Sex Specific Microscopic Analysis of Canidae Breed - An Evidentiary Tool in Forensic Science.
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Abstract

Close association of man and dog in localities contributes commonly to discovery of dog hair mostly in cases of homicidal crime scenes, as ‘Locard’s Principle of Exchange holds that whenever perpetrators enter or leave a crime scene, they will leave something behind and take something with them or it can be shortly said every contact leaves a trace, similarly hair can be transferred through direct or indirect contact. This paper focuses on the study of micro-metric and microscopic features of hair of 16 Canidae which concentrate on 8 different breeds of dog specifically emphasizing on differences between male and female individual of the same breed. Micrometry contributes very less in individualization as our results notify therefore microscopic features are given more importance. Hair is a trace evidence of great evidentiary value since it is found frequently on crime scenes. It cannot be destroyed easily and also serve as a link between perpetrator, victim and scene of crime but still is not a conclusive evidence in court of law, therefore more work on trace evidence is needed to keep up the value of such evidences.

Objective of this study:
• To link hair evidence to scene of crime, perpetrator and victim
• To distinguish between sexes of same Canidae breed
• To aid in individualization and identification of dog breed by using micrometry and microscopic feature such as cuticular index, medullary index, medullary patterns, medullary margins and cuticular patterns.
• To enforce more such studies to uplift trace evidence in court of law

Keywords: Canidae, Medullary patterns, cuticle patterns, indices

Introduction

Hair analysis is of great importance in forensic science for crime investigations, whether to use it for species identification, race, age, sex determination for making a personal profile of a human or non-human hair samples, so that it can serve as main or corroborative evidence in linking crime with crime scene, criminal and victim. [1] Presence of hair is one of the characteristic features of mammals [2]. Hair is an outgrowth of epidermal layer of dermis that
grows out of an organ known as the hair follicle. [3]

The anatomy of hair consists of three sections:
1. the outermost layer known as the cuticle,
2. the innermost layer which possesses medulla and
3. the medial layer i.e. portions between medulla and cuticle known as the cortex.[2]

Broadly, there are three stages of hair cycle anagen referred to the period of active growth, including development of the follicle and subsequent synthesis of the hair, the rapid growth in anagen abruptly ceases and the follicle is largely destroyed in a brief phase, termed by Dry as catagen and the follicle remnant and associated hair are in a phase of apparent inactivity known as telogen. [4]

Hair analysis includes mainly microscopic observation of morphological features such as cuticle pattern, medulla pattern, medullary margins, cuticular margins shape and the micro-metric analysis includes measurements of diameter of hair shaft and medulla, cuticle cell length, medullary index (ratio of average diameter of medulla to the average diameter of hair shaft), cuticle index (ratio between average cuticle cell length to the diameter of hair shaft) and hair shaft length. Besides these characteristics hair also possess DNA (whether nuclear DNA or mitochondrial DNA) present in the follicle tissues attached to root and can only be extracted from hair if they have roots with tissue or a greater number of hair strands and DNA has high degree of individualization [5]. But hair microscopic and micro-metric analysis are more feasible and may show variations which would contribute in discriminating between breeds, sex, age, and other factors.

Dogs are one of the common pets and stray animals found near humans and their hair coat is so thick, as being light in weight and due to hair cycle’s telogen stage, hair are easily shed and exchanged. According to Locard’s principle of exchange every contact leaves a trace, similarly hair is also obvious to transfer directly or indirectly from crime scene to suspect, victim or vice versa. [6] Hence, it would help in an investigation involving presence of dog or dog hair. And will narrow down the search of investigator related to identification and individualization of dog on the basis of age, sex, DNA profile, breeds, etc. which will further help in linking crime with crime scene, criminal and victim eventually leading to proving or disproving facts to achieve justice. [7, 3]

For sex determination DNA profiling has always been a good method but it is time consuming and is not always feasible. Also, for extracting DNA, a large quantity of hair strands is required with the presence of hair root and follicular tissue attached to it. Follicular tissue is needed so that nuclear DNA extraction can be done but it is not always found at the crime scene, considering this, our study is based on micro-metric and microscopic analysis of hair as a tool to discriminate between male and female dogs using smaller number of hair strands.
Animal hair found at the scene of occurrence are mostly the shed hair which fell due to being in telogen stage that is why it is rare to find a hair sample with a root bulb and follicular tissue. [3]

The hypothesis of this study is analysis of Microscopical and micrometrical characteristics of hair are workable alternative for sex discrimination between dogs. Hence, using stereomicroscope, compound microscope, light microscope and ocular micrometer we studied morphological and micro-metric differences between male and female dogs of 8 different breeds.

**Methods and Materials**

**Exclusion and Inclusion Criteria**

Our study design is of a cross- sectional study in which the hair was collected using random sampling method by taking care of following things in the breed;

All the samples were collected from a healthy dog, no samples were collected from unhealthy or allergic breed of dog, age of dog breed was between 6 months to 10 years as the older dogs of age more than 12 years are prone to infections and allergies. Here, we have studied the micrometry and microscopic features of hair of 16 Canidae which concentrate on 8 different breeds of dog specifically emphasizing on differences between male and female individual of the same breed.

**Collection of Hair Samples**

Samples from different dog breeds were collected randomly from neighbors and dog sellers, all the hair samples were taken from the dorsal portion of the dog’s body. Hair samples were collected in a plastic zip lock bag so it can prevent any damage of hair. [3]

**Slide preparation**

Four hair from each breed was examined macroscopically as well as microscopically - three samples were examined by mounting on a slide using water and 1 sample was kept for bleach with reagent Hydrogen Peroxide for 4-6 hours, since hydrogen peroxide is a strong oxidizing agent which helps to observe hair characteristics more clearly and then examined. [8]

**Laboratory testing**

1. **Stereomicroscope**

All sample slides were analyzed under stereomicroscope (stereo zoom microscope - model no. RI-90-03) for qualitative examination to observe if the sample hair consist of hair characteristics such as medulla, hair shaft and root.

2. **Compound Microscope**

Compound microscope with magnification of eye piece 5X and objective lens 40X for micro-metric or quantitative examination, it was done using ocular and stage micrometer. Ocular and stage micrometer were calibrated using magnification at 200X. [9] Size matters: the use of the ocular micrometer in diagnostic parasitology. [9]
Medullary Index and cuticular index were calculated for each hair sample using micrometers.

3. Light microscope

Light microscope with magnification of eye piece 10X and objective lens 45X was used for studying the medullary patterns, medulla margins and cuticular patterns of each hair, which can significantly help in identification of dog breed and help us to narrow down the search in such criminal cases.

Hair type (Profile), color, and length of all the four samples from each breed were examined macroscopically and other microscopic observations such as Cuticle - scale margins, scale pattern and scale distance, Medulla - type, medullary index (ratio between medullar average diameter and hair fiber average diameter) and medullary margins was examined.

**Hair Casting**

For analyzing cuticle patterns, thin cast was made using a nail paint, it was brushed over a glass slide very cautiously and hair is kept and pressed on the cast and was removed slowly after the cast was dried. Quantitative measurements such as cuticular index and scale distance is measured using micrometer. Cuticular patterns was noted using light microscope with magnification of eye piece 10X and objective lens 45X. [10]

**Results**

Identification of dog can be done by using microscopic and macroscopic analysis. Characteristic taken into consideration here are;

Medulla is present at the center of the hair strand; medullary index is the distinguishing feature between animal and human hair. Medullary index of animal hair is always greater than human hair that is more than 1/3. The medullary index is defined as the ratio between the diameter of medulla and the diameter of the entire hair, medullary index = (medulla diameter) / (hair diameter) (this is a dimensionless unit). [11]

There are distinct patterns of medulla that can be observed in dog samples such as unbroken, broken, ladder, and miscellaneous patterns. Medullary margins are also one of the characteristics used for identification, such as straight, fringed and scalloped patterns of margins.

Cuticle - The cuticle, or the outermost layer, comprises a large number of mostly transparent and overlapping scales. The shape, size, margin, and arrangement of the scales along the hair shaft vary across species and are used for species characterization and identification. Cuticular index can be defined as the ratio of the free proximo-distal length of a scale to the diameter of the hair shaft. There are different cutimo patterns that can help in identification of a dog breed such as coronal and imbricate. Imbricate pattern consists of petal, wave, mosaic and transitional patterns. Wave pattern consist regular and irregular wave patterns, similarly mosaic

[^10]: Reference to the specific literature or study.
[^11]: Reference to the specific literature or study.

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pattern consist of regular and irregular mosaic patterns. [12] All patterns observed were imbricate scale patterns. Scale patterns is the arrangement of scales along longitudinal axis of hair shaft.

<table>
<thead>
<tr>
<th>BREEDS</th>
<th>MEDULLA PATTERNS</th>
<th>MEDULLA MARGINS</th>
<th>CUTICULAR PATTERNS</th>
<th>CUTICULAR MARGINS</th>
<th>DISTANCE BETWEEN CUTICULAR MARGINS</th>
</tr>
</thead>
<tbody>
<tr>
<td>German Shepherd (M)</td>
<td>uniserial ladder</td>
<td>scalloped+ straight</td>
<td>Regular Mosaic</td>
<td>Smooth</td>
<td>Distant</td>
</tr>
<tr>
<td>German Shepherd (F)</td>
<td>broken narrow fragmented + simple medulla</td>
<td>fringed</td>
<td>Irregular wave</td>
<td>Crenate</td>
<td>Near</td>
</tr>
<tr>
<td>Labrador (M)</td>
<td>uniserial ladder + light amorphous</td>
<td>fringed</td>
<td>Irregular wave</td>
<td>Crenate</td>
<td>Close</td>
</tr>
<tr>
<td>Labrador (F)</td>
<td>simple amorphous medulla</td>
<td>scalloped+ straight</td>
<td>Regular Wave</td>
<td>Crenate</td>
<td>Near</td>
</tr>
<tr>
<td>Pekingese (M)</td>
<td>unbroken aeriform lattice</td>
<td>scalloped</td>
<td>Irregular wave</td>
<td>Crenate</td>
<td>Near</td>
</tr>
<tr>
<td>Pekingese (F)</td>
<td>broken fragmented</td>
<td>fringed straight</td>
<td>Irregular wave</td>
<td>Crenate</td>
<td>Near</td>
</tr>
<tr>
<td>Shih Tzu (M)</td>
<td>unbroken dense aeriform lattice</td>
<td>fringed</td>
<td>Regular Wave</td>
<td>Crenate</td>
<td>Near</td>
</tr>
<tr>
<td>Shih Tzu (F)</td>
<td>unbroken dense aeriform lattice</td>
<td>fringed straight</td>
<td>Irregular wave</td>
<td>Crenate</td>
<td>Near</td>
</tr>
<tr>
<td>Stray dog (M)</td>
<td>simple medulla</td>
<td>irregular</td>
<td>Irregular wave</td>
<td>Crenate</td>
<td>Close</td>
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<tr>
<td>Stray dog (F)</td>
<td>Miscellaneous</td>
<td>scalloped</td>
<td>Irregular wave</td>
<td>Crenate</td>
<td>Near</td>
</tr>
<tr>
<td>Spitz (M)</td>
<td>broken interrupted+ uniserial ladder</td>
<td>irregular</td>
<td>Irregular wave</td>
<td>Crenate &amp; Smooth</td>
<td>Near</td>
</tr>
<tr>
<td>Spitz (F)</td>
<td>fragmented (long length)</td>
<td>fringed</td>
<td>Regular Wave</td>
<td>Smooth</td>
<td>Near</td>
</tr>
<tr>
<td>Cane Corso (M)</td>
<td>uniserial ladder</td>
<td>scalloped</td>
<td>Irregular wave</td>
<td>Crenate</td>
<td>Near</td>
</tr>
<tr>
<td>Cane Corso (F)</td>
<td>simple interrupted</td>
<td>irregular</td>
<td>Flattened Irregular Mosaic</td>
<td>Smooth</td>
<td>Near</td>
</tr>
<tr>
<td>Dalmatian (M)</td>
<td>simple medulla</td>
<td>fringed</td>
<td>Irregular wave</td>
<td>Crenate</td>
<td>Distant</td>
</tr>
<tr>
<td>Dalmatian (F)</td>
<td>simple medulla +uniserial ladder</td>
<td>fringed</td>
<td>Regular Wave</td>
<td>Smooth</td>
<td>Near</td>
</tr>
</tbody>
</table>

*Table 1: This table shows all the microscopic characteristics examined such as; Medulla patterns, Medulla margins, Cuticular patterns shape, margins and distance between the scales.*
<table>
<thead>
<tr>
<th>BREEDS</th>
<th>CUTICULAR INDEX</th>
<th>MEDULLARY INDEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>German Shepherd (M)</td>
<td>0.23</td>
<td>0.5</td>
</tr>
<tr>
<td>German Shepherd (F)</td>
<td>0.14</td>
<td>0.4</td>
</tr>
<tr>
<td>Labrador (M)</td>
<td>0.15</td>
<td>0.48</td>
</tr>
<tr>
<td>Labrador (F)</td>
<td>0.17</td>
<td>0.69</td>
</tr>
<tr>
<td>Pekingese (M)</td>
<td>0.2</td>
<td>0.39</td>
</tr>
<tr>
<td>Pekingese (F)</td>
<td>0.17</td>
<td>0.5</td>
</tr>
<tr>
<td>Shih Tzu (M)</td>
<td>0.4</td>
<td>0.55</td>
</tr>
<tr>
<td>Shih Tzu (F)</td>
<td>0.2</td>
<td>0.64</td>
</tr>
<tr>
<td>Stray dog (M)</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Stray dog (F)</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Spitz (M)</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Spitz (F)</td>
<td>0.2</td>
<td>0.56</td>
</tr>
<tr>
<td>Cane Corso (M)</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Cane Corso (F)</td>
<td>0.16</td>
<td>0.4</td>
</tr>
<tr>
<td>Dalmatian (M)</td>
<td>0.21</td>
<td>0.72</td>
</tr>
<tr>
<td>Dalmatian (F)</td>
<td>0.2</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Table 2: This table shows all the micrometry values examined such as; Medullary index and cuticular index
<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. German Shepherd (M) - Uniserial ladder medulla</td>
<td>2. German Shepherd (F) - Simple medulla</td>
</tr>
<tr>
<td><img src="image1.jpg" alt="Image" /></td>
<td><img src="image2.jpg" alt="Image" /></td>
</tr>
<tr>
<td>3. Labrador (M) - Uniserial ladder medulla</td>
<td>4. Labrador (F) - Amorphous &amp; fragmented medulla</td>
</tr>
<tr>
<td><img src="image3.jpg" alt="Image" /></td>
<td><img src="image4.jpg" alt="Image" /></td>
</tr>
<tr>
<td>5. Pekingese (M) - Aeriform Lattice</td>
<td>6. Pekingese (F) - Fragmented medulla</td>
</tr>
<tr>
<td><img src="image5.jpg" alt="Image" /></td>
<td><img src="image6.jpg" alt="Image" /></td>
</tr>
<tr>
<td>7. Shih Tzu (M) - Aeriform Lattice</td>
<td>8. Shih Tzu (F) - Aeriform Lattice</td>
</tr>
<tr>
<td><img src="image7.jpg" alt="Image" /></td>
<td><img src="image8.jpg" alt="Image" /></td>
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</tr>
<tr>
<td><strong>9. Stray Dog (M)- Simple continuous medulla</strong></td>
<td><strong>10. Stray Dog (F)- Miscellaneous shape of medulla</strong></td>
</tr>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>11. Spitz (M)- Uniserial medulla (Type-1)</strong></td>
<td><strong>12. Spitz (F)- Broken (Fragmented) medulla</strong></td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
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<tr>
<td><strong>Spitz (M)- Broken (Interrupted) medulla (Type-2)</strong></td>
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<tr>
<td><img src="image5.png" alt="Image" /></td>
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<tr>
<td></td>
<td>Medulla Patterns - male vs female dogs</td>
</tr>
<tr>
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</tr>
<tr>
<td>13. Cane Corso (M)</td>
<td>Uniserial Ladder</td>
</tr>
<tr>
<td>14. Cane Corso (F)</td>
<td>Broken (Interrupted) medulla (Type-1)</td>
</tr>
<tr>
<td>15. Dalmatian (M)</td>
<td>Simple continuous medulla</td>
</tr>
<tr>
<td>16. Dalmatian (F)</td>
<td>Uniserial Ladder (Type-1)</td>
</tr>
<tr>
<td></td>
<td>Cane Corso (F) - Simple continuous medulla (Type-2)</td>
</tr>
<tr>
<td></td>
<td>Dalmatian (F) - Simple medulla (Type-2)</td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>1. German shepherd (M) - Regular Mosaic</td>
<td>2. German Shepherd (F) - Irregular Wave</td>
</tr>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>3. Labrador (M) - Irregular Wave</td>
<td>4. Labrador (F) - Regular Waves</td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>5. Pekingese (M) - Irregular Wave</td>
<td>6. Pekingese (F) - Irregular waves</td>
</tr>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>
7. Shih Tzu (M) - Regular waves

8. Shih Tzu (F) - Irregular Wave

9. Stray dog (M) - Irregular Wave

10. Stray Dog (F) - Irregular Wave

11. Spitz (M) - Irregular Wave

12. Spitz (F) - Regular Wave
Table 4: Cuticle Patterns - Male vs Female Dogs

As per the data assembled in the tables, micrometry cannot be administered of much importance as an evidence to the scene of crime, observations in this study can be indicative of the meaningful variation between both the sex of Canidae breed. We observed Medullary patterns in which only two pairs of different sexes have same medullary pattern that is only 25% different sexes have shown similarity in their medulla patterns, similarly in case of medullary margins 25% of different sexes have shown similar medullary margins. In case of cuticular pattern only Pekingese breed and stray dog were observed to have similar cuticular patterns. Table 3 and table 4 shows the images of medullary and cuticular patterns observed under microscope.

Previously, Mirela E. CADAR in 2015 used SEM to study hair cuticle pattern in canidae breeds, he studied various parameters in cuticle cells such as width and height of cuticle cells, and he also examined hair fiber diameter, medullar diameter and indices. He concluded micro-metric results as non-value for examination and medullary index and patterns as a valuable source for identification. [11].
**Conclusion**

Micro-metric analysis is a type of preliminary analysis so its data is not as much reliable as the microscopic and macroscopic analysis. Different breeds of same or different sex may have same cuticular index as observed in case of pekingese (M), shihtzu (F), stray dog (F), spitz (M) & (F), cane corso (M) and Dalmatian (F) and Medullary index as observed in case of German Shepherd (F), stray dog (M) and cane corso (F) i.e. cuticular and medullary indices help in species identification and may give an idea regarding the sex to which the hair belongs. Result of the study indicate that with the examination of microscopic characteristics, it may be possible to individualise the hair from different sex of dog and the base of distinction of sex can be the medulla patterns combined with other microscopic characteristics. There are very less studies conducted on trace evidence such as Hair in India, this study also promotes more researches in this section of study for better advancements and improvement in conducting investigations.

**References**

11. Mirela E. CADAR* SEM Study of Hair Cuticle in Some Canidae Breeds. (october 2015)Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary, Source : ResearchGate
Comparison between the Binary Method and Modern Day Software Used For Mixed DNA Interpretation (A Review)

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²Research scholar at Galgotias University,
³MBA in Forensic Accounting, Gujarat Forensic Sciences University,

Abstract

A mixed DNA sample has multiple contributors along with touch DNA, which are transferred to a surface when it comes in contact. These samples are very difficult to interpret due to the complex band obtained during DNA profiling. With the gradual technical advancement, we can now derive the DNA profile from trace amount as compared to the bulk evidence which was needed earlier. These complex DNA mixtures were always available at the crime scene but were undetected or neglected. As they had more contributors and a high degree of degradation which means they were very difficult to interpret. DNA matching is done by matching the allele peaks, where a low amount of DNA gives a smaller peak and can have some might drop out or drop in. When matching the profile obtained with multiple contributors, there is difficulty in predicting which peak belongs to which contributor. So, a better way to express it is in terms of probabilities. Computer software used for predicting this probability uses statistical and biological models. They consider drop-out as well as drop-in along with all other possibilities like commonality of alleles among the population. This final probability is called likelihood ratio. This review aims at comparing the old or binary method of mixed DNA interpretation with the results of the modern-day software like kongoh, STR mix, EuroForMix, LRmix, etc., to predict the better and more efficient method.

Keywords- mixture DNA sample, DNA profiling, likelihood ratio, STR, binary method

Abbreviation- DNA- deoxyribonucleic acid

Introduction

With the advent of Next generation sequencing, the identification using DNA samples in forensic investigation has given great results and has proven to be a boon. As DNA has always been the major part of all the evidence collected from a crime scene, this method has transformed the future of forensic investigation. The only difficulty faced by the investigators was the interpretation of mixed DNA samples,
which had multiple contributors. When the allele peaks are obtained from the mixed sample by DNA sequencing, the prediction regarding which peak belongs to which individual contributor became a tedious job for the forensic professionals.\[1\] The mixed DNA sample which has been found on the crime scene since time indefinable, where initially interpreted using binary models and have gradually moved on to the probability model.

The software used for the probability model takes into consideration the stochastic effects such as drop-out alleles and drop-in alleles and also becomes sensitive to low amounts of DNA present in the sample, giving more accurate results. The software uses either continuous or semi-continuous models, which includes qualitative and quantitative evaluation. The final probability of each contributor is expressed in terms of likelihood ratio.\[2\] The introduction of these methods into a laboratory requires testing, validation, training and documentation. With the introduction of each new software in line, there has been upgradation in their user friendliness, calculation time, bugs fixation and introduction of new features, making them more efficient for degraded and low amount of mixed DNA samples.\[3\]

To this end we present a thorough comparison of the basic old method and some new software functionality, which are used for probability models of mixed DNA interpretation. The modelling of various software including EuroForMix, Kongoh, STR mix, LRmix, GenoProof mixture 3 and true allele has been discussed in detail, indicating their positive points as well as their drawbacks. This will give a complete insight to the investigators as to which software will be a better suit for the interpretation of mixed DNA samples, as this will take them down the lane of advancement of techniques in this field.

**Binary Method of Interpretation**

The traditional ‘binary’ approach used for interpretation of mixed DNA samples uses a stochastic threshold and parameters like mixture ratio, stutter ratio and heterozygote balance. In the initial approach the statistical evaluation of mixed DNA samples were done using the methods including combined probability of inclusion (CPI) and Random Match Probability (RMP).\[1\] The RMP gives the probability of the chances of a DNA profile to match within a given population, whereas CPI estimates the unrelated individuals which could include the prediction of possible contributors in a population. CPI does not focus on each individuals’ DNA profile but works on a general outcome. The initial studies related to mixed DNA were made on samples mainly containing only two contributors, which did not highlight the problems like allele drop-out and drop-in that were prominent in cases with multiple contributors.\(\text{Ref 2}\) But with the recognition of ‘touch DNA’ as the main component of evidence collected, the quality and quantity of DNA samples have decreased and thus has led to the exposure of various difficulties in their interpretation.\[4\]

The difficulties faced during interpretation of mixed DNA samples were acknowledged by International Society for
Forensic Genetics (ISFG) and they provided guidance and recommendations for handling of such mixed DNA samples.[5] The autosomal STR interpretation uses PCR to amplify targeted alleles and the product obtained is then analyzed using two approaches—Quantitative, where the peak heights are compared and the Qualitative approach. The genotypes of the PCR product which are excluded are assigned zero and the one which are included are assigned one, the binary digits. A weak peak obtained in the product indicates a low amount of DNA, while the incomplete or missing area indicates degraded DNA sample. These were all the indications of stochastic effect.[6] The peak height was considered proportional to the amount of DNA and its location indicates the presence of a particular allele. The comparison of peak height was used for identification, which did not give a satisfying result as the peaks were confused among individuals. Thus, the stochastic threshold determines which loci should be included and which should be excluded, indicated by the binary numbers assigned to them. On the other hand, the CPI method calculates the probability of occurrence of each allele and also helps in the identification of allele drop-out. [7]

The DNA fingerprinting is based on the restriction fragment length polymorphisms (RFLP) of DNA using amplification of short tandem repeats (STR) along with some DNA markers like CpG sites. Some commonly used alleles in forensic DNA fingerprinting are NRCAM, ABLIM1, LRRN3, NELL2, NOG, D5S818, D7S820, D13S317, TH01, TPOX, HUMFES, HUMTPOX and HUMTH01.[6,7,8] The binary method used for mixed DNA interpretation was not found to be very successful in achieving its goal, as it failed to give the assured number of contributors with their correct identification. The uncertainty of the result decreased its value as an evidence in the court of law and thus, a demand for an improved and advanced approach of mixed DNA profiling arose.[8]

**Mixed DNA Interpretation Softwares**

- **Euroformix Software**

The STR technique which has been widely used for DNA fingerprinting is not found efficient in case of degraded DNA. The shift from STR to single nucleotide polymorphism (SNP) has to be done specially in cases of degraded DNA. Massively parallel sequencing (MPS) technique can analyze a large sample of degraded DNA detecting both SNP and STR genotype markers simultaneously in a single run. MPS has been observed as a suitable and efficient technique while dealing with multiple contributor samples in forensic investigation. While working on this technique a large amount of data is generated and the most efficient software for interpretation of all minor variations is EuroForMix, an open-source software.[9]

The DNA samples collected are first genotyped for all the targeted STRs and SNPs. The library of these samples were prepared and the MPS was applied using a MiSeq sequencer. The STR and SNP
analysis are presented on a continuous scale. The quality and reliability of data was insured by calculating the stutter, allele coverage and depth of coverage ratio. Keeping the drop-in probability as 0.05, the likelihood ratio for the major and minor contributors are calculated based on the autosomal (STR and SNP) and Y-STR analysis.[10] The number of the compatible and non-compatible loci were determined and their contribution to the identification of minor and major contributors was established. The comparison of expected and observed number of individuals was made using Fisher's exact test and was confirmed on the basis of Hardy-Weinberg equation. [11]

- **Kongoh Software**

For calculating the likelihood ratio

1. Select the number of contributors which varies from 1 to 4
2. Set the hypothesis- the person of interest is a contributor or not
3. Select a genotype combination and a locus
4. Calculate the expected peak height (G)

\[
\text{Likelihood ratio} = \frac{\Pi_i \Sigma_i W_{i,l} \Pr(G_{i,l}|H_p)}{\Pi_i \Sigma_i W_{i,l'} \Pr(G_{i,l'}|H_d)}
\]

- The value of \(W_{i,l}\) (goodness of fit) is determined by comparing the observed and expected peak height.
- \(\Pr(G_{i,l}|H)\) represents the frequency of genotype combination.

**Fig 1. The process of calculating likelihood ratio is as following [12]**

The kongoh software estimates the number of contributors more efficiently by using either allele or locus specific effect as compared to other quantitative approaches.
There are some problems which one can face when the number of contributors exceeds four, as the difference between the likelihood value of contributors decreases making the identification difficult.[1]

- **STR Mix Software**

STRmix is a fully continuous quantitative method and is based on the Markov chain Monte Carlo method. The likelihood ratio is based on the Bayes’ theorem, which expresses it as posterior odds = LR x defense odds.[13] As this method involves the assigning of highest posterior density value and the coancestry coefficient value, it has become biased.[4] The relatedness between known and unknown individual’s data can be established using the Balding theory. The results produced by this method have high reproducibility and accuracy.[14] The stutter value is automatically assigned in this case making it a better approach than others who require manual values to be assigned.[15]

- **Lrmix Software**

It utilizes the value which is plug-in rather than the integral value. This approach is based on the maximum likelihood estimation method. This is a semi-quantitative method which requires an input for drop-out value and also does not account for degradation. It utilizes the Balding method to overcome the size related bias observed in this process. This method requires the stutter determination which increases the subjectivity and reduces the evidentiary value of the report.[16]

- **Geno proof Mixture 3 Software**

The sample DNA is typed and the raw data collected is directly fed to the GenoProof mixture 3 for interpretation. This software is based on a continuous model, which requires a statistical value for the hypothesis for the interpretation process to start. This model provides both a probabilistic and non-probabilistic approach of data interpretation.[17] It takes into consideration biological parameters like genotype weight, height of peaks, stutter peak, amplification efficiency, strength of degradation, allele size, drop-in and drop-out alleles for making the likelihood ratio. The method that it uses as a base is the Markov chain Monte Carlo method, which helps in identification of minor and major contributors. There is also a sub-population consideration feature in GenoProof mixture 3 software which reduces the dependency of alleles belonging to same sub-population and increases the chances of individual discrimination. Since it is a quantitative fully continuous process and needs initial consolidation, it has a touch of subjectivity in its nature. This approach produces a reproducible and highly accurate results by deconvoluting the genotype of the given mixture.[17]

- **True Allele Software**

The TrueAllele calculator computes the DNA data collected to interfere with the number of contributors by estimating the probability of each allele and making
comparisons to determine the likelihood ratio. This software is based on the mixture weight, genotype and the distribution of data. It requires the stutter peak value and takes into consideration the drop-out alleles while calculating the likelihood ratio[1]. The quantitative calculation are based on the comparison of the STR peak height with genotype model pattern. The probability is based on the Bayesian inference, with a good reproducibility. The assigning of manual hypothesis value initially is required to calibrate the system, which add a subjective nature to this method. As, this method is based on likelihood probability approach, it has a greater productivity, more accurate information and can infer the genotype of an unknown individual even without standard for comparison.[18]

<table>
<thead>
<tr>
<th>Properties</th>
<th>STRmix</th>
<th>True Allele</th>
<th>EuroFor Mix</th>
<th>Kongoh</th>
<th>LRmix</th>
<th>GenoProof Mixture3</th>
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<td>Bayes MCMC</td>
<td>ML Bayes Monte Carlo</td>
<td>ML estimation</td>
<td>Markov Chain MC</td>
<td></td>
</tr>
</tbody>
</table>

A comparison of different software discussed[10]

Discussion
The likelihood ratio that is being used in all the above mentioned methods is the ratio of prosecution hypothesis and the defense hypothesis. If the value of likelihood ratio is greater than one, the prosecution hypothesis holds true while if it’s value is less than one the defense hypothesis is proved to be true. The binary method does not prove to be a beneficial method for the identification of various contributors of a mixed DNA sample. As in this method the allele peak height is not used for calculation, a value of one or zero is simply assigned to denote the presence or absence of an individual's DNA. In this method some allelic peaks do not appear as they have a height below the threshold value, these are known as drop-outs. Thus, the binary method cannot be used for the interpretation of mixed DNA samples.

Other than the binary method, researchers use the qualitative and quantitative continuous modern approach using various software. The qualitative method does not use the allele peak height but has to assign stutter peak, making this method subjective in nature and has loopholes for biasness. The qualitative continuous approach that we have discussed is the LRmix software. Thus, this method is not very preferable for the investigation purpose. On the other hand, the quantitative approach calculates the likelihood ratio using the peak height of alleles and thus, there is no need for assigning stutter peak. This method also takes into consideration the drop-out and drop-in alleles along with the probability of other interference, giving all possible genotypes. Thus, by eliminating the scope for subjectivity it estimates the number of contributors specifying the major and minor contributors.

Among the different open source quantitative software that we have discussed above. Some software has to be fed with manual estimation of number of contributors before starting the software, for example EuroForMix, STRmix, TrueAllele software, thus, making them less user friendly and efficient. On the other side, software like Kongoh, are the one which automatically calculate the value of both hypotheses predicting the number of contributors without any manual input are considered highly efficient for the forensic investigation process. This software is easily available to all unlike the previous software.

**Conclusion**

The biggest challenge faced by forensic investigators in analysis of DNA is the interpretation of mixed DNA samples. There are two approaches for this, the conventional or binary method which uses the combined probability inclusion and can be used for the identification of maximum two individuals and the modern software based approaches, which uses the likelihood ratio and can be used for the proper identification of multiple contributors of a mixture. The more credible approach for elucidation of complex mixture samples of DNA are the ones using the likelihood ratio for interpretation. Among the various qualitative and quantitative continuous methods, the quantitative methods are more cogent in nature, as they do not give way to subjectivity by the researcher. Now looking...
at the various open source software, the software which are more readily available and those which are more user friendly are preferred over others. In this manner software like Kongoh are available on GitHub, making it accessible to all, while other software are not easily available on public portals. The automatic estimation of hypothesis value is a preferred feature over those in which manual entry is required. (Ref 9) Thus, we can say that the development of quantitative continuous approach based software have proven to be a boon for the forensic DNA examiners, as they give a more informative and consistent results and can now interpret the readily available and early ignored trace mixed DNA evidences, to either confirm or eliminate the presence or involvement of an individual in a crime.

For the future research scope, one should look towards development of methods which will be more efficient when the number of contributors exceed four with a greater difference between their likelihood ratio for proper identification.

Reference


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Acquittal or Conviction–A big question mark on the Criminal Judicial System due to failure of Viscera Report

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Abstract

Viscera report (Chemical Examiner’s Report) is the most important scientific report which plays the major role of evidence on behalf of death due to poisoning in the Criminal Judicial System (CJS). Hon’ble Supreme Court has made the viscera report mandatory in poisoning cases and also in the cases where is suspicion of death due to poisoning. In India, failure of the viscera report (negative report) is routinely a problem because of some reasons. There are lots of cases and judgments pending due to failure of viscera report that's why conviction or acquittal? Is been a most important question in front of the Criminal Judicial System (CJS). The primary objective of the viscera report is to find out the cause and manner of death. This is conducted by the senior scientific officer (Chemical examiner) at the Forensic Science Laboratory (FSL). Hon’ble Supreme Court has made it mandatory to bring such reports on record in court trials. Even there is various corroborative evidence, the failure of the viscera report makes the jury and investigating agencies let in confusion. Viscera report is very important in criminal cases, where the witness shows a tendency to turn hostile. This paper discusses the various factors that cause failure to viscera report such as, in India, forensic officials are not the first responders at a crime scene it is the drawback. There is no characteristic or significant finding in PM report as much of poisons have silent features and can be falsely interpreted and tends the finding to negative report, in many of the cases viscera report is not brought on record. This paper briefs about the study of the last 10 year cases (2011-2020) and discuss the stand of the viscera report in the Criminal Judicial System (CJS) in India.

Keywords: Viscera report, Criminal Judicial System (CJS), Poisoning, Hon’ble Supreme Court, Forensic Science Laboratory (FSL), etc.

Introduction

Viscus is the singular of viscera meaning in Latin, “an organ of the body”. The viscera report (Chemical Analyser’s report) is necessary for scientific evidence in death due to poisoning cases brought by Forensic Science Laboratory (FSL). Poison plays an important role in crime it’s a silent weapon of destroying life secretly and mysteriously. Poisoning cases are mostly suicidal but often homicidal and accidental also. The
viscera analysis is subjected not in every case but whenever there is suspicion of poisoning it must be done. It is very important to produce reliable evidence in front of the judicial system so that they can come on a proper judgment hence, viscera report plays a major role in Criminal Judicial System (CJS), as it gives scientific evidence to believe court on that the death has been occurred to the deceased by poison.

The current scenario in India is very challenging about the viscera report. The thousands of jars containing viscera lie dusted at hospitals. Delay in the analysis of viscera as it is not sent to the FSL by police, sometimes report not brought on record by police or prosecutor, and ultimately it negatively affects the judgment of the case. The cases are pending and go cold because of the forgotten viscera sample and its analysis. Sometimes, the viscera report is not produced during trials, and hence, in many cases, the accused/appellant has given the benefit of doubt and said to be the acquittal of all the labelled charges.

Hon’ble Supreme Court has ordered that the cases where death due to poisoning is suspected, the viscera should immediately send to the Forensic Science Laboratory (FSL) after the post-mortem for chemical analysis. The prosecuting agency/investigating officer should ensure that the viscera examined immediately and report sent to the investigating agency or court. Hon’ble SC also said that the trial court must ensure that there must be such a viscera report brought on record. If not brought on record, the concerned examiner of the FSL must ask for an explanation about the viscera report. Bringing a viscera report on record in trials is necessary because the witnesses who spoke about the poisoning generally show a tendency to turn hostile. [1, 2]

Viscera report served as a scientific report along with post mortem report. Under Sec 45 IEA, which is for the opinion of experts, Forensic Toxicologist as a Chemical Examiner at FSL is supposed to answer the questions and give his opinion on the viscera report and its analysis when asked by the court. The sections in the Indian Penal Code related to poison are Sec 324, 326, 328, and 284 IPC. Even there is corroborative evidence; the absence of a viscera report took the case entirely on the different stand in court. Circumstantial evidence plays a vital role in the Criminal Judicial System (CJS) in poisoning cases where viscera report not brought on record which is enough to produce the guilt of the appellant/accused.

Failure to present viscera report in court has many reasons, firstly there is no responsible duty is taken by police officers to transport viscera from post mortem house to the FSL immediately after the post mortem. Delay in sending viscera to the FSL ultimately delayed the report hence report not brought on record at the time of trial and judgments got pending. FSL is already overloaded with viscera samples. The chemical analyzer already dealing with huge analysis, unlike other cases chemical analysis of viscera needs at least 15-20 days. Lack of manpower in Forensic Science Laboratories results in a delayed analysis of viscera samples. The absence of
the viscera report in court weakens the case of the prosecution.

The factors causing indirectly or directly failure to viscera report are; the forensic experts are not the first officials at the crime scene. As police (head constable or PSI) are the first responders at the crime scene, they don’t have proper knowledge and training about the collection and preservation of evidence related to poisoning cases. Such as in many cases police forgot to collect the stains of vomiting or vomited remains at the crime scene which is a drawback to the Criminal Judicial System. Sometimes prosecution fails to prove that accused had administered the poison to the deceased and hence given the benefit of doubt. Poisons are the silent killer, some poisons did not detect in an analysis of viscera hence the report came to be negative in many cases of poisoning. These all factors are studied from the poisoning cases of the last ten years which leave the impact on the judgments and let the court in confusion about the acquittal and conviction of the accused.

Laws related to the word poison in India

In India, there is no such precise definition of poison but is defined in law as, "any stupefying, intoxicating, or any wholesome drug or thing which is poisonous to the human body when administered". The word poison in Indian Penal Code, 1860 is mentioned under sec 324, 326, and 328 also in 284 IPC.

The importance of viscera report is not only in the case u/s 328 IPC but also in the cases u/s 299, 302, 304-A, 306, 307, 309, 324, 326 and 498-A IPC and also in 376 IPC, these offenses deal with the administration of poisonous substances. [3]

When viscera analysis is supposed to do?

As per the study of the last ten years of cases, the viscera report is asked to produce in a court of law, when there is a suspicion of death due to poisoning or when the cause of death was not ascertained at the time of post-mortem by the concerned medical officer. At the time of post-mortem, if there are injuries present on the dead body, which indicates the forcible administration of poison then the viscera is must be sent to FSL. When the dead body is brought in the mortuary with a history of poisoning in the inquest report then the viscera is must preserved. The external signs on the dead body like, if nails and mouth turned bluish-colored, frothy discharge from mouth and nostrils, swallowing of the face shows there is a possibility that the deceased had died due to poisoning. Internal signs like organs (liver, spleen, kidney) congested are possible in poisoning. After the post-mortem examination, the viscera are supposed to forward immediately to the FSL.

Collection and forwarding of viscera samples to FSL

The viscera must be collected as mentioned under the norms of forensic science laboratories, with proper labelling in separate viscera bottles, with a proper seal.
Bottle no. 1: stomach contents (10-20ml)
Bottle no. 2: Whole stomach, pieces of small intestine with its content
Bottle no. 3: liver, spleen, and kidney
Bottle no. 4: Blood from the heart (50-100 ml, more if available)
Bottle no. 5: vitreous humor with 2% sodium fluoride as a preservative
Bottle no. 6: Urine
Bottle no. 7: Skin piece along with tissue beneath (in case of injectable poison/animal bite)
Bottle no. 8: preservative used

The collected viscera bottles with proper tag and seal then forwarded to the FSL with the sign of competent authority on forwarding letter which must include a copy of FIR/DD, copy of PM report, copy of police inquest report given to the doctors before PM, copy of suicide note or copy of the statement given by relatives of deceased if available. [4]

**Importance of viscera report to the Criminal Judicial System (CJS)**

As per the study, lots of confusing cases running in a court of law, such as generally the criminals try to wash out the evidence of poisoning, hence criminal hang the dead body or throws dead body in a river and try to convince the court that deceased committed suicide by hanging and drowning. In such cases viscera report is the “key evidence” in the court of law, which is enough to convict the accused and prove his guilt.

"Fact doesn't lie, men can do" believing this principle in forensic science, viscera report as forensic evidence in a court of law has given huge evidence in a court of law has given huge importance. As the case history or back story of the case suggests the eye witness to the court in trials who made the statement about the poisoning in case, but many of the reasons the witness shows a tendency to turn hostile. This weakens the case of the prosecution that's why presenting a viscera report in court strongly supports the case.

The viscera report not only helps to convict the accused but also helps to the acquittal of an innocent person who is wrongly implicated in the case. Sometimes there are charges made against the person in the poisoning case, that time viscera report supports the innocence of the person, if not any type of poison detected in viscera. Sometimes, in absence of viscera report the Modi's medical jurisprudence and Medical jurisprudence by Lyson these books play a vital role in the Criminal Judicial System (CJS). The signs and symptoms are shown by the deceased before dying can help to link the court that it is the case of poisoning. In many of cases, when a medical officer is asked to give his opinion on the cause of death, he had explained very finely that the symptoms shown such as vomiting, fainting, etc. is possible in poisoning case as per the toxicology books and it is accepted in the court of law.

**Current scenario of viscera analysis in India**
Viscera report as scientific evidence in court is the key to proving the guilt of accused in the case and helps in conviction. Even after knowing the importance of the viscera report, the current scenario regarding the viscera analysis in India is not so good and it directly affecting the Criminal Judicial System (CJS). Hundreds of medicolegal cases are pending in a court because the police did not send the viscera to FSL for examination.

There is a big conflict between police and medical officers about the transportation of viscera samples. The health dept. wants the police to deliver the viscera to the forensic science laboratory (FSL) while the police claim that this is the job of medical officers and midst of this dispute the viscera is locked up in a room at hospitals for decades. The thousands of viscera containing jars even do not have the proper labels to identify the name of the victim. Some viscera jars only have the number marked on that with chalk. The viscera preserved in 10% formalin or with a saline solution or with saturated common salt for preservation at least 45 days, but the viscera are rotting in the dusted bottles at the mortuary as it preserved years ago.

Forensic Science Laboratories (FSL) is already overloaded with viscera samples. The lack of manpower at the FSL directly affect to the delay of the viscera report. Unlike the analysis of other forensic evidence, viscera analysis needs at least 20 days. While analysis, when the viscera sample opened it discovered in condition to unfit for examination as the liquid had turn stinky and dusty also the tissues decayed. Because of these all reasons the current scenario in India regarding the viscera report is very challenging. [5-7]

**Reasons that cause failure to viscera report**

Many reasons are responsible for the failure of the viscera report, few of it as follows:

- There is an issue regarding the dispatch of the viscera sample to the FSL.
- Forensic experts are not the first responders to the crime scene it's the drawback, as police don't have the proper knowledge and training about the collection and preservation of evidence. E.g. In poisoning cases, vomit stains on cloth, bed sheets, etc. vomit remain at crime scene not collected by police officers in many cases in the table below.
- Viscera report is not brought on records (delay in analysis/ viscera not sent to FSL)
- The sample sent to FSL for analysis has less quantity which cannot accomplish the whole analysis.
- Gastric lavage has a significant role in the case of poisoning but in many cases, the authority failed to send gastric lavage or vomit along with the viscera sample.
- While in analysis poison not detect in viscera, it has many reasons as, certain poisons have a very short life in a biological system, when the patient is hospitalized for treatment there must be chances that the poison metabolized completely, repetitive stomach wash can also give negative results.
- Many vegetables poisons not detected in viscera analysis, due to improper
preservation organic poison also might get decompose.

- Sometimes, the viscera sent for analysis is not suitable, as the liquid turned stinky, organs get decomposed.
- Some drugs have the property to rapidly metabolize hence, it can’t detect in routine analysis.
- Lack of instrumentation, expertise, an experienced and qualified toxicologist at FSL.
- No mention of the proper words about the analysis and opinion in the viscera report by Chemical analyzer. The viscera report when submitted to the court, not mentioned properly about the fetal and lethal dose of poison and that poison particularly is responsible for the cause of death. [8-11]

The stand of viscera report in the Criminal Judicial System (CJS)

The stand of the viscera report as scientific evidence in the Criminal Judicial System (CJS) plays a vital role in conviction and acquittal of the accused/appellant. This paper presents some of the case studies from last 10 year, in which the viscera report failed to convict the accused because of some drawbacks. Some of those cases are mentioned in the table given below. Discussion about Some challenging fact in the cases regarding viscera report as follows;

- **Kinnariben Narendrabhai Patel Vs. State of Gujarat** – In this case, the dead body of the deceased not been able to send for the post-mortem analysis because the cremation was already done, the body of another deceased after reporting FIR exhumed and viscera sent for analysis. As per the viscera report, no poison detected in viscera. Accused stated in court while investigation that, she had administered poison to both of the deceased. The poison was datura seeds boiled with water and gave to the victim mixing with glucose water. [12]

- **Kamalakar Ganesh Deshpande @ Babaso Ganpat Patil Vs. State of Maharashtra** – This is the case of datura poisoning but no poison was detected in viscera as per chemical analyzers report. PM report suggested that death is due to myocardial infarction. Also, the drawback of the case was that police failed to collect vomit from the scene. [13]

- **Virender Singh Vs. State of Haryana** – This is the case of Aluminium Phosphide (sulphas) poisoning as detected in viscera. But the drawback of the case was that the witness turned hostile and viscera report was not properly mentioned about the poison was fetal and responsible for the death of the victim and hence accused acquitted of all charges labelled against him. [14]

- **Roop Kishore Vs. State of U.P.** – Viscera report was not brought on record. When questioned asked about the viscera report in the trial court the police deposed that he did not try to collect the viscera report and in anticipation of receipt of the viscera
report he submitted the final report and suggested there was no evidence regarding the cause of death hence, accused acquitted. [15]

- **Anil Kataria Vs. State** – Viscera report confirmed the presence of Aluminium Phosphide poison as the cause of death of the victim. The prosecution failed to prove the guilt of the accused in court. The case confused that the poison was in coffee but the poison was present in chutney. The prosecution believed that poison administered through coffee and no coffee mug or coffee sample was collected by police. [16]

- **Dev Kanya Tiwari Vs. The State of U.P.** – This case was registered as the deceased had died due to consuming poison but the post-mortem report showed that it was a case of hanging. Even there were signs of poisoning like nails were bluish, blisters were present on the body, and organs were congested. It was expected that doctors will preserve viscera for chemical analysis but the backdrop of the case was doctor failed to perform his duty. Hence, the benefit of the doubt given to the accused. [17]

- **Ganesh @ Premnath Dattu Deore Vs. State of Maharashtra** – Viscera report given by chemical analyzer was insufficient to prove that the deceased had died due to account of consumption of poison as there was no mention about the lethal dose in the viscera report. Accused entitled to given the benefit of doubt. [18]

- **Vinod Kumar Arora Vs. State** – after the post mortem, viscera sent to CFSL for analysis, the report stated that no opinion about the exact nature of poison detected in viscera. But the medical officer suggested that there is a strong possibility of death due to poisoning, hence viscera resubmitted for analysis, and the final opinion gave on viscera report that no poison detected in viscera. [19]

- **Venkatappa Vs. State res. By the inspector of police, Krishnagiri** – The appellant acquitted of all said charges, as there was no poison detected in viscera. The cause of death ascertained that the death was due to asphyxia (smothering) but at the post-mortem examination doctor noticed a frothy discharge from mouth and nostrils, nails of both hands and tongue was turned bluish coloured. [20]

- **Suvarna Jyotiram Chavan & Another Vs. State of Maharashtra & Another** – This is the case of homicide poisoning. After the death of the deceased instantly cremation was done. By taking permission of the concerned authorities exhumation performed by police and Postmortem done on spot, the body was highly decomposed, no poison found as per CA report. [21]

- **Maya Devi & ORS. Vs. The state & ANR.** – In this case, no poison detected in the viscera report, cause of death ascertained as per PM report, "septic shock consequent to lung infection". The deceased was non-smoker nor suffering from any respiratory disease. While investigation, in photographs of
the deceased it was observed that face was turned bluish. It is strong possibility that there is involvement of organic poison, because it not detected easily in analysis. [22]

**Joshinder Yadav Vs. State of Bihar –**
Viscera report was not brought on record. This case implicated that the deceased had died due to drowning but it was the actual case of poisoning. Doctors did not send the viscera to FSL even on the request and leave a disastrous effect on the Criminal Judicial System (CJS). [23].

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<td>Prabhas Mandal Vs. The state of Bihar – HC Patna (04/09/2015)</td>
<td>304B IPC</td>
<td>Acquittal</td>
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<td>17.</td>
<td>Basheer Palliyali Vs. The state of Kerala – HC Kerala (09/07/2015)</td>
<td>302 IPC</td>
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<td>19.</td>
<td>Joshinder Yadav Vs. State of Bihar – Supreme Court of India (20/01/2014)</td>
<td>302 &amp; 201 IPC</td>
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<td>20.</td>
<td>Vinod Kumar Arora Vs. State – HC Delhi (16/05/2014)</td>
<td>304B &amp; 498A IPC</td>
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<td>22.</td>
<td>Suvarna Jyotiram Chavan &amp; Another Vs. State of Maharashtra &amp; Another – HC Bombay (09/01/2013)</td>
<td>302 IPC</td>
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<td>24.</td>
<td>Pappu Rabi Das Vs. State of Bihar – HC Patna (03/05/2013)</td>
<td>306 &amp; 201 IPC</td>
<td>Acquittal</td>
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<td>27.</td>
<td>Sahabuddin Vs. State of Assam – Supreme Court of India (13/12/2012)</td>
<td>304B &amp; 302 IPC</td>
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Table of a few criminal cases studied form the last 10 years (2011-2020) in which viscera report not brought on record.

**Conclusion**

This paper focuses on the fact that caused directly or indirectly failure to the viscera report leaving an impact on the Criminal Judicial System (CJS) as discussed in the paper. It also founds some loopholes from...
the study of last ten years of cases and will help to overcome from it in the upcoming judgments. This paper also focuses on the current scenario of viscera analysis in India which must have to change and upgrade.

References


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Forensic Implications of Paint, It’s Use & Detection

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Abstract

There are many evidences that have a great significance in forensic investigations to solve civil and criminal cases. Paint is one of them. Paint as an evidence is found in many cases many in vehicular accidental cases. In this review, the general introduction, uses and detection methods of paint as an evidence is discussed.

Introduction

Like other evidences such as glass, hairs, fibres, blood etc paint is considered as powerful forensic trace evidence to solve the crime. The forensic analysis of paints, more properly called coatings to encompass any surface coating intended to protect, aesthetically improve, or provide some special quality, is one of the most complex areas of the forensic laboratory.

What is Paint?

Paint is a thin coating of liquid solution that after application on a substrate converts into a solid film. Paint gives color, protection & texture to the different objects. Paint has its own physical & chemical properties. The types of paint comprises oil paints, synthetic paints, emulsion paints, cellulose paints, varnishes, water paints & some special paints such as aluminium paints, anti-condensation paints, bituminous paints, chlorinated rubber paints, fire-resistant paints, fungicidal paints, texture paints etc. Paint is usually applied on a surface by brushing or spraying techniques. There are some painting defects that involves chalking, bleeding, blistering, blooming, brush marks, cracking etc.

Composition of Paint –

Generally, paint consists of three primary components: binder, pigment & solvent. Binder is a support medium for pigments & additives. Pigment provides color, texture & opacity. Solvents are the suspension of binders & pigments for application. The paint used in automobiles is called automotive paint. Automotive paint is a powder coating colored with a dye. It is a layered paint that comprises
electrocoat primer, primer surface, basecoat & clearcoat. Electrocoat primer is electrocoated on steel body is epoxy based resin whose color ranges from black to grey. Primer Surfacer is a powder consists of highly pigmented epoxy-modified polyesters or urethanes. It helps to bind the color basecoat to primer. Basecoat is acrylic based polymers that provides color & clearcoat is also acrylic or polyurethanes unpigmented powder that provides gloss, durability & etch resistance.

**Forensic significance**

In forensic science, paint can be categorized into two main groups. The first one involves coatings applied to all means of transportation such as automobiles, bicycles, motorbikes boats, and aircraft. The second category involves coatings on household objects such as walls, doors, window frames, and tools. Paint is forensically significant. Paint as an evidence is usually found in hit – and-run & burglary cases. Hit – and – run cases involves transferring of paint from one vehicle to another & fabric impressions while burglary cases involves fingerprints & shoe impressions in a wet paint, tool marks & tire impressions. Paint chip can be found on the body of victim of hit - & - run. From the sequence of the paint evidence found on the suspect vehicle, the investigators could predict that the suspected vehicle had hit the victim’s vehicle. Moreover, Investigators should search for several things like the edges of the paint sample, and whether or not there are multiple layers of paint present beyond the surface layer. This could be helpful for police to determine if the culprit’s car is newer or older, as a paint chip from a car that has been painted several times over the years is likely to have many paint layers. Thus, Paint is a very useful evidence in the investigation of crimes specially those involving automobiles.[1-3]

**Case Study**

One hit and run accident case was reported in district Jind, Haryana. In this case, one motorcycle was found in a worst conditions at the scene of accident. In this incident, the person who was riding on the motorcycle found dead. The motorcycle was seemed to be having red transfer of paint on its damaged and detached battery cover which was then collected for comparison purposes with the accused vehicle. A number of vehicles were picked up later on which were suspected to be involved in the collision. Out of these, one tanker truck was determined to be having red and white transferred paint. This white and red paint was then analysed and found to be matching physically and chemically with paint of number plate. The red paint found on motorcycle was analysed and found to be matching physically and chemically with the red paint of the tanker truck. Hence it concluded that it was the paint traced as physical evidence that linked the collision of accused vehicle with victim vehicle and hence the case could be solved beyond any doubt.[3]. Thus, this case study shows the significance of paint as forensic evidence in hit & run collisions.

**Detection**

The aim of a forensic paint analysis will depend on the case itself and will be determined according to the needs of the client and the capabilities of the laboratory.
Generally, the paint analysis involves visual examinations, chemical tests & physical match. Beside these, the instrumental methods involve Optical microscopy, micro-infrared spectroscopy and micro-spectrophotometry in the visible range that are routinely used techniques in the detection, identification and differentiation of various paint samples.[4]

1. Characterisation of paint samples by infrared and Raman spectroscopy: [4-5]

Infrared micro spectrometry and Raman microscopy can apply in characterisation of paint coatings such as in identification of pigments and for the differentiation between paint samples of similar colour and shade. It involves the use of different excitation lasers that enable to reduce the fluorescence of the sample and thus identify the main pigments present in the sample. It was shown that Raman mapping has more potential for the study of paint samples. It is useful because paints are usually chemically complex and heterogeneous mixtures. Spectroscopic images allow characterization of the chemical heterogeneity of a sample in terms of the spatial distribution of the molecular constituents. On the other hand, Infrared spectrometry is sensitive to molecular structure and therefore able to give much information about the chemical composition of a paint sample, like about its polymer binder and inorganic fillers and pigments. However, the identification of organic pigments found in such a trace is usually not possible as a result of their content is simply too low for detection. Raman spectroscopy proved to be a promising good technique in the area of forensic examination of paint pigments. The major advantage of Raman spectroscopy is the non-destructive nature of the analytical procedure, which can often be applied regardless of the form of the sample and its preparation for analysis. It is considered as a non-invasive method that can be used in situ. Raman spectroscopy measure the inelastic scattering of light, depending on the vibrational modes of non-polar bonds of a molecule when goes excited by an intensive monochromatic source such as a laser. These vibrations tend to give a far higher signal than those involving polar bonds. However, some difficulties occur concerning strong fluorescence produced by paint components that may overwhelm the weaker Raman scattering peaks. To minimise fluorescence, some of the recent Raman studies of paint have used a Fourier transform Raman instrument, which uses near infrared excitation. Other authors propose use of several excitation lasers in measurements of paint samples for the same purpose. Thus, both IR and Raman spectroscopy seem to be good methods for characterisation of multilayered car paint chips enabling identification of inorganic components and organic pigments.

2. By Using Pyrolysis–Gas Chromatography–

Pyrolysis is the breaking up of chemical bonds by using thermal energy. Paint binders are macromolecules that will decompose into smaller volatile fragments. Under controlled conditions such as under specific temperature, heating rate, and time, the same distribution of smaller molecules can be produced. The fragments are then be separated by GC and identified or
characterized using MS. A minimum of 10 mg sample is necessary for the analysis, and the samples must be prepared under controlled conditions. Each layer of a paint flake should be isolated before analysis. This technique is used to identify the monomers used in binder systems, but it might also characterize some additives or pigments if reference pyrograms and/or MS spectra are available. An extensive advantage of Py–GC/MS is its high discriminating power and its potential to detect and compare minor constituents & its disadvantages are its destructive nature and time-consuming analysis.[6]

3. Elemental Analysis (Scanning Electron Microscopy/Energy Dispersive x-Ray Analysis, Micro x-Ray Fluorescence)

Elemental analysis of paint sample includes the measurement of the atomic emission of the elements present in it. Commonly used methods are based on the atomic emission of a particular x-ray lines from a material that has been excited by high-energy x-rays. In this category, scanning electron microscopy coupled with energy dispersive spectrometry (SEM/EDS) is the most commonly used method in forensic Labs. Micro x-ray (mXRF) fluorescence instruments could also be a choice, mostly for homogeneous samples. Many layered samples must be prepared such as by either of embedding, polishing or sectioning. If a high-vacuum SEM is used, a metallic coating of the samples is needed. This is not the necessary for low-vacuum SEM or mXRF. Elemental analysis is mainly used for comparison in qualitative or semi quantitative purposes. Elemental analysis also gives an indirect identification of the inorganic content of paint by the detection of the elements present in it. This technique will mostly identify the extenders and inorganic pigments as well as some organic pigments. The analyses are rapid and rather sensitive (_0.1 wt. %) and give complementary information as against FTIR, Raman, and Py–GC/MS, which mostly characterize the organic components of a paint.[6]

4. Micro spectrophotometry

A micro spectrophotometer is one that measures the light intensity which is transmitted, absorbed, or reflected by a sample at a specific wavelength of the visible or ultraviolet region of the spectrum. It permits an objective measurement of the color. Paints are basically sustained in situ and in the reflectance mode using dark field optics. If required, transmittance spectra in the UV or visible range can also be recorded. It needs some sample preparation and quartz microscope slides. MSP is mostly used to compare samples. Pigment identification through this is rather not easy for several reasons: most of the paints contain two or three pigments and thus the resulting spectrum will be a combination of all their spectra like mostly the major ones; also, the MSP spectra of pigments are often not very characteristic, especially in reflectance mode. MSP can give an objective measure of the colour and might be able to differentiate between optically identical specimens (metamers). However, this method is more problematic for effect coatings for which there is large variation in the spectral response and measurements should be carried out on clean and undamaged sample areas of similar size and morphology. [6]
5. Fourier transform infrared spectroscopy (FTIR)

The most popular and powerful technique for paint characterization & identification is infrared spectroscopy. Almost all substances absorb specific infrared radiation and produce characteristic infrared spectra. The measurement with the IR instrument includes comparing of the IR radiation energy that transferred through the sample with the energy transferred through the reference. IR method makes it possible to examine even small samples with a little or without any loss that forms the basis of forensic investigation. Samples for infrared analysis are obtained from mid-layer to avoid any chances of surface contamination and weathering effects or to prevent the possible contributions of component migration from substrate materials and adjoining layers, especially in wet-on-wet applications. Through This method, it is possible to identify polymer binder, main pigments and fillers in each layer of the paint coat of sample. The small peaks thus visible in the spectra are signals from pigments and fillers. These signals are mostly intensive in the region of 400 – 1000 cm⁻¹. Signals such as from TiO₂ and ZnO are intensive even at a low concentration of these compounds. Signals from BaSO₄, CaCO₃, talc and kaolin can also clearly visible if present. Organic pigments are difficult to identify due to their low concentration in the given analysed samples. Signals from many pigments are overlapped by signals specific for resins. In most cases, characterization of polymer type is based on the comparison of the obtained spectra with the spectra from library.

Thus, FTIR is employed for comparison and identification of various paint film binders. Also, some of the inorganic components of a paint sample can be determined. There are mainly two FTIRs used: - Perkin-Elmer 1725X with a SpectraTech IR-Plan microscope, Perkin-Elmer 2000 with a PE microscope 1. Paint samples can be prepared for FTIR analysis by slicing a thin cross-section or by slicing thin peels of each layer separately. The samples are then rolled out on a glass slide with the help of the roller end of a roller knife. The prepared samples are then transferred to the surface of a KBr plate. The KBr plate with the prepared paint sample is placed on the stage of the FTIR microscope and transmission IR data is collected & obtained in accordance with the instrumental procedure for the particular FTIR employed.[7-8]

6. Trace elemental analysis of automotive paints by laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS)

Paints and coatings are oftenly encountered as types of materials that are submitted to forensic labs whenever a trace evidence transfers. A laser ablation method is used to simultaneously sample several layers directly prior to introduction into an inductively coupled plasma–mass spectrometer for the identification and quantification of the trace metals present in the layer(s). Time-resolved analysis plots thus shows the elemental response and quantification of selected metals that are compared to associate/discriminate paint samples. Laser ablation–inductively coupled plasma–mass spectrometry (LA–ICP–MS) has potential to detect elements
present in the paint matrix, but unlike SEM–EDS, the technique has the ability to trace elemental analysis. Elemental profiling by ICP–MS leads greater power of discrimination between glass fragments from different sources and to excellent power of association between fragments that originates from the same Source.[9]

**Conclusion**

Thus all the above mentioned techniques are used to solve cases such as mainly road accidents in which paint is found as an evidence. In some cases, microscopic examination is not sufficient then the instrumental techniques are preferred that are rapid & sensitive & thus give accurate results.

**References**