Gender Identification through Barr Body Analysis in Salivary Deposits on Chewing Gum: A Forensic Approach

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***Abstract*—Gender identification plays a pivotal role in forensic investigations, particularly in scenarios where biological evidence is limited or degraded. Among various biological traces, saliva is one of the most frequently encountered at crime scenes, often found on objects such as cigarette butts, envelopes, bite marks, and chewing gum. This study explores the forensic applicability of identifying gender through the detection of Barr bodies in buccal epithelial cells present in saliva deposited on chewing gum. Barr bodies, representing inactivated X chromosomes, are typically observed in female somatic cells and absent in normal male cells, thus serving as a reliable cytological marker for sex determination. Saliva samples were indirectly collected from chewing gum chewed by individuals of known gender. Buccal epithelial cells embedded in the gum matrix were carefully extracted, smeared onto slides, fixed, and stained using appropriate staining techniques to enhance nuclear visibility. Microscopic examination was conducted to identify the presence or absence of Barr bodies. The study demonstrates that chewing gum serves as a viable and practical source of biological evidence for cytological analysis. The findings reinforce the significance of Barr body detection as a cost-effective, rapid, and reliable method for gender identification in forensic science, especially in resource-limited settings where advanced DNA analysis may not be readily accessible.**

***Index Terms*—Gender Identification, Barr Body, Saliva, Chewing Gum.**

I. INTRODUCTION TO SALIVA AND ITS FORENSIC RELEVANCE

Saliva is a complex biological fluid that plays a crucial role not only in physiological processes but also in forensic investigations. It is secreted by major and minor salivary glands and consists predominantly of water, along with enzymes, proteins, electrolytes, and epithelial cells. The presence of desquamated buccal epithelial cells within saliva makes it an invaluable biological specimen for forensic analysis, particularly in the identification of individuals and determination of gender.

From a physiological standpoint, saliva aids in digestion, lubrication, and oral hygiene. Enzymes such as salivary amylase initiate carbohydrate digestion, while antimicrobial agents like lysozyme and immunoglobulin A protect against microbial invasion. However, beyond its biological functions, saliva has significant forensic importance due to its frequent presence at crime scenes.

In forensic contexts, saliva is commonly found on objects such as chewing gum, cigarette butts, envelopes, and bite marks. These traces often contain epithelial cells rich in nuclear DNA, enabling both cytological and molecular analyses. Even when DNA degradation occurs, cytological methods such as Barr body identification can still provide crucial information regarding the biological sex of the individual.

The non-invasive nature of saliva collection and its widespread availability make it particularly useful in

forensic investigations. Unlike blood, which may not always be present, saliva is frequently deposited unknowingly, making it a valuable form of trace evidence. Furthermore, advancements in forensic cytology have enabled scientists to utilise saliva not only for DNA profiling but also for preliminary screening techniques such as Barr body detection. This method is especially useful in cases where rapid results are required or when laboratory resources are limited. Thus, saliva serves as a bridge between biological science and forensic application, offering both molecular and cytological insights. Its role in gender identification, particularly through the detection of Barr bodies in buccal epithelial cells, underscores its significance as a reliable and accessible forensic tool.

II. COMPOSITION AND ENZYMATIC PROPERTIES OF SALIVA

Human saliva is a multifaceted biological fluid composed of approximately 99% water and 1% organic and inorganic substances. Despite its high-water content, the dissolved components play essential roles in maintaining oral health and facilitating forensic investigations. The organic constituents of saliva include enzymes, proteins, mucins, and immunological components. Salivary amylase is the most prominent enzyme, initiating the breakdown of starch into simpler sugars. Lingual lipase contributes to lipid digestion, while antimicrobial enzymes such as lysozyme and peroxidase protect against bacterial infections.

Inorganic components include electrolytes such as sodium, potassium, calcium, phosphate, and bicarbonate ions. These ions regulate the pH of saliva, which typically ranges between 6.2 and 7.6, and help in maintaining dental integrity through remineralisation processes. From a forensic perspective, the most significant component of saliva is the presence of buccal epithelial cells. These cells contain nuclei with chromosomal material, making them suitable for both DNA analysis and cytological examination. The presence of enzymes like amylase also serves as a biochemical marker for identifying saliva stains at crime scenes.

The enzymatic composition of saliva further enhances its forensic value. For instance, amylase detection tests are commonly used in forensic laboratories to confirm the presence of saliva. This is particularly useful in cases involving bite marks or objects suspected of containing salivary traces. Additionally, the antimicrobial properties of saliva contribute to the preservation of cellular material to some extent, especially when embedded in substances like chewing gum. Although environmental factors may degrade the sample over time, the initial protection offered by saliva can be crucial for successful analysis. Thus, the composition and enzymatic properties of saliva not only support essential physiological functions but also significantly contribute to its utility in forensic investigations, particularly in gender identification studies.

III. CHEWING GUM AS FORENSIC EVIDENCE

Chewing gum is a unique and highly valuable form of trace evidence in forensic science. Its physical properties, including elasticity, adhesiveness, and semi-solid consistency, make it an ideal medium for retaining biological material such as saliva and epithelial cells. When an individual chews gum, prolonged contact with the oral cavity results in the deposition of buccal epithelial cells. These cells become embedded within the sticky matrix of the gum, preserving them for later analysis. The process of mastication enhances cell transfer due to continuous friction between the gum and oral tissues.

One of the key advantages of chewing gum is its ability to preserve biological material. As the gum dries, enzymatic activity decreases, slowing down the degradation of cellular components. Additionally, the gum matrix provides a protective environment that shields the cells from external environmental factors such as wind and minor disturbances. Chewing gum is also commonly discarded in public places, making it readily available at crime scenes. Its presence can provide crucial forensic clues, particularly when other forms of biological evidence are absent.

Moreover, chewing gum allows for both cytological and molecular analyses. It can be used for DNA profiling as well as Barr body detection, making it a versatile forensic tool. The ease of collection and storage further enhances its practicality in forensic investigations. Thus, chewing gum serves as a reliable and accessible source of biological evidence, significantly contributing to the field of forensic science.

IV. CHROMOSOMAL BASIS OF GENDER DETERMINATION

Gender determination in humans is fundamentally based on chromosomal composition. Normal human cells contain 46 chromosomes, including one pair of sex chromosomes. Females possess two X chromosomes (XX), while males possess one X and one Y chromosome (XY). The presence of the Y chromosome, specifically the SRY gene, initiates male development. In its absence, female development occurs by default. However, the presence of two X chromosomes in females necessitates a mechanism to prevent genetic imbalance.

This is achieved through X-chromosome inactivation, a process in which one of the X chromosomes becomes transcriptionally inactive. The inactivated chromosome condenses into a structure known as the Barr body, which is visible under a microscope. In males, since only one X chromosome is present, Barr bodies are typically absent. This fundamental difference forms the basis for cytological gender identification.

Chromosomal abnormalities such as Klinefelter syndrome (XXY) and Turner syndrome (X) highlight exceptions to this rule, emphasising the need for careful interpretation in forensic analysis. Thus, chromosomal composition provides a reliable biological foundation for gender determination, particularly through the identification of Barr bodies.

V. BARR BODIES: STRUCTURE AND IDENTIFICATION

Barr bodies are condensed, inactive X chromosomes found in female somatic cells. Microscopically, they appear as dense, dark-staining structures located at the periphery of the nucleus. They are typically observed in interphase cells and can be identified using various staining techniques. Approximately 20–60% of female epithelial cells exhibit visible Barr bodies under optimal conditions.

The identification of Barr bodies serves as a rapid and cost-effective method for determining biological sex. However, factors such as staining quality, sample preservation, and observer expertise can influence detection accuracy. Thus, Barr body analysis remains a valuable tool in forensic cytology.

VI. METHODOLOGY OF BARR BODY DETECTION

The methodology involves collecting saliva samples from chewing gum, extracting epithelial cells, preparing smears, fixing and staining the samples, and examining them under a microscope. Staining techniques such as Papanicolaou and Feulgen staining enhance nuclear visibility. Proper slide preparation and staining are crucial for accurate identification.

Microscopic examination focuses on identifying the presence or absence of Barr bodies in the nuclei of epithelial cells. Thus, the methodology is simple, cost-effective, and suitable for forensic applications.

VII. RESULTS AND DISCUSSION

The study demonstrates that Barr bodies are present in female samples and absent in male samples. The detection rate varies depending on sample quality and time intervals. Environmental factors and sample handling influence cell recovery and visibility. Despite limitations, the method proves effective for gender identification. Comparison with previous studies supports the reliability of Barr body analysis. Thus, the findings reinforce the forensic applicability of this technique.

VIII. FORENSIC SIGNIFICANCE AND LIMITATIONS

Barr body analysis plays a crucial role in forensic investigations by aiding in sex determination, narrowing suspect pools, and supporting legal evidence. However, limitations include chromosomal abnormalities, sample degradation, and observer bias. Despite these challenges, the method remains valuable, particularly in resource-limited settings.

IX. CONCLUSION

Gender identification is a fundamental aspect of forensic science, serving as a critical step in establishing biological identity and aiding criminal investigations. This study highlights the significance of saliva as a readily available biological sample and chewing gum as a practical medium for preserving epithelial cells. The detection of Barr bodies in buccal epithelial cells provides a simple, rapid, and cost-

effective method for determining biological sex. The findings demonstrate that chewing gum can effectively retain salivary cells, enabling cytological analysis even after a certain period. The presence of Barr bodies in female samples and their absence in male samples confirms the reliability of this method.

While advanced DNA profiling techniques offer higher accuracy, Barr body analysis remains an important preliminary tool, especially in situations where resources are limited. It provides valuable insights that can guide further investigation and reduce the scope of analysis. However, the method is not without limitations. Factors such as environmental conditions, staining quality, and chromosomal abnormalities can affect results. Therefore, Barr body analysis should be used in conjunction with other forensic techniques for more accurate conclusions. In conclusion, the study underscores the importance of integrating simple cytological methods with modern forensic practices. The use of chewing gum as a source of biological evidence opens new avenues for investigation, making forensic science more accessible and efficient.

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