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DNA Quantity and Quality Assessment Using Vwa and D18S51 Primers and Morphological Comparision of Fresh and Soil Incubated Human Hair

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Abstract

A crime scene is encountered by several types of evidence. Hair is common evidence that we mostly encountered in maximum of crime scenes. Being important and common evidence, hair found in any crime scene helps in a forensic investigation. Hair can be obtained from 6 different parts of body like head hair, eye brows and eyelashes hair, beard and moustache hair, underarm hair, body hair and pubic hair. Hair is a well-known target to identify the age, sex, colour, race, species identification, disease profile, environmental exposure, metal poisoning, and both nuclear and mitochondrial DNA analysis. But in this study we have taken 2 parameters for the examination, the morphological difference and nuclear DNA quality & quantity difference. Here we have compared between the fresh and soil stored hair with the above parameters. Upon the analysis of the hair samples under electronic compound microscope with the mentioned parameters, it is found that there is negligible or no difference in case of morphology. While determining the quantity and quality of the DNA with the help of UV-Spectrophotometry, we found a significant difference in DNA quality and quantity between fresh and soil stored hair within a specific time gape. We have also compared between Vwa and D18S51 primer by using these fresh and stored hair sample's DNA. This study on hair would be helpful in further forensic examination and research.

Keywords: Hair • Forensic investigation • Morphology • DNA quality and quantity • Soil stored • Primers

Introduction

A crime scene witnessed several types of biological evidence, among those evidences hair is considered as a valuable one and helps the investigation in various ways. As we all know growth, loss and replacement of hair is a continuous phenomenon from birth to death of an individual. Due to this continuous loss and replacement phenomena they get easily transferred during the act of a crime. In a forensic investigation hair helps to identify age, sex, species identification, poisoning, DNA analysis and many more [1]. But morphological study and DNA analysis are two important criteria that forensic expert looks for and different analyses have been performed for hair investigation.

In case of hair analysis scientists give first priorities for microscopic study and examine characters like length, colour, curliness, cortex, medulla, scales, pigmentation, dye etc. of hair. Species identification from hair whether it's a human hair or animal, is an important part in forensic investigation [2]. Hence morphological study of hair is a basic step of hair analysis as well as a significant part. Hair is also a good source for both nuclear and mitochondrial DNA where major part of nuclear DNA is located in root portion of hair and shaft of the hair is enriched with mitochondrial DNA. But in forensic point of view nuclear DNA analysis is always the first priority than mitochondrial DNA [3].

In forensic scenario it's very obvious to deal with degraded and contaminated evidences. Biological evidences undergo rapid degradation after contamination which creates complications in further examination process. So it's very common for hair to get contaminated with foreign objects and environmental factors, especially soil in the crime scene. Recent study shows that total DNA in hair estimated at <10ng DNA in recently shed hair decline to <1ng after a few months of storage [4]. And for forensic scientists it's very obvious to deal with degraded samples [5]. STR markers are widely accepted tool for DNA profiling in forensic for many advantages over other techniques [6]. The robustness and correctness of result even with the lower amount of DNA making STR typing more preferable one in the field [7]. There are several STR loci have been operated for human identification purpose but CODIS system for STR analysis is widely accepted worldwide. The CODIS core loci are CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11 [8].

So here in this study we have stored the shed hair with roots in soil to check and compare both the morphology and DNA quality quantity with fresh ones. Again we have taken two STR primers vWA and D18S51 and compare them with fresh and stored samples and observe the band pattern difference for the same.

Materials and Methods

Sample collection

Hair samples were collected from 15 volunteers of different age groups of both male and female from Visakhapatnam, Andhra Pradesh, India. The total no of hair was 150 (i.e. 10 from each). All samples were put in marked separate paper envelopes immediately after the collection. Then we collected the soil samples also from the area of Visakhapatnam for the exposure of the hair to mimic the actual crime scene. We divided the hair samples into two groups. One for fresh sample and rest for stored in soil for 30 days. All the required permission was taken by ethical committee. The purpose of the study were informed to all the subject with maintaining privacy of their personal information and acknowledgement were signed by all the subject for sample collection and analysis of the sample for the same.

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Morphological study

Morphology of hair samples were studied under electronic compound microscope. Before sampling both the fresh and stored samples were washed with distilled water 2-3 times then treated with 50% alcohol solution. After dry mounted on microscope slide and studied under microscope [9].

DNA extraction from hair sample

For fresh samples DNA extraction is done within 1 day of the collection of samples. For stored samples extraction is done after 30 days of incubation in soil. First the soil stored samples were washed with distilled water 2-3 times then followed by 70% alcohol then made them to dry. For the extraction of DNA, phenol chloroform process is used [10].

Procedure

- Took 5 rooted hair and cut 1-2 cm portion length of hair from root end, and then chopped them.
- Grinded the hair sample in 1ml of lysis buffer (50 ml 2% SDS, 10 ml 1 M Tris buffer, 4 ml of 0.5 M EDTA, 28 ML 5 M NaCl, DTT to make it 100 ml).
- Added 10 ul of proteinase-K to it.
- Incubate homogenate at 65C for 30 minutes, then immediately incubate at -20 °C for 15 minutes. Repeat this process for 2 times. Equal volume of chloroform and isoamyl alcohol is added at 24:1 ratio and mixed it well.
- Centrifuged it for 2 minutes @13000 rpm.
- Collected the aqueous phase and repeated the above step.
- For aqueous phase 15 ul ammonium acetate of 3M is added and 1 ml of isopropanol is added and incubated at 20 °C for 1 hour.
- After incubation, it is centrifuged at max rpm for 5 min, which yield the DNA pellet.
- The DNA pellet was washed with 70% ethyl alcohol then pellet was dried and added to TE buffer to it.

Quantity and quality of DNA

UV spectrophotometer is used for the quantity and quality of DNA. DNA concentration is estimated by measuring the absorbance at 260 nm and ratio of the absorbance at 260 and 280 nm is used access the purity of DNA. The ratio is commonly used to access the amount of protein contamination since proteins absorb at 280 nm. The ratio from ~ 1.7 to ~2 will be of good quality of DNA [11,12].

PCR and gel electrophoresis

Both the fresh and stored DNA was performed in PCR with both the primers vWA and D18S51. Here D18S51 had having greater amplicon size with 290-310 bp than vWA which one 140-180 bp amplicon size. After the successful amplification of the primers with fresh and soil stored samples agarose gel electrophoresis was performed for both the primers separately [13].

Results

After analysis of the hair samples, following results were obtained on the hair analysis of both fresh and stored samples on the 2 parameters, morphology and DNA quality and quantity.

Morphology

The hair samples were studied under electronic compound microscope. Photos of microscopic study from both fresh and soil stored were taken compared with each other. Figures 1 and 2 are showing the difference of fresh and soil incubated hair of same origin under compound microscope. Here we can observe that there are no significant differences in morphology of fresh and stored hair. The morphological structure remains same in both the samples.

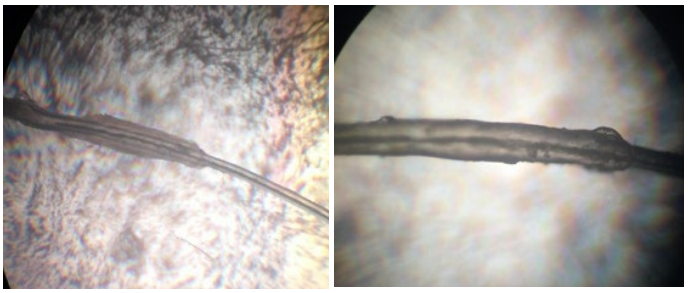

Figure 1. Fresh hair under microscope and soil stored hair under microscope.

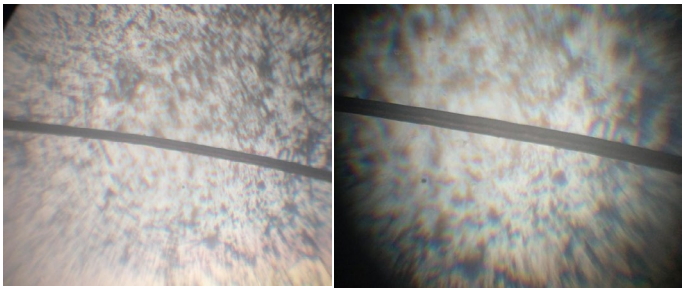

Figure 2. Fresh hair under microscope and soil stored hair under microscope.

DNA quality and quantity

After the extraction, DNA quality and quantity is done by using UV spectrophotometer for both fresh and soil incubated hair samples. After analyzing both the samples with UV Spectrophotometry the data were obtained. Tables 1 and 2 are showing the data that obtained from UV spectrophotometer for quantity and quality for fresh and soil stored hair samples accordingly.

Analysis of Primers

After the successful PCR of two primers with fresh and stored samples, agarose gel electrophoresis was done. Figure 3 shows the result with vWA primer and Figure 4 shows the result with D18S51 primer. Both the primers show good bands with fresh samples than stored samples. But we can say vWA shows better bands for stored samples than D18S51.

Discussion

The cuticle, medulla and cortex of hair's morphology structure are major microscopic study to do which helps in identifying of species also plays an important role in forensic comparison of human hair. In our above study it's very clear that there are no significant differences in morphological structure between fresh and soil stored human hair. The main building block of hair is keratin which is degradable by proteolysis of keratin lytic fungi found in soil, which is a very long process and also this process influence by the environmental factors. Due to the resistance structure of keratin it is too difficult to degrade or deform the morphology of hair. If we talk about the DNA quantity and quality it shows a drastic change between fresh and soil stored hair sample. While in case of fresh the quantity of DNA is from 1ng – 6ng with 1.7 – 2.0 purity but in case of soil stored sample the DNA quantity is decreased to <1ng with not so good purity. It has been studied that shed hair with root may contain 1 ng -10 ng of DNA. Here the shed samples were collected for the study because maximum of crime scene encountered with shed hair samples. So technically the PCR amplify of two primers was done with fresh and degraded samples so as the gel electrophoresis. And from the result it's clearly showing that both the primers amplify perfectly for fresh samples but vWA shows better amplify for degraded samples than D18S51. Here we have taken the amplicon size of vWA (140-180 bp) smaller than D18S51 (290-310 bp). So the smaller amplicon size primer goes well with degraded samples than larger one.

Table 1. DNA quantity and quality of fresh samples.

SL. No	Sample ID	DNA Quantity (ng/uL)	DNA Quality
1	S1 F	2.8	1.96
2	S2 F	4.9	2.05
3	S3 F	3.41	1.99
4	S4 F	5.4	2.00
5	S5 F	4.4	1.70
6	S6 F	5.7	1.56
7	S7 F	3.36	1.62
8	S8 F	3.8	1.61
9	S9 F	2.19	1.73
10	S10 F	6.5	2.04
11	S11 F	1.9	1.78
12	S12 F	4.9	1.58
13	S13 F	5.58	2.06
14	S14 F	6.3	1.99
15	S15 F	4.8	1.95

Table 2. DNA quantity and quality of soil stored samples.

SL. No	Sample ID	DNA Quantity (ng/uL)	DNA Quality
1	S1 S	.165	3.37
2	S2 S	.640	2.29
3	S3 S	.390	3.34
4	S4 S	.46	2.53
5	S5 S	.390	2.00
6	S6 S	.545	1.59
7	S7 S	.32	2.91
8	S8 S	.295	2.97
9	S9 S	.160	2.42
10	S10 S	.930	2.48
11	S11 S	.110	2.75
12	S12 S	.505	2.68
13	S13 S	.585	3.56
14	S14 S	.710	2.56
15	S15 S	.495	2.78

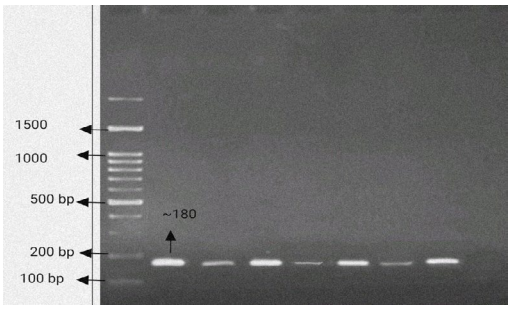

Figure 3. Result of vWA primer with both fresh and soil stored DNA.

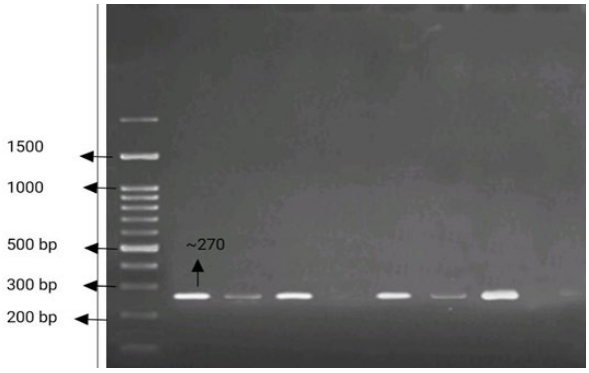

Figure 4. Result of D18S51 primer with both fresh and soil stored DNA.

Conclusion

The results of this study concluded that it takes time to decomposition of hair, so it remains stable in environment for several years. Even the morphology remains unchanged for few years of burial of hair or dispose in environment. This study also suggests that it's always good to choose fresh hair samples for any DNA related work than older hair samples or samples disposed in soil. Because hair undergoes rapid DNA degradation in environment and even more in medium like soil. Also the environmental conditions are also affecting the degradation rate. Now a day degraded DNA is a challenging problem for forensic scientists, but STR analysis handling well with this kind of problem. While it shows smaller amplicon size primers able to successfully amplify the degraded DNA and larger amplicon size primer fails to do. Here in this study vWA having smaller bp so shows good amplify with degraded DNA while D18S51 has larger bp than vWA and fails to amplify with degraded DNA. It suggests using mini STR for degraded DNA in forensic uses.

Conflict of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

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