conditions

Submitted in Partial Fulfillment of the Requirement for the

Degree of M.Sc. Forensic Science

Submitted By

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CERTIFICATE

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This is to certify that the Project entitled **"COMPARATIVE ANALYSIS OF DNA FROM FINGERNAIL (EXOGENOUS DNA) SAMPLE STORED IN DIFFERENT CONDITIONS"** submitted in partial fulfillment for the award of the Degree of **M.Sc. (FORENSIC SCIENCES)** from Galgotias University, Greater Noida, is a record of research work carried out by **MS. SHAILY SHARMA** from 13th January 2020 to 16th March 2020 under my guidance and supervision.

All the help and assistance received during the course of this investigation have been duly acknowledged.

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CANDIDATE DECLARATION

I hereby declare that the dissertation entitled "Comparative Analysis of DNA from fingernail (Exogenous DNA) Sample stored in different conditions" submitted by me in partial fulfillment for the degree of M.Sc. Forensic Science to the Division of Forensic Science, School of Basic and Applied Science, Galgotias University, Greater Noida, Uttar Pradesh, India is my original work. It has not been submitted in part or full to this University of any other Universities for the award of diploma or degree.

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SHAILY SHARMA

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ABSTRACT

To prove the involvement of a person in criminal cases become difficult if there is shortage of physical evidences and evidences may get degraded. There are various cases of homicide, assault cases increases day by day in whole world and cases of physical assault occurs, mostly and mainly female is victim. To identify or individualize a perpetrator in assault cases, DNA is very helpful evidence. During any physical assault either it is sexual or non-sexual, there are always transfer of biological material such as saliva, blood, tissue, epithelial cell, semen etc. between victim and assailant. As according to Locard's Exchange Principle in Forensics, "There is always transfer of material between victim and perpetrator during any crime, and criminal always leaves a clue behind it". For instance, if a victim scratches the assailant by fingernail forcefully in order to save herself during assault, so biological material or debris from the assailant may get intact inside or beneath the fingernail as foreign material. Thus, nails are important source which acts as carrier of foreign DNA in culprit identification. Therefore, fingernail are examined for the exogenous DNA present in the foreign material located beneath the fingernail of victim. It provides evidence of occurrence of any physical contact between victim and suspect or was possibility of occurrence. In this study we discuss about the exogenous DNA result obtained from analysis of debris found under hyponchium (an area of epithelium of nail) of fingernail of victim in different condition like may deceased body found in submerged water, soil etc. and protocol of about the best method for the collection of fingernail samples. Process of extraction of exogenous DNA from sample, purification of it to applies Y-STR technique to find evidentiary material in order to identify assailant regarding criminal cases.

INTRODUCTION

DNA is the hereditary material found in each cell of organisms (except erythrocytes). It is a double standard molecule, which twisted like a helical structure or called “helix”. It has useful biologically information to make protein on the basis of genetic code. Even we all are unique although every human being shares 99.9% of their DNA but there is only 0.1% every person differ from another person and this difference between organisms due to the order of base pair. DNA profiling which is also known as the DNA fingerprinting. It is a process used to determine the DNA characteristics of an individual or show specific DNA pattern obtained from a person tissue. For example:- The different sequences is the same as the word “POST” has different meaning from “STOP” or “POTS” even though they use same letters (14). DNA profiling are taking popularity day by day because evidence found on crime scene like bones, skin, skull and files etc. forensic DNA plays an important role in criminal justice system, because **DNA itself a secret to solve the all secret.** During any physical assault either sexual or non -sexual, biological evidence may be transferred between assailant and victim during victim struggle. Any material such as fibers, nails, hairs, blood, and epithelial cells etc. can be transferred between them (12, 13, 41). We can collect many trace evidence during assault cases but among them one is collection of fingernails of victim. Nails contain genomic DNA that can be used for genetic analyses, which is important for forensic studies 12, 40). The DNA present in nail clippings originate from a germinal matrix cell in the nail root. Nails are important tissues for personal identification of human because of their morphological traits as well as bio-molecular information (24, 25, 35, 36). Nature of nail growth also provides unique chronological information along the length of the nail and biological inspection of recent life history (35). Nails are easy and cheap to obtain and store as compared to other biological evidences such as saliva or blood. For a case, if victim tries to save herself during assault, victim could have scratched the assailant over his forearm or face (12, 13, 40). So, there is some cell of assailant or blood of assailant may be found as foreign material beneath the fingernail from which foreign DNA can be extracted and leads to assailant. Although, the use of hair sample for DNA extraction is well-known but there are many practical problems encounter with the use of hair sample because there is very insufficiency of DNA in hair, provided follicle which implies that hair should be pulled from the root not broken one (8, 25) Hence hair sample found at crime scene may not be necessarily pulled from hair root and it is so, it is necessary it would be sufficient for the extraction of DNA to aid the criminal investigation. In contrast to this, the department of neurology at Baylor College of medicine in its analysis program STOP SUDEP (Sudden

Unexpected Death in Epilapsy) reported that largely hair samples found at crime sites are often without follicles and they could also be treated with thermal and chemical process with varied hair products (8, 19). Violent crimes occur in the United States in every 26.2 seconds, according to the Uniform Crime Report (2011) (12), During any assault or homicide cases, biological material may be transfer between assailant and victim during victim's struggle (any material such as blood, saliva, epithelial cells, skin part, fiber, hair, etc.), can be transfer between them (7, 12, 13, 39). We can collect many trace evidences during assault but among them one is collection of fingernails of victim, because nails are easy and cheap to obtain and store as compared to other sample as saliva or blood. Biological evidence contain foreign material may originate from more than one source and as consequences a mixed DNA profile will be produced during analysis and provide evidential material which help in police investigation (8, 35, 39, 40). Sexual violence against women have been studied in many populations in which finding of foreign DNA profiles beneath the fingernail sample, which vary between 12%-71% in DNA positivity depends on the scratching intensity and analytical method which will be used (41). A potential source of DNA is through the collection of debris (foreign material) from fingernail hyponchium (an area of nail epithelium) of victim (39, 41). For instance, after assault assailant may throw the body of victim in water or may deceased body found under submersion of water or placed in soil etc. in cases of homicide (13, 21, 40). The method for both collecting and processing nail evidences generally vary among practitioners depending upon the what type of analysis carried out by means for identification of drug (toxicological analysis) or identification of individual by DNA analysis (serological analysis). Fingernail from hands are removed with the use of sterile nail clippers, nail swabbing, and scraping of nails with wooden applicator (12, 13, 40, 41), usually if the victim does not want to cut nails or if the nails are already submitted by themselves in laboratory they can scraped, swabbed, or clipped and directly put into the lysis buffer (12). Foreign DNA located inside the fingernail might be a significant evidence for criminal investigations or forensic casework for individualization of a person (33, 41). DNA transfer can be occurred due to any previous intimate physical contact may not related with crime (12, 30, 40). The chances of foreign DNA profiles located under the fingernail samples from donors varies from 6% to 24%, hence amount of exogenous material found beneath the fingernail depends on number of evidential samples occurred or collected in sexual assault cases (13, 30, 33). It is found that exogenous DNA presence is rare with exception of couples having an intimate between them (39, 40). DNA isolation method and analysis method may change depends on what type of foreign material (like blood, saliva, skin piece etc.) recovered from the crime scene. Previously includes, organic extraction method or commercial kit use and other automated processes for the latter, DNA analyzed by PCR technique to get multiple copies of DNA sample(1, 2) and autosomal STRs or Y-STRs, to prove or disprove the involvement of

male attacking a victim in assault case (2, 12, 13). Because limited amount for foreign materials occurs from fingernails sample, should tries to extract the maximum amount of exogenous DNA while minimize the removal of endogenous DNA in order to obtain a complete DNA profile of assailant from exogenous DNA because endogenous DNA can be collected by donor. (12, 13).

For example: - if blood is found beneath the female fingernail we can analyses it and apply Y-STR technique over the sample. During the process of collection of fingernails from donors it may contains exogenous and endogenous DNA but we should try to collect the maximum amount of exogenous material to found foreign DNA because donor DNA we can get from any other source (Saliva , Blood) from donor , So minimizes the detection of endogenous DNA.

The all the items of trace evidences collected by experts , in some cases often collected are fingernails, because the victim could have scratched the assailant or any other act so that exogenous material was deposited beneath the nails. These nails are then passed along to the Forensic laboratory, where they may be analyzed.

The methods for collection and analyzing nail evidence vary widely between analysts. It vary due to choose different methods for extracting foreign material and identification of DNA from biological tissue by using different techniques (like Laser induced breakdown spectroscopy, X-ray technique, PCR etc.) (4, 5, 8) by the practitioners.

Sample are collected to put in digestion buffer for 5min. to 10 min. and detergents like SDS , NaCl etc. because the digestion buffer and strong detergents release the exogenous fingernail DNA, thus increasing the chance of obtaining a DNA mixture in which the exogenous DNA extract.

The goal of this study is to compare three different sampling methods (swabbing, clipping, and scraping) in order to identify the maximum amount of foreign/exogenous male DNA recovered. The discussion will focus on the comparison of the detection of foreign/ exogenous male DNA from fingernail sample in different conditions. It also helpful to provide personal identification of victim and identification of culprit on the basis of Y –STR in assault cases.

OBJECTIVES

- Collection of fingernails by different methods i.e.
  a) Nail clipping
  b) Swabbing
  c) Scraping.
- DNA isolation and visualization from different fingernail samples.
- Analysis of the DNA isolated using Y-STR - PCR technique.

REVIEW OF LITERATURE

- In December 2015, David Foran *et al;* worked on Trace DNA from Fingernails: Increasing the Success Rate of Widely Collected Forensic Evidence. His work result showed that three different method of collecting exogenous DNA from fingernail sample (clipping, swabbing, scraping), which produced significant difference in DNA yield. He further analyzed the sample by using Y-STR. He also generated endogenous DNA from fingernail sample and compare the Y-STR analysis of both endogenous and exogenous DNA and which results strong exogenous profile were produced. His result of different collection method showed that exogenous DNA collected by clipping gives good result, by swabbing gives intermediate result and by scraping resulted in the least exogenous DNA (12).

- In 2018, Oorschot *et.al;* in their paper, DNA transfer in forensic science: a review, explain about DNA transfer as a trace evidence and how it is useful in criminal investigation. The study shows that how the DNA transfer between two individuals (means unidirectional transfer, bi or multi step transfer), persistency and prevalence of DNA in all aspects of different cases (homicide or assault cases etc.), how material for DNA recovered from different type of tissues (blood, saliva, fingernail, skin or any other biological material), how sampling is done, what the impact on samples to apply different kind of extraction, collection and storage techniques, and interpretation of DNA profiles. Hence, they studied about what are the variable impact of DNA transfer, persistency, prevalence and recovery (DNA-TPPR) in different investigation of criminal activities and review mainly, focuses on the current situation related to DNA-TPPR to assist the casework in criminal investigation by different samples (40).

- In 2017, Chander Grover and Shikha Bansal in their work, the nail is an investigation tool in medicine: what a dermatologist ought to know explain about why nails use for forensic and clinical analysis over the any other tissue such as saliva, blood etc., they also explain about nail structure and how to use nail as a source of DNA in investigations. They also gave brief description about exogenous or foreign material found intact in nails from which foreign DNA may isolated for the identification of a person in forensic investigation. The examination of nail depends on the type of investigation carries out means, nails are used for the examination of drug (toxicological analysis) or for the identification of the person by exogenous and endogenous DNA (serological analysis) and brief description about the

techniques used for examination of nails in different roles depends on analysis type (clinical, toxicological or serological analysis etc.) (8).

- In 2019, Denna D. Hayden, Jeanette M. Wallin in their research work, a comparative study for the isolation of exogenous trace DNA from fingernails, performed three different methods (clipping, swabbing and scraping) for sampling and compared them in order to identify which method is suited well to extract the minimum amount of endogenous DNA and maximizes the amount of exogenous DNA from fingernail sample and their result shows that swabbing method is best method for the recovering of Foreign DNA and minimizes the amount of exogenous DNA from the fingernail (13)

- In May 2017, Loveleen, in her paper , role of nail striation in forensic identification compare inter and intra striation of fingernail in which inter nail striation does not show any matching result and similarly same result occurs when compare the toe nails striations, the work also explain about uniqueness and persistency of nail striations so nail clippings are useful as evidence for the purpose of identification because fingernail striations also possess unique characteristics as that of fingerprints (28).

- In September 2017, Alessandra luvaro *et al;* in their paper male DNA under female fingernail after scratching: transfer and persistence evaluation by RT-PCR and Y-STR typing discussed about the analysis of male DNA found underneath the fingernail female after scratching experiment by using RT-PCR and Y-STR technique. They gave observation on the basis to perform practice of sample they collected on the same day and 5 hours of the collection. Their result show significantly decrease in Y-STR profile quality and Y-STR typing of exogenous DNA may provide genetic information about male suspect but in after 5 hours sample scratching profile male DNA were lost in between parts of samples (2).

- In September 2008, Simon Malsom, Lindsey Dixon, in their work compared female fingernail foreign DNA sample with both male suspected and her spouse reference sample by using PCR or Y-STR technique on there all sample (exogenous DNA)collected by swabbing of fingernails of females in order to detect the presence of Y-chromosome. It was found in samples (male suspect) that 63% of samples shows full or partial profile for Y-

chromosome. Then compared with her partner's profiles and searched through two halotype databases to determine evidential value of that samples (39).

- In December 2012, Pragnesh Parmar, Gunvanti B. Rathod in their Review research paper "Forensic Onychology : An essential entity against crime explain about nail nature, structure, nail DNA , exogenous DNA, recovering of it and what techniques has been used if drug found as foreign material inside the nail, to identified it and brief explanation of how to DNA extraction and said nail is important trace as compare to other tissues, it may use to determine pattern of drug and recent crime history and relatively survive well as compare to soft tissue in the decomposition environment. Therefore, nails provide useful genetic information for identification (35).

MATERIAL AND METHODOLOGY

MATERIAL

- Firstly, all the equipment's and chemicals were sterile.
- Number of Eppendorf's (2ml), micropipette's 100µl-1000µl, 10µl-100µl, 1µl-10µl, 1000rpm centrifuge, water bath incubator, vortex, ice, electrophoresis unit and other chemicals as follows in table 1 :-

1. Saliva DNA lysis buffer	50Mm Tris-Cl, 10Mm EDTA, PH-8, 10% SDS (mix in 50ml sterile distilled water)
2. 5M NaCl	14.5g NaCl in 50ml sterile distilled water
3. RBC lysis buffer	0.60g Tris HCl, 0.37g KCl, 1.01g $MgCl_2$, 0.37g EDTA (mix in 500ml autoclaved distilled water)
4. WBC lysis buffer	0.12g HCl, 0.07g KCl, 1.2g $MgCl_2$, 0.07g EDTA, 0.46g NaCl (mix in 100ml autoclaved distilled water)
5. 6M NaCl	8.76g NaCl in 25ml autoclaved distilled water
6. Triton X	0.2ml 100% Triton X in 19.8ml autoclaved distilled water
7. 10% SDS	10g SDS in 100ml autoclaved distilled water
8. Agarose gel	0.35g agarose in 100ml distilled water, added 1ml TAE buffer
9. 50XTAE buffer	200ml distilled water, 60.5g Tris base, 18.88g EDTA (mix), 57.2ml Glacial acetic acid

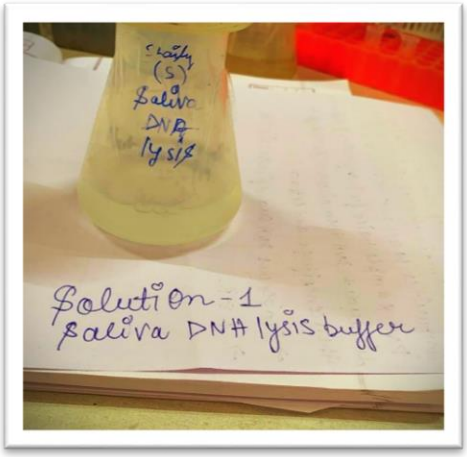

Fig. 1 Saliva DNA lysis buffer

fig. 2 Blood DNA extraction solutions

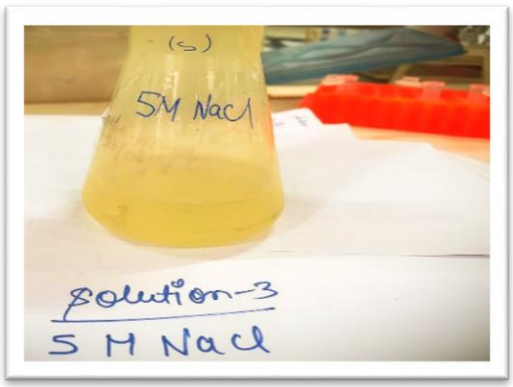

Fig. 3 5M NaCl

fig. 4 Centrifuge

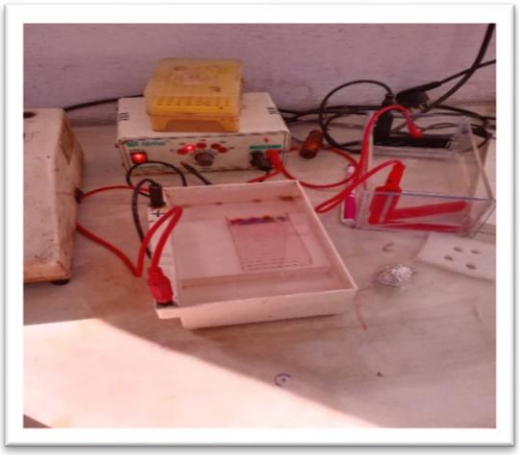

Fig. 5 Electrophoresis unit

METHODOLOGY

Collection of samples

A number of fingernail samples of females were collected from female donors and stored them at -20ºC until use. All control samples of male blood collected from one male volunteer and saliva sample were collected from another male donors. The sample collected from female donors to extract exogenous DNA from debris or foreign material.

Collection of fingernails could be done by three methods:

- **Clipping**: Clipped the nails with sterile clipper to avoid any contamination and packed in small packets separately for further extraction and identification.
- **Swabbing**: Debris were swabbed beneath the fingernail by using wet cotton bud.
- **Scraping**: It is done beneath the nail generally using a wooden applicator and collecting the debris.

Condition provided to samples as follow:-

- Fingernail Samples stored under normal condition.
- Fingernail Samples in submerged water (as found in homicide cases).
- Provide burn condition to fingernail.
- Observe by placing under soil (secondary crime scene cases).

Initial experiments, collection of samples

Collection of fingernails by clipping method

Initially, fingernail samples from female donors collected by clipping method. Nails were clipped by using sterile nail clipper and transferred them on a paper and stored in sterile box to prevent it from contamination.

Collection of blood and saliva from male donor

Blood sample donated by from single male donor in anticoagulant coated vacutainers and saliva sample were collected by a two male volunteers by spitting in a sterile flask and stored at 4ºC for further use.

Preparation of nail

Firstly, fingernail clippings cleaned with sterile water and then known amount of male blood were placed on fingernail clippings as exogenous material for the extraction of exogenous DNA. Similarly, saliva sample of known amount placed on fingernail clippings. Now, nail sample ready

for comparison with control sample. 6 Control sample were taken by using blood and saliva sample without providing any condition.

DNA EXTRACTION AND PURIFICATION

Two DNA isolation methods were used one for the extraction of DNA from blood and another from saliva in different conditions.

By using saliva as a debris

Condition applied on fingernail samples

Normal condition with different amount of saliva

Procedure:

- Saliva samples of different amount taken in Eppendorf tubes.
- Saliva DNA lysis buffer was added in all Eppendorf tubes, mixed by inverting the tubes several time.
- The samples incubated at 65ºC for 30 minutes. Then cooled the samples at room temperature.
- Added 5M NaCl in samples and vortexed at high speed.
- Then incubated on ice for 8 minutes and centrifuged at high speed (10000 rpm) for 5 minutes.
- Transferred the supernatant in new Eppendorf tube and added chilled ethanol to chelate the extract DNA and inverted the tubes several time (50 minutes), centrifuged at high speed (10000 rpm) for 5 minutes.
- Discarded supernatant carefully without dislodging the pellet of extract DNA in each tube, air dried the tube until smell of ethanol vaporized.
- Add 20µl TE buffer in each Eppendorf tube to mixed the DNA pellet and stored at -20ºC till used, run the DNA on agarose gel to visualize the DNA band concentration.

  The following table 2 shows the amount of different chemical used with different amount of saliva:

Saliva Amount	Saliva DNA lysis buffer	5M NaCl	Chilled Ethanol
300µl	750µl	300µl	500µl
150µl	375µl	150µl	250µl
100µl	250µl	100µl	167µl
50µl	125µl	50µl	84µl

Condition: Saliva sample Submersion in water

Control sample without water

Procedure:

- Taken 300µl saliva sample of different amount in each of two Eppendorf tubes.
- Added 750µl saliva DNA lysis buffer in all Eppendorf tubes, mixed by inverting the tubes several time and followed by the isolation procedure as same mentioned in normal condition in different amount of saliva.

Submersion of sample in water for 0 day

- Taken 300µl saliva in each of five Eppendorf tubes containing fingernail clippings and taken 100µl of water in another five Eppendorf's and transferred the nail clipping containing saliva in these tubes.
- Centrifuged at 10000 rpm for 5 minutes and transferred the supernatants into new Eppendorf's and isolate the exogenous DNA with same procedure as mentioned earlier.

Submersion of sample in water for 2 days

- Taken 300µl saliva in each of five Eppendorf tubes containing fingernail clippings and taken 100µl of water in another five Eppendorf's and transferred the nail clipping containing saliva in these tubes and kept for 2 days.
- After two days, centrifuged at 10000 rpm for 5 minutes and transferred the supernatants into new Eppendorf's and further followed isolation procedure.
- Then added 20µl TE buffer in each Eppendorf tube to mix the DNA pellet, then transferred all the extracted exogenous DNA into one Eppendorf tube and stored at -20ºC till used.

Then, 20µl of DNA from control sample and 20µL of each from 0 and 2 days sample extracted and other kept in -20ºC and run the samples on agarose gel to visualize the DNA band concentration through gel electrophoresis.

Condition: Saliva sample placed in soil

Control sample without soil

Procedure:

- Taken 300µl saliva sample of different amount in each of two Eppendorf tubes.

- Added 750µl saliva DNA lysis buffer in all Eppendorf tubes, mixed by inverting the tubes several time and isolated exogenous DNA same as proceed earlier.

Sample placed in soil for 0 day (PH-6.45)

- Taken 300µl saliva in each of five Eppendorf tubes containing fingernail clippings and taken few amount of soil in another five Eppendorf's and transferred the nail clipping containing saliva in these tubes.
- Centrifuged at 10000 rpm for 5 minutes and transferred the supernatants into new Eppendorf's followed by isolation procedure to extract exogenous DNA.

Sample placed in soil for 2 days

- Taken 300µl saliva in each of five Eppendorf tubes containing fingernail clippings and taken few amount of soil in another five Eppendorf's and transferred the nail clipping containing saliva in these tubes and kept for 2 days.
- After two days, centrifuged at 10000 rpm for 5 minutes and transferred the supernatants into new Eppendorf's and afterwards extract DNA by isolation process as mention earlier.

Then, 20µl of DNA from control sample and 20µL of each from o and 2 days sample extracted and other kept in -20ºC and run the samples on agarose gel to visualize the DNA band concentration through gel electrophoresis.

Condition: saliva sample placed in weak acid (PH-2.32)

Control sample without acid

Procedure:

- Taken 300µl saliva sample of different amount in each of two Eppendorf tubes and proceed with isolation process of saliva as mentioned earlier.

Sample placed in acid for 0 day

- Taken 300µl saliva in each of five Eppendorf tubes containing fingernail clippings and taken 50µl weak acid (glacial acetic acid) in another five Eppendorf's and transferred the nail clipping containing saliva in these tubes.
- Centrifuged at 10000 rpm for 5 minutes and transferred the supernatants into new Eppendorf's followed by isolation process.

Sample placed in acid for 2 days

- Taken 300µl saliva in each of five Eppendorf tubes containing fingernail clippings and taken 50µl of weak acid (glacial acetic acid) in another five Eppendorf's and transferred the nail clipping containing saliva in these tubes and kept for 2 days.
- After two days, centrifuged at 10000 rpm for 5 minutes and transferred the supernatants into new Eppendorf's and then, performed isolation as mentioned earlier.
- Add 20µl TE buffer in each Eppendorf tube to mix the DNA pellet, then transferred all the extracted exogenous DNA into one Eppendorf tube and stored at -20ºC till used.

Then, 20µl of DNA from control sample and 20µL of each from o and 2 days sample extracted and other kept in -20ºC and run the samples on agarose gel to visualize the DNA band concentration through gel electrophoresis.

Blood sample used as debris

Condition blood sample submerged in water

Control sample without water

Procedure:

- Taken 300µl of blood sample in 2 ml of each three Eppendorf tube, added 900µl RBC lysis buffer in all Eppendorf tubes.
- Mixed by inverting the tubes several times and then added 50µl Triton X in each tube and mixed by inverting several times.
- Incubated at room temperature for 5 minutes, centrifuged at 10000rpm for 5 min. and discarded the supernatant from all tubes.
- Again added the 900µl of RBC lysis buffer in all Eppendorf tubes, 30µl of Triton X in the above pellet, mixed it several times in all tubes and incubated at room temperature for 5 minutes.
- Then, centrifuged at 10000rpm for 5 minutes and discarded the supernatant. Then, RBC's completely lysed and white color WBC pellet appeared.
- In WBC pellet, added 300µl of WBC lysis buffer and mixed by inverting these tubes several times. Then added 40µl 10% SDS in all tubes, mixed properly by inverting the tubes several times. Incubated at room temperature for 5 minutes.
  At the end of incubation, added 100µl NaCl in all Eppendorf's and mixed properly and centrifuged at 10000 rpm for 5 minutes.

- Transferred the supernatant into new tubes and then, added 300µl chilled alcohol (ethanol), mixed it properly by many times and centrifuged at 10000 rpm for 10 minutes.
- Discarded the supernatants and mark the pellet at end carefully and kept at room temperature until the pellet completely dry.
- Add 20µl TE buffer in each Eppendorf tube to mix the DNA pellet, then transferred all the extracted DNA into one Eppendorf tube and stored at -20ºC till use.

Sample submersion in water for 0 day

- Placed the nail clipping in each of five 2ml Eppendorf tubes and added 300µl blood on it in all tubes. Taken 100µl of water in another five 2ml Eppendorf tubes and transferred the nail sample containing blood into it.
- Centrifuged the samples for 5 minutes and transferred the supernatant into new Eppendorf's.
- Added 900µl RBC lysis buffer in all Eppendorf tubes and further followed by isolation process of DNA from blood similarly as performed in control sample.

Sample submersion in water for 2 day

- Placed the nail clipping in each of five 2ml Eppendorf tubes and added 300µl blood on it in all tubes. Taken 100µl of water in another five 2ml Eppendorf tubes and transferred the nail sample containing blood into it. Kept it for 2 days and after 2 days…
- Centrifuged the samples for 5 minutes and transferred the supernatant into new Eppendorf's and performed isolation of DNA as mentioned above
- Then, 20µl of DNA from control sample and 20µL of each from o and 2 days sample extracted and other kept in -20ºC and run the samples on agarose gel to visualize the DNA band concentration through gel electrophoresis for comparison.

Condition: Blood sample placed in soil (PH-6.45)

Control sample without soil

Procedure:

- Taken 300µl of blood sample in 2 ml of each three Eppendorf tube, added 900µl RBC lysis buffer in all Eppendorf tubes and performed isolation.

Sample placed in soil for 0 day

- Placed the nail clipping in each of five 2ml Eppendorf tubes and added 300µl blood on it in all tubes. Taken few amount of soil in another five 2ml Eppendorf tubes and transferred the nail sample containing blood into it.
- Centrifuged the samples for 5 minutes and transferred the supernatant into new Eppendorf's and further followed by the steps of isolation as mentioned above.

Sample placed in soil for 4 days

- Placed the nail clipping in each of five 2ml Eppendorf tubes and added 300µl blood on it in all tubes. Taken few amount of soil in another five 2ml Eppendorf tubes and transferred the nail sample containing blood into it. Kept it for 4 days and after 4 days…
- Centrifuged the samples for 5 minutes and transferred the supernatant into new Eppendorf's.
- Added 900µl RBC lysis buffer in all Eppendorf tubes and performed all the steps of isolation as mention earlier.

Then, 20µl of DNA from control sample and 20µL of each from o and 2 days sample extracted and other kept in -20ºC and run the samples on agarose gel to visualize the DNA band concentration through gel electrophoresis.

Condition: blood sample placed in acid (PH-2.45)

Control sample without acid

Procedure:

Taken 300µl of blood sample in 2 ml of each three Eppendorf tube, added 900µl RBC lysis buffer in all Eppendorf tubes and performed isolation process of DNA as mentioned above.

Sample placed in acid for 0 day

- Placed the nail clipping in each of five 2ml Eppendorf tubes and added 300µl blood on it in all tubes. Taken 50µl of acid in another five 2ml Eppendorf tubes and transferred the nail sample containing blood into it. After addition of acid samples in all the Eppendorf's got freeze then kept the all samples boiling water bath but it did not get melt, therefore, was not performing the process of isolation for 0 day and for 2 days samples.

Comparison of exogenous DNA (placed in different conditions) with control sample DNA

After isolation of DNA from samples placed in different conditions compared with the control sample (through agarose gel electrophoresis) to observed that DNA got degraded or not and variation in amount of concentration of DNA (good concentration or dilute concentration) in comparison of control samples.

Result and Discussion

Initially, sample were collected by using clipping method from female volunteers and collection of external material (blood and saliva) from male donors which were use as debris for the experiment and placed biological material over the fingernails clipping collected from females to prepare nail sample for extraction of exogenous DNA. 6 control samples were also created by both biological tissue (blood and saliva) donated by male donors for making comparative result.
Nail samples placed in different conditions such as submersion of water, buried in soil etc. for different period of time and after applying condition performed the isolation procedure to extract exogenous DNA and after gel electrophoresis, the following observation has occurred:-

When saliva used as debris

1. For the normal condition (without applying any condition) when different amount of saliva has been used their result as follow:

Fig. 6 normal condition sample of different saliva amount (left to right)
Well 1: 300µl
Well 3: 150µl
Well 5: 100µl
Well 7: 50µl

Observation Table 3:

WELL	AMOUNT OF SALIVA	DNABAND CONCENTRATION
Well 1	300µl	DNA band observed in good concentration
Well 3	150µl	DNA band observed in good concentration
Well 5	100µl	DNA band observed in dilute concentration
Well 7	50µl	DNA band observed in very dilute concentration

By using different amount of saliva it would be observed that in the wells (1, 3) DNA band observed in good concentration and dilute concentration of band observed in well 5 and 7 as compare to two other wells. So, it indicates that it does not matters what amount of biological tissue recovered from crime scene if matter did not get contaminated it will gives good result but concentration of DNA band may vary depends on amount of biological sample.

2. Condition: sample submerged in water

   The fingernails samples submerged in water for 0 day and 2 days for the experiment to check DNA band concentration and observation as follows:

Fig. 7 saliva control sample

Fig. 8 nail sample containing saliva (Submerged in water for 0 day)

Fig. 9 nail sample containing saliva
(Submerged in water for 2 days)

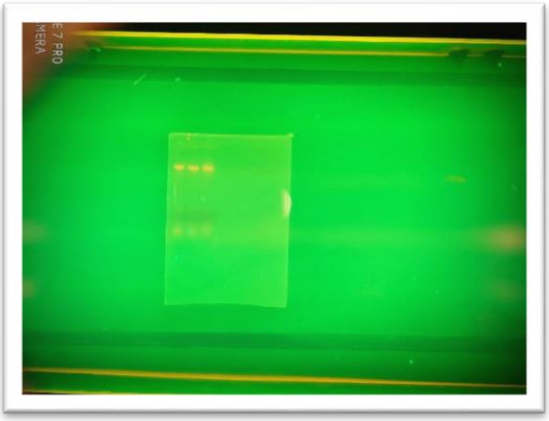

Fig. 10 saliva sample, submersion in water (left to right)

Well 1: control sample

Well 2: 0 day sample

Well 3: 2 day sample

Observation Table 4:

WELL	**DNA BAND CONCENTARTION**
Well 1	DNA band observed in good concentration
Well 2	DNA band observed in good concentration
Well 3	DNA band observed in slightly dilute concentration as compare to well 1 and 2

After submersion of samples in water condition when compared with control sample it has observed that control sample gives good DNA concentration and 0 day sample DNA also found in good concentration but when we compare 2nd day sample with 0 day sample it get slightly dilute in terms of concentration.

3. Condition: sample buried in soil

   There are many condition when we found the victim's body buried inside the soil, in that case DNA get depleted or not. So the condition was artificially created in laboratory to place the sample in soil (PH 6.45) for 0 and 2 day, performed the experiment which result as follows:

Fig. 11: control sample

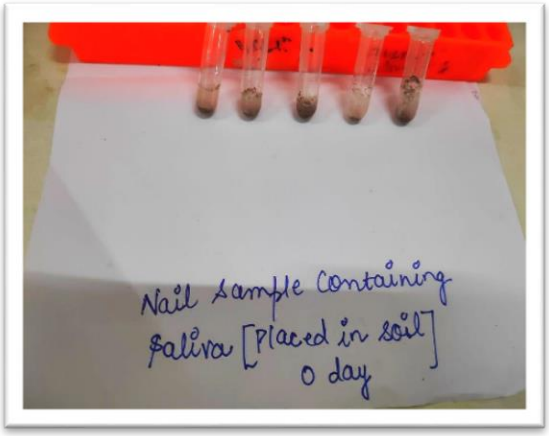

Fig. 12: nail sample containing Saliva (placed in soil for 0 day)

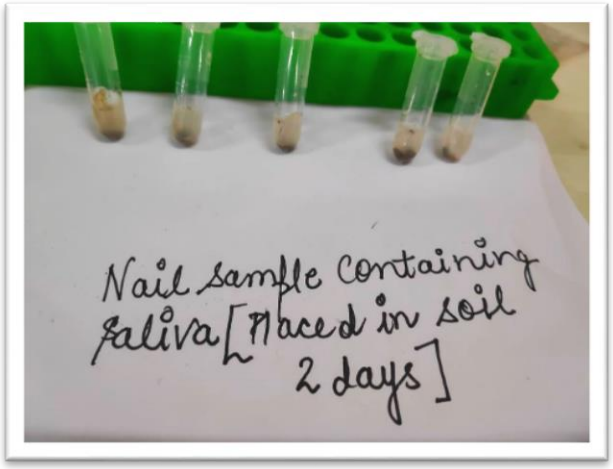

Fig. 13: nail sample containing saliva (Placed in soil for 2 days)

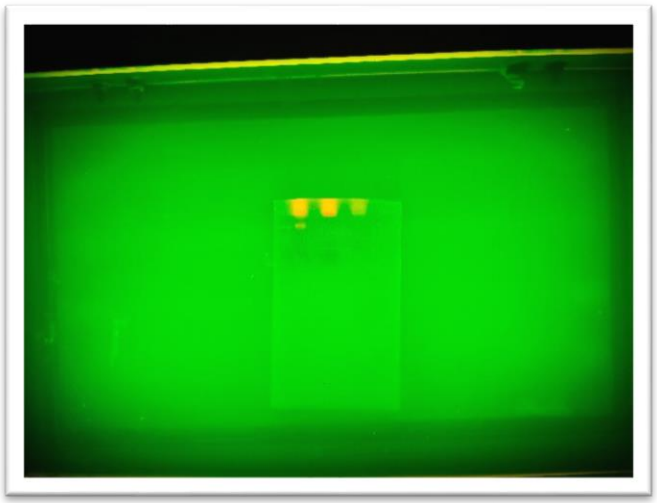

Fig. 14: saliva sample placed in soil (left to right)
Well 2: control sample
Well 4: 0 day sample
Well 6: 2 day sample

Observation Table 5:

WELL	**DNA BAND CONCENTRATION**
Well 2	DNA band observed in good concentration
Well 4	DNA band got degraded
Well 6	DNA band got degraded

On the basis of observation, it has found that control sample gives good amount of DNA band concentration but when we compare well 4 and 6 which contain 0 day and 2 days sample respectively, with control sample DNA band got degraded.

4. Condition: sample placed in acid

   In some acid attack cases or any homicide cases in which victim's body get burns hence, fingernails may useful source for personal identification. We provide the burning condition with the use of weak acid (PH-2.32) to fingernails sample contain saliva for 0 day and 2 day and observed result as follow:

Fig. 15: nail sample containing saliva

(Placed in weak acid for 0 day)

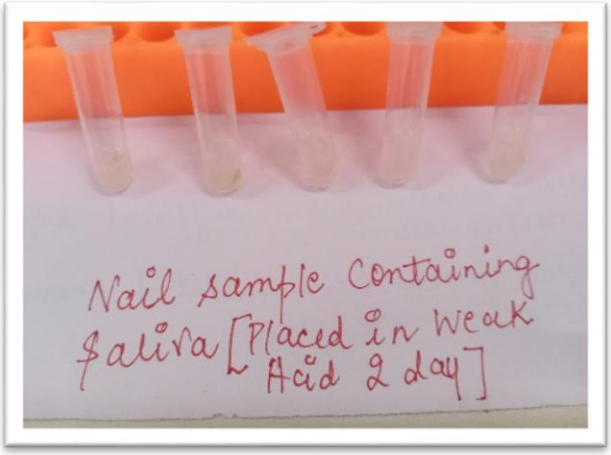

Fig. 16: nail sample containing saliva

(Placed in weak acid for 2 day)

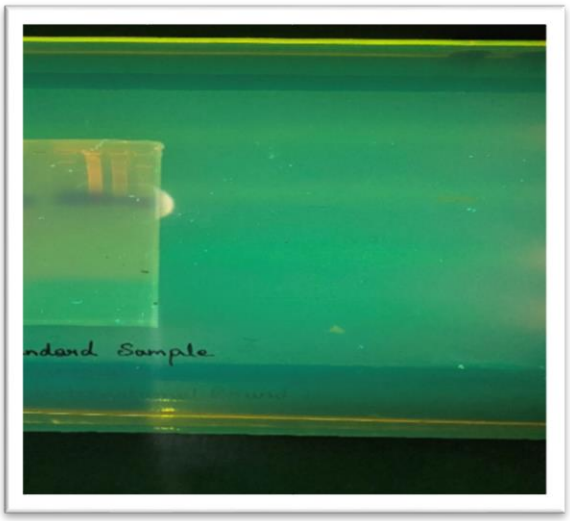

Fig. 17 saliva sample placed in acid (left to right)

Well 3: control sample

Well 4: 0 day sample

Well 5: 2 day sample

Observation Table 6:

WELL	**DNA BAND CONCENTRATION**
Well 3	DNA band observed in good concentration
Well 4	DNA got degraded
Well 5	DNA got degraded

On comparison of 0 day and 2 day fingernail sample with control sample we found that the DNA get degraded in both 0 and 2 day samples while control sample gives good amount of DNA band concentration. So, it is clear by above observation there are very less chances or no chances to identify a victim or suspect if body get completely burn by acid.

When blood use as debris

1. Condition : fingernail containing blood submerged in water
   By placing the sample in tap water for 0 day and 2 days the following observation occurred:

Fig. 18: Control sample of blood

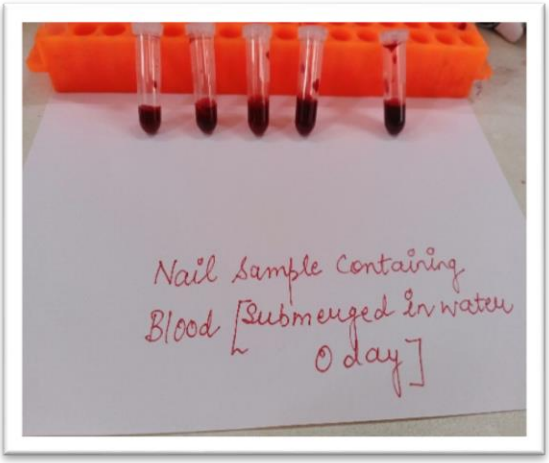

Fig. 19: Nail sample containing blood
(Submerged in water for 0 day)

Fig. 20: Nail sample containing blood

(Submerged in water 2 days)

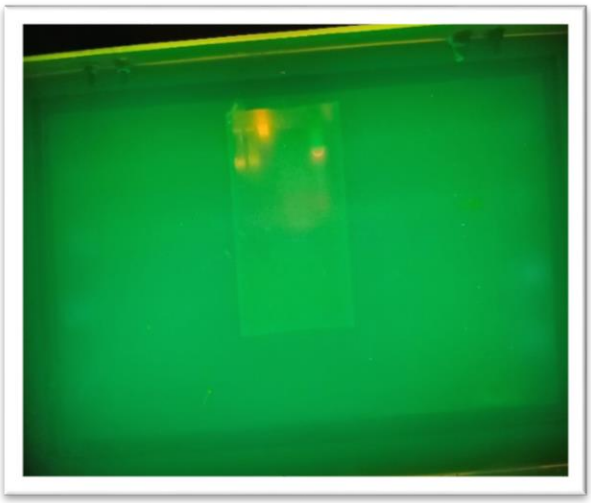

Fig. 21: Blood sample containing water (left to right)

Well 1: Control sample

Well 2: 0 day sample

Well 3: 2 day sample

Observation Table 7:

WELL	**DNA BAND CONCENTRATION**
Well 1	DNA band observed in good concentration
Well 2	DNA band observed in slightly dilute concentration
Well 3	DNA band observed in light concentration

On the basis of observation, we found control sample gives good DNA band concentration and on comparing 0 day sample with control sample DNA band concentration found slightly depleted but when compared 2 day sample with 0 day sample it get much more degraded as shown in figure 21. It indicates that blood as exogenous material if found underneath the fingernail found in water there are little chances to know the identity of a person related to crime.

2. Condition: fingernail sample buried inside the soil

By placing the sample in soil (PH- 6.45) for 0 day and 4 days the following observation occurred:

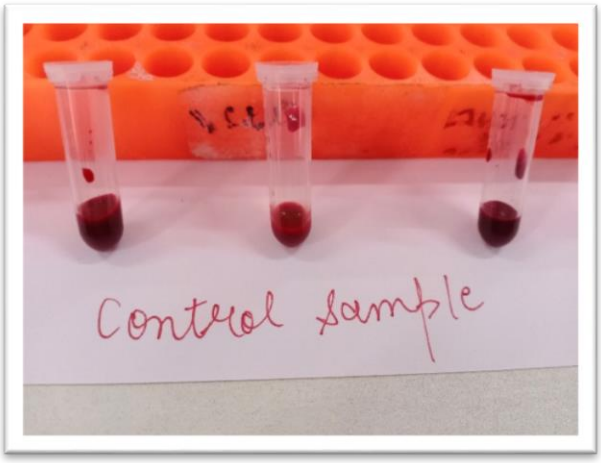

Fig. 22: Control sample blood

Fig. 23: Nail sample containing blood

(Placed in soil for 0 day)

Fig. 24: Nail sample containing blood

(Placed in soil for 4 days)

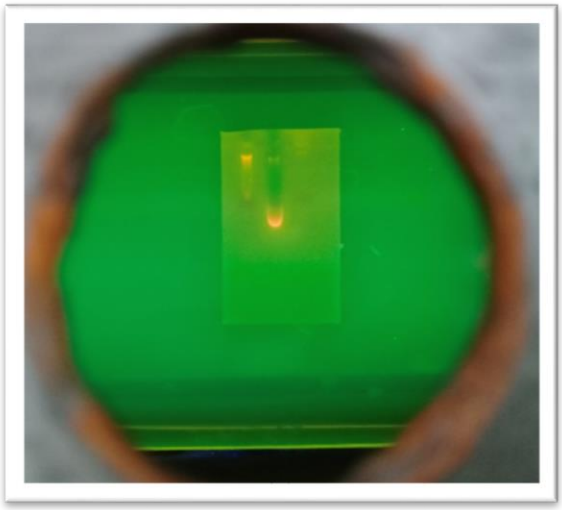

Fig. 25: blood sample placed in soil (left to right)

Well 2: control sample

Well 4: 0 day sample

Well 6: 2 day sample

Observation Table 8:

Well	**DNA BAND CONCENTRATION**
Well 2	DNA bands observed in good concentration
Well 4	DNA bands observed but in dilute concentration
Well 6	DNA get degraded.

On the basis of observation, when provide the condition to the sample (buried in soil), we found control sample gives good DNA band concentration and on comparing 0 day sample with control sample DNA band concentration found slightly depleted but when compared 2 day sample with 0 day sample it get much more degraded as shown in figure 25. Similarly there are also few chances to know the identity by isolating DNA depends upon the passage of time.

3. Condition: sample placed in acid

   Provided burn condition to fingernail sample by placing the samples in weak acid (PH-2.32) for 0 day and following observation has observed:-

Fig. 25 Blood sample placed in weak acid

(Sample got freeze)

On the basis of observation it has resulted that all the samples in Eppendorf tubes would be freeze and even after kept under boiling water bath for 30 minutes it would not be got melt. Therefore, did not perform this condition further and for 2 day set. Hence, in such type of cases if the sample completely got degraded it is not possible to reveal the identity of any person.

CONCLUSION

The proposed study will help to get different result by comparing samples of fingernails (suspected sample) with control sample which were taken as sample of victim and suspects. It may help to identify best extraction for different type of biological material found beneath the fingernail and collection method if body found in different condition like body found in water, in burning cases or buried inside soil etc., This study may helpful in identification of assailant and also give clue about involvement of single assailant and number of assailants (in gang rape cases) and identification victim or personal identification of an individual.

FUTURE SCOPE

DNA fingerprinting is one of the most important discovery of the late 20 century, which has revolutionized forensic investigations in order to solve crime. It is quite possible to know the identity of a person (personal identification) by fingernail DNA (exogenous or endogenous DNA) analyses. In the future, we may use fingernail DNA obtained from crime scene to create description about suspects or unidentified victims. Sometimes, mixed DNA profiles also obtained from the exogenous material found underneath the fingernail will helpful in effective analyses of male-male mixed DNA samples to identify multiple perpetrator in gang rape cases or in homicide cases, fingernails have also been recovered from the dead or decomposed body (within limited time) and may analyzed successfully.

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