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Detection of Aged Bloodstains by using Different Techniques- A Review

Prince Singla, Rupinder Singla

Abstract:- **With an increase in criminal activities, a large number of crime cases are reported every day. These violent crimes are usually suicidal, homicidal, or accidental in nature. In forensics investigations, blood is the most significant and valuable biological evidence, which is found very frequently at crime scene. The main motive of the collection of biological samples from the crime scenes is to get the genetic information from collected samples and use that information for the identification of the suspect. A DNA profile indicates the person's identity and the pattern of the streaks reveals the crime scene position, which means both DNA profiling, and bloodstain patterns serve as a significant tool for the identification of the perpetrator. The blood found at the crime scene in the form either blood pool or stain, needs to be collected, preserved, and analyzed by the examiner on the spot or in the laboratory. The dried bloodstains can give an indication of the relative age of a trauma and timing of its occurrence , which can be extremely helpful to investigators in determining whether the stain was related to that crime scene or not. Numerous researchers gave their contribution in the development of a plethora of methods for the determinations of age of dried bloodstains. In the present study, an attempt has been made to review all those conventional and modern techniques used by various scientists to estimate the age of the bloodstains.**

Keywords:- *Criminal, Suicide, Homicide, Biological Evidences.*

I. INTRODUCTION

In forensic investigations, every evidential material can play a significant role in the reconstruction of crime and finding out the culprit. The crime scene investigators mainly deal with physiochemical properties of these evidential substances, which might have changed with the passage of time. To study these changes in the characteristics of the evidence, various scientists studied some measureable parameters and tried to establish the relationship between these parameters and time. These studies were used to calculate the age of questioned trace material in the form of blood. Few decades ago, a question about the time elapsed since bloodstain deposition was raised and this question generated a curiosity in the mind of several researchers to conduct research to answer the question.

Blood is one of the most common fluids encountered at the crime scene. Few decades ago, with advancement in technology, the forensic expert were able to discover one of the important tools that is DNA profiling, to tackle the queries related to criminal offences involving blood as evidence. Nowadays, the research is underway in quest of the best method that can be used to determine the age of bloodstains.

A. Nature of Blood

The human blood, the connective tissue, is composed of 55% of plasma and 45% of cellular components. It consists of 91.5% of water, 7% of proteins and 1.5% of other solutes present in the plasma. It also has a coagulation factor known as fibrinogen, which is a glycoprotein. This fibrinogen helps in the formation of fibrin/clot during blood coagulation. Because of coagulation, all the cellular components of blood are separated from the plasma by the action of fibrinogen, and the liquid component left without fibrinogen is known as serum. Three types of cells are present in the blood are called erythrocytes (red blood cells), leukocytes (white blood cells) and thrombocytes (platelets). Out of these three cellular components of blood, leukocytes are primarily involved in the immune response being generated by producing antibodies, which recognize and respond to foreign substances (antigens). Erythrocytes help in the transportation of gases such as oxygen and carbon dioxide from the lungs to the cells as well as from the tissues to the lungs of organisms because of the presence of hemoglobin into it. Hemoglobin also imparts color to the blood. Blood is in the liquid form when it circulates into the body. Whenever blood is exposed to the external conditions, it begins to dry, and the time it takes depends on the size, volume, nature of the surface and the inclusion of different components. A little, lighter and thin bloodstain pattern on non-permeable surfaces dries quickly and effectively under the usual conditions of the temperature and humidity. In contrast, bloodstains, which are larger in size and volume, require additional time to dry. The drying process of bloodstains begins from the peripheral area and moves towards the center. The chipping at the center, combined with the intact periphery provides a skeletal appearance to the dried bloodstains.

B. Stain Pattern of Blood:

The bloodstains pattern plays a significant role in interpretation and reconstruction of crime scenes. The study of bloodstain patterns requires a thorough knowledge of position, direction, dropping distance, angle and shape of blood pattern

with respect to the origin and trajectory that can be analyzed by a well experienced examiner. The analysis is based on various experiments, which require comparable surfaces materials with respect to the crime location.

- Surface texture helps in the interpretation of bloodstain patterns. Unknown samples are compared with the standards only when there is a presence of identical surfaces. If the surface is hard or less porous, then the spatter of blood is less.
- Shape of the bloodstain is useful in the determination of direction of travel of blood. In general, the pointed end of the bloodstain is always shows the direction of blood travelling.
- Angle of bloodstain is determined by measuring the circular distortion of stain on flat surfaces. For instance, if a drop of blood strikes the surface at right angle, then the bloodstain formed is of circular shape. When the angle decreases, the bloodstain becomes elongated in shape.
- The origin of a blood disperses in a two dimensional plan that can be set up by characterizing straight limits through the long center of several individual blood stains. The combination or point of gathering of the lines depicts the direction where the blood comes from.

C. Aging of Bloodstains:

In crime scenes investigations, estimating the age of bloodstains provides a huge, valuable amount of information. During the aging process, blood undergoes various chemical reactions. When it take place inside the body (in- vitro) then, red blood cells get ruptured after completing the lifespan of 120 days and are replaced by the new cells. However, conditions are different outside the body (in vitro). In the external conditions, the coagulation process occurs separating the cellular components of blood from liquid. After the process of evaporation, the blood gets dry and becomes a stain. With the passage of time, the color changes can also be observed in the blood. The change in the color of the blood takes place by the degradation of oxy-hemoglobin into met-hemoglobin followed by hemi chrome. When blood oozes out from the body at first, it interacts with air and gets oxygenated, which means hemoglobin converts into oxy-hemoglobin. Secondly, this oxy-hemoglobin degrades into met-hemoglobin, which further gets degraded into changes in the color of dried bloodstains. Primarily, it appears radiant red due to the presence of oxy-hemoglobin, followed by bluish brown due to the conversion of hemoglobin into met-hemoglobin and dark brown because of the appearance of hemi chrome. It is the oxidation process of oxy-hemoglobin, which results in the change of the color of dried bloodstains and ultimately provides the age of bloodstains. This approach is not reliable because it is useful to get accurate results. During the visual examination, the outcomes vary from individual to individual. So, to overcome this issue, a number of other experts have incorporated several new techniques to accomplish the best results.

Bloodstains are something mixed with other body fluids, and then different strategies are required to analyze. Some of them are –electron paramagnetic resonance spectroscopy, reflectance spectroscopy, atomic force microscopy, oxygen electrode, hyper spectral image analysis, electron spin resonance spectroscopy and others. The main objective of the present study is to sum up all the strategies and methods, which are utilized by various researchers for the determination of the age of age of bloodstains. This review study also evaluates the impression and problematic consequences of numerous procedures. Furthermore, it covers the new turn of events, the future difficulties and other factors that can impart the outcome of the experiments.

II. DISCUSSION

After reviewing the literature related to the age estimation by using different techniques, it was found that numerous researchers have used several techniques for the determination of age of bloodstains. Firstly, **Patterson 1960** used photoelectric colorimeter, a non-destructive spectrometric method to determine the color changes of the bloodstains and concluded that maximum changes occur up to the first three days, which may be due to the effect of external conditions such as temperature, humidity etc. Secondly, **Miki et al. 1987** utilized Electron Spin Resonance technique to study the met-hemoglobin, non-heme irons and organic radicals of degraded blood and inferred that although the ESR signals increases in blood with the passage of time but the ESR signals of non-heme gives good results for age estimation of bloodstains. Thirdly, **Sakurai et al. 1989** took the same technique that is Electron Spin Resonance to study the protein levels of bloodstains and concluded that accurate observations can be made up to 120 days. Further, **Inoue et al. 1992** developed a high performance liquid chromatography to work on an experiment involving alpha- globin to heme ration, which decreases as the age of bloodstain increases. It was figured out that good outcomes could be achieved up to 20 weeks old bloodstains. Furthermore, **Matsuoka et al. 1995** described the use of an oxygen electrode method to estimate the age of bloodstains, they worked on decaying of oxy-hemoglobin at different temperatures with respect to time. This approach can provide precise results up to 10 days. In addition, **Bauer et al., 2003** used RT-PCR to study the degraded RNA to estimate the age of 15 years old bloodstains. **Sharma et al. 2004** used UV-Visible Spectrophotometric technique to determine the age of bloodstains, kept in two different conditions. It was found that the level of absorbance decreases with the increase in the age of blood stains. Apart from it, **Anderson et al. 2005** utilized RT-PCR to estimate the age of bloodstains by studying different types of RNAs (mRNA and rRNA) and concluded that meticulous results can be achieved up to 150 days. **Fujita et al. 2005** used electron paramagnetic resonance to study the denatured hemoproteins under controlled conditions and observed that this technique is suitable for the determination of age of bloodstains that should not be older than 2 months. In addition to it, **Strasser et al.**

2007 worked on the effect of time frame on the elasticity of erythrocytes by using Atomic Force Microscopy and concluded that the elasticity of bloodstain tends to decrease with the passage of time. **Hanson and Ballantyne 2010** used UV- Visible Spectrophotometric to study the Sorbet band of hemoglobin and concluded that this technique is helpful to distinguish between the bloodstains that were deposited minutes, hours, days, and weeks before**. Li et al. 2011** employed reflectance spectroscopy, a non-destructive technique for the estimation of age of bloodstains. It was observed that there is an effect of baseline variation and simple scattering on the generated results. Besides this, **Bremmer et al. 2011** used diffuse reflectance spectroscopy to study the three components (oxy-hemoglobin, met-hemoglobin and hemi chrome) of blood up to 60 days and found that the fraction of these components changes with respect to the time. **Edelman et.al. 2012** used near infrared spectroscopy for the age estimation of bloodstains present on dark backgrounds. Next, **Edelman et al. 2012** used hyper spectral imaging to record the visible reflectance spectra of relative fractions of different components of bloodstain up to 200 days and found that with this technique the rate of occurrence of error increases for the bloodstains that are older than 200 days. Further, **Nakao et al., 2013** used real-time PCR for the identification of microRNAs (miR-16 and miR- 451) and the concentration of ethanol, amphetamine sulfate and methamphetamine hydrochloride in bloodstains for blood stain age estimation and concluded that concentration of microRNAs decreases as the bloodstains gets older. From the same study, it was also noticed thatthe analysis of concentration of drugs in bloodstains are not significant as microRNAs because of the fast evaporation rate of ethanol which becomes undetectable after 180 minutes and the concentration of other drugs remain constant.

Despite it**, Li et al. 2013** utilized visible wavelength hyper spectral image for age estimation of bloodstains up to 30 days under control conditions with an error of (+) (-) 1.17 days. In addition, **Thanakiakral et al. 2013** employed the usage of smartphone cameras in combination with low- cost illumination system for estimating bloodstain ageing. In addition, with the help of Random Forest's classification technique the age of bloodstains was predicted up to 42 days with 12% error. In addition, forty blind samples and 83% of mock casework samples were classified correctly by using this technique. **Edelman et al. 2016** used optical reflection spectroscopy to study the optical properties of various substrates on blood by converting one-dimensional light-transport model into two- layered model and later using this model to calculate the relative amounts of oxy-hemoglobin, met hemoglobin and hemi chrome on colored surfaces by observing their reflectance spectra.

Doty et al. 2017 used Raman spectroscopy for the determination of age of one week old bloodstains and for the distinction of fresh and old bloodstains. In the same year, by coupling the same technique with PLSR and PCR models. It was noticed that with this collaborating technique, the age of bloodstains up to 2 years old can be calculated with 70% accuracy. More than that, **Bergmann et al., 2017** utilized absorption spectroscopy for the distinction between human and non-human bloodstains and for the estimation of age of bloodstains which were 2b-3 weeks old. **Shine et al., 2017** developed a new method for the calculation of Time since Deposition (TSD) of Bloodstains. Fluorescence was used to estimate the age of bloodstains after 91h of deposition and found that the fluorescence lifetime measurements decreased with the increase in age of bloodstains. **Lin et al., 2017** used ATR-FTIR technique combined with chemometric methods to estimate indoor and outdoor bloodstains up to the age of 107 days. Two PLSR models provided excellent outcomes with small root mean squared error of 5.83 and 4.77, high R values of 0.94 and 0.96, and RPD of 4.08 and 5.14, respectively. Two more PLSR models were used to enhance the precision of results. **Kumar et al. 2020** utilized ATR-FTIR combining with new generation chemometric methods for the calculation of age of bloodstains up to 175 days. In this technique, the results obtained from three models such as curve estimation, partial least squared regression, multiple linear regression were compared and it was observed that the outcomes of PLSR and MLR are better than CE because of less error rate in bloodstain ageing estimation using PLSR and MLR models.

III. CONCLUSION

By observing the literature, it is concluded that some techniques gave results of short term and long-term changes in the bloodstains. Out of all the above-mentioned techniques, study of RNA by using RT-PCR was found capable of providing accurate results up to 15 years and an oxygen electrode method gave results up to 10 days. Reflectance spectroscopy is a non- destructive technique involving light source and spectrometer, which does not destroy the sample and the same samples, can be used for other experiments. Hyper spectral imaging and Near Infrared spectroscopy method is usually used when the bloodstains are deposited on colored surfaces. ATR-FTIR coupled with advanced chemometric methods can be employed to get better results with minimum error rate. Nowadays, various models such as Curve Estimation (CE), Partial Least Squared Regression (PLSR) and Multiple Linear Regression (MIR) are being used in the ATR-FTIR technique.

Due to sensitivity of these methods, these can be carried out on the samples, which are prepared in control condition, but outcomes could vary with the effect of external conditions such as temperature, humidity, and etc. to use these techniques in situations when the bloodstain already dried out in the outside environment. There is a requirement to improve these methods in order to get the most accurate results which may help the forensic investigators in selecting the relevant technique to evaluate blood stain ageing even from a very small amount of forensic samples.

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