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Overlooked Aged Blood Stain Resolved a Tangled Unsolved Case

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Abstract

Stains of body fluids as evidence are commonly found at almost every crime scene, especially in violent crimes and its significance in forensic investigation is a well known fact. Different analysis of these stains found at crime scene has always been helpful in forensic case works. Especially, blood sample in the form of dried blood stain as blood gauze piece is the most used practice in forensic case works as these stains are easy to store and transport. Although DNA extraction from these blood stained gauze is a little laborious process but it is still preferred in India like place where the climate is adverse at times and particularly when it has to be transported to laboratories for DNA analysis situated at far off places. At times, blood stain samples observed at crime scene may be scanty in amount but should not be ignored by experts as even it helps to establish the identity of an individual.

Keywords: Blood gauze piece; blood stain on plastics for DNA Finger printing; Dried blood stain; Forensic DNA analysis.

Introduction

In forensic investigation, different types of human origin stains are found that connect a suspect with the crime scene. Human Blood is considered as a major connective tissue. An adult human body consists of around 60% of body fluid and around 7% of blood of the total body mass. Blood plays a very important role in the immune complex [1]. Besides oxygen carrier capacity by RBC's used for day to day routine of human being, WBCs present in blood plays a very important role in forensic human identification. Blood stains commonly found commonly at crime scene is important in forensic investigation by studying their pattern, to reconstruct the crime scene and DNA analysis from blood stain for identification purpose. However, study of blood stain pattern to understand the exact sequence of crime at scene of crime by forensic scientists, is not a new idea [2]. DNA analysis of blood stains is especially used for forensic identification purpose to link the perpetrator with the crime scene. DNA analysis from blood stain depends on the quality and quantity of blood stains, even presence of small Low Copy Number (LCN) of DNA demonstrates accurate results to establish identification as the techniques used now a days are advanced and improved [3,4].

DNA analysis is cogent method for biological identification by matching leftover biological traces with a suspect at crime scene [5]. There are two different conditions in which blood stains may be found, either dried or in liquid condition [1]. But dried blood stains are the ideal sample for DNA analysis in forensics as blood stains ensure in ease of its collection, storage

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and transportation in almost all environmental conditions. The only problem with dried blood stain is that it requires additional extraction steps for DNA extraction procedure [6].

Although it has been reported that sufficient amount of DNA has been obtained for genetic profiling from cotton and nylon fabrics washed at 60°C while sufficient amount of DNA could not be obtained from the ones washed at 90°C [7]. The DNA obtained from blood stains on plastic surface has resulted in higher amount of transferred DNA but depending upon the relation of blood volume to included area [8]. It is suggested that not even a minute amount of blood stain on any surface should be ignored for forensic work.

Case study

A case of rape was registered by an investigating agency. Sample of vaginal swab and under wear of victim were collected. Penal swab along with control swab, fresh blood in guaze piece of suspect, pant of suspect were also collected and sent for DNA analysis to forensic laboratory. The first forensic examiner reported human blood and human semen from blood stained gauze and the undergarments respectively. After analysis, out of six exhibits submitted for DNA analysis, DNA profile was observed only from two exhibits i.e. Gauze piece of suspect and under wear of victim. First Forensic examiner concluded that DNA profile generated from gauze piece of suspect did not match with the DNA profile generated from under wear of the victim. Hence, no conclusion was obtained and this lead to the acquittal of suspect.

A fresh appeal was filed by an investigating agency in this case and the exhibits were re-examined by board members consisting of Forensic DNA examiners. Board members suggested to collected fresh blood sample of the suspect along with previous submitted exhibits. After analyzing the said samples along with the fresh blood sample of the suspect, board of forensic DNA examiner opined that DNA profile generated from under wear of victim matches with the fresh blood sample of the suspect. This created a difference of opinion regarding DNA profiles generated by two different forensic examiners.

Again, investigating agency decided to examine the exhibits by third forensic examiner to overrule the confusion/chaos. The third examiner reported that the blood stained gauze did not yield any DNA for analysis. However, it was reported that the DNA profile generated from the pant of the suspect share the same DNA alleles as observed on under wear of victim and fresh blood of suspect collected by board of members of forensic examiner. However, it was different from the alleles as generated from the blood stained gauze piece by the first examiner. DNA profile generated by all the three forensic examiners from under wear of victim was similar.

Only the first forensic examiner was able to generate DNA profile from gauze piece of suspect which was different from fresh blood of suspect collected by the second team. Thus, the investigating agency wanted the fourth examiner to generate DNA profile from gauze piece of suspect collected initially and then match it with the DNA profile of the fresh blood sample of suspect which was collected again and also match with the DNA profile generated from gauze piece by first examiner.

It is important to mention that it was very important to generate DNA profile from the blood stained gauze (collected initially) so as to rule out the intension of the first examiner or of the person who collected and sealed the blood stained gauze sample. Thus, was submitted to the fourth examiner, i.e. our laboratory.

It was very important to generate DNA profile from gauze piece of suspect (collected initially), as it was challenging to generate DNA profile from gauze piece being almost eight years old gauze piece and left over sample was very less almost negligible (as consumed by earlier experts).

Finally during thorough examination of the exhibits, in our laboratory, it was observed that there were some small dried and old stains in the inner surface of the container in which gauze piece was kept. It is quite obvious that these stains were generated while blood gauze piece was being kept in the container. It was overlooked by the earlier examiners.

Material and method

Gauze piece was cut into small pieces and dipped them in forensic extraction buffer to elute blood cells. Dried blood stain was taken using sterile cotton buds dipped in forensic extraction buffer. Both tubes were incubated at 56°C and squeeze them with the help of sterile syringe. DNA extraction was done by organic method. Extracted DNA were quantified using Qubit™ fluorometer and subjected to PCR amplification using AmpFlSTR™ Identifiler™ plus PCR amplification kit followed by analysis using automated DNA sequencer.

Results

DNA was successfully extracted from Gauze piece and dried blood stain collected from the inner wall of the container. Gauze piece did not generate any DNA profile as it demonstrated highly degraded DNA where as dried blood stain collected from the inner wall of the container amplified at 12 out of 16 loci.

The DNA profile generated from the blood stain collected from the inner wall of the container matched with the DNA profile generated from the fresh blood sample of the suspect.

Discussion

Stains from different biological fluids are most common in forensic cases and their storage at different time intervals were studied and discussed time to time. Researchers successfully extract DNA and performed multiplex STRs analysis using a variety of STR kit from 15 to 30 years old stains [9,10,11].

Storage of blood in form of blood stains is a routine practice for DNA analysis in medico legal situations. A lot of research work has been done related to blood stains. Blood stains on different texture and type of cloth (i.e. Cotton, jeans and spandex) revealed different DNA concentration when stored outdoor for 9 weeks. All type of clothes showed loss of allele after 5 weeks of storage. Results suggests that storage condition and type of clothes for preservation of stains have a great effect on PCR amplification [12,13].

Also, previous studies have observed that blood stained denim cloth is not ideal for DNA analysis as indigo dye extracted along with DNA may act as PCR inhibitor. However, the method was improved later on to extract DNA from blood stained denim for STR analysis [14]. Analysis of DNA on washed items as well as the DNA transfer has become a major concern in recent years, especially after the detection of minute traces of biological samples. Though Janine *et al* has observed transfer of DNA from blood and saliva stains and demonstrated complete DNA profile from hand washed smooth surface kitchen utensils [15].

Amanda & Reena studied Y-STR amplification of body fluids on various substrates but not on plastic surface [16].

Conclusion

In medico legal cases, blood stains can be found on every possible surface. However, we can choose a reference sample according to our choice. It is already studied that DNA can be efficiently extracted and amplified from very old blood stains. This case study also supports the previous studies that DNA can be efficiently amplified from blood stains stored on plastic surfaces.

Present study suggests that one should carefully explore the exhibits in medico legal cases so that justice can be prevailed.

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