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Research Article

Contact Lens as Evidence in Crime Scene - A Way to Identify Gender

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Abstract

Introduction: Identifying Barr bodies' presence has a significant diagnostic value in multifaceted science disciplines. Testing Barr bodies was important in diagnosing infertility, a syndromic association such as Klinefelter and psychopathic disorders, and disorders of sex development (DSD). It also plays a role in cancer detection in the uterine cervix, identifying transplanted retinal pigment epithelium in porcine models. Identifying the gender of victims or criminals becomes a fundamental requirement in any forensic analysis of a crime scene. The current study hypothesizes identifying the gender using Barr body detection from collected contact lens samples and tries to establish disposed soft contact lenses to consider as evidence found at the crime scene.

Methods: A total of 120 (60 males and 60 females) were included in the study; from each subject, contact lens and Buccal samples were collected using sterile wooden toothpicks and soft contact lenses after insertion and removal. Both the buccal and contact lens samples were built into two smears staining with Saffranine and Methylene blue stains. The smears underwent cytological assessment by two examiners using a binocular microscope at 40X. The details of findings were graded on a scale of 1 to 5 based on visualization of Barr bodies seen.

Results: The mean rank and median grading scores for higher using saffranine among females across both the samples. The sensitivity is higher at 100% for both the stains among contact lens samples, and specificity is higher among buccal samples, 93% for saffranine and 90% for methylene blue.

Conclusion: Overall, it is conspicuous that contact lenses can be considered as evidence found at the crime scene in identifying the gender using Barr body detection.

Keywords: Barr Bodies; Contact Lens; Crime Scene; Saffranine; Sex Determination

Introduction

Visualization of Barr bodies presence has an immense scope in multidisciplinary research and practice. Barr bodies are specific to gender. In females, X chromosomes become inactivated and form Barr bodies. This process of inactivation is known as Lyonization. Due to Barr body formation, the nucleus in a female cell tends to be smaller than in a male [1]. Barr bodies bear 0.8 X 1.1 microns and show the morphology of V, W, X or S shapes, spherical, triangular, Plano-convex or concave, rectangular shapes visualized under a microscope [2]. Barr bodies bear a surface antigen and aid in identifying the molecular basis of the inactivation of the X chromosome [3]. Murray L Bar and Ewart G Bertram first identified Barr bodies as nucleolar satellites in male and female cat neurons with the help of a basic compound microscope and the Nissl method of staining. According to their findings, 30-40% of female cells bear Barr bodies [4].

Testing Barr body presence has significant diagnostic value in multifaceted science disciplines. Barr bodies testing is essential in diagnosing infertility and syndrome association in males such as Klinefelter with cognitive impairment and subnormal intelligence [5]. The process of X -Chromosome inactivation (used as XCI hereafter) is very random in early life. However, non-random inactivation is very prominent in tumours, recurrent pregnancy loss, X-linked diseases and older women [6]. The presence of many Barr bodies also helps in cancer detection from the uterine cervix. Double Barr bodies are seen in cases of Carcinoma in situ, and Invasive Carcinoma, which also correlates positively with measured nuclear DNA content [7]. The number of Barr Bodies is also dependent on the Ploidy of cells. One Barr body is seen in a diploid individual, whereas in a triploid individual, it could be one or more [8]. Multiple X syndromes are associated with defective genes leading to psychopathic disorders, indicating the probability of criminal tendencies. In a study, among 100 male jail inmates tested for Barr bodies with Peripheral blood smears (PBS) and Buccal smears (BS), 60% of PBS and 36% of BS showed the presence of Barr bodies [9].

Barr Body staining is also more effective in detecting the transplanted porcine RPE and differentiating between the donor and host RPE in the opposite gender [10]. Gender determination became more prominent in 1960 among Olympic athletes to remove the unfair gender advantage of winning under the women category with male characteristics. The crude physical examination of externalia has revealed possessing female external genitalia among suspected athletes with delayed diagnosis of Disorders of Sex Development (DSD) [11]. Gender misrepresentation in the 1968 Olympics is addressed by Barr body detection through dignified cytological assessment of buccal smears [12].

Decimated brutal human mass disasters lead to severe loss of lives. The gender of Victims or criminal remains can be identified in forensic investigation via both morphological and molecular analysis. The morphological analysis includes reconstruction of post-mortem dental profiles, dimensional tooth analysis, Cheiloscopy, Rugoscopy, and identifying morphological traits of the skull. The molecular analysis includes the extraction of DNA and visualizing the presence of Barr bodies, F-bodies and SRY genes in various body specimens [13,14]. Gender determination serves as the prime source of massive disasters. In a few cases, it also reduces the chance of tampering with evidence by perpetrators helping to establish the initial search of either victim or criminal. Gender identification is based on multiple factors. Using dental records, 93%, 44% by physical characteristics of the body, 42% by physical belongings, and 30% by personal documents and fingerprints [15].

Most recent literature has identified gender using Barr bodies with the help of Buccal and odonatological samples. In addition, gender can be found using samples like amniotic fluid, nails, hair, vaginal cells, surface epithelial cells, urinary residues, and blood [16]. Moreover, the cytological assessment of the above samples is performed using Aceto-orcein, Methylene blue, May-Grünwald Giemsa (MGG), Papanicolaou, diamond fuchsin, Acridine orange, haematoxylin and eosin, carbol fuchsin stains so far.

Gender identification can also be identified using PCR and fluorescence assays, but it might take 4-8 hours and be less cost-effective [17]. On the other hand, cytological assessment using basic staining procedures is highly cost-effective and less time-consuming.

Most studies have performed gender identification using various samples and stains through Barr body identification. In the current study, we hypothesize that if a criminal is a soft contact lens user and disposes of the contact lenses at a nearby crime

scene, we anticipate finding the gender using the disposed contact lens. On the other hand, sex estimation is never assessed through saffranine in Barr body detection in most of the earlier studies, which is considered a positive stain and can easily stain -ve charged DNA. Moreover, saffranine is a readily available stain that can stain the samples collected from the ocular surface.

Aims and Objectives

The current study aims to establish contact lenses as evidence in the scene of an investigation by finding the gender of the individual via a disposed soft contact lens. The study's objective is to assess the efficacy of saffranine stain in sex estimation using cytological assessment of Barr bodies among contact lens samples.

Materials and Methods

The current study is a prospective comparative analytical study design. This study is performed under the declarations of Helsinki and approved by the institutional committee. The study was conducted at the Department of Optometry, Centurion University of Technology and Management, Andhra Pradesh. Students from various departments were invited to explore contact lens wear experience. Students who volunteered to experience the soft contact lens wear were assessed for ocular health by performing visual acuity assessment, refraction and slit lamp biomicroscopic examination.

All subjects free from ocular and ocular surface diseases and infections were considered samples. Subjects with topical cosmetic application on lids or eyelashes were excluded. All the volunteers were also screened for oral health. Subjects with any oral ulcers, infections or diagnosed to have any other systemic conditions or social history of tobacco chewing or smoking have been excluded from the study.

Contact lens sampling

After oral and ocular health screening, each subject was given study participant information and informed written consent. Subjects who agreed to participate were inserted with a single contact lens in either volunteer's preferable eyes with an Optometrist's help. All the soft contact lenses were made of the same Hydrogel material, i.e., Omafilcon A and are daily disposables with an 8.6mm Base curve, 58% water content, and 14.2 mm diameter. Enough time was given to experience each subject's wear and waited until the lacrimation subsided. The lenses were removed and placed back in the blister packs containing contact lens solution, and the lenses were suspended in the solution.

With the help of a micropipette, 15 µL solution from blister packs having removed contact lens samples suspended were drawn and retained on two dry microscopic slides. One of the slides is treated with Methylene blue, and the other with saffranine stain. Using Whatman filter paper, the excess stain was blotted out and carried out for cytological assessment.

Buccal smear sampling

All the selected volunteers have undergone preprocedural rinsing using distilled water to avoid the presence of mucus, debris or any other contamination in the sample. With the help of sterile wooden toothpicks, buccal scraping was taken from every individual and suspended onto two dry microscopic glass slides. Similar to smears prepared from contact lens solution, one of the buccal scrape samples was treated with methylene blue stain and the other with saffranine.

Sampling

60 healthy volunteers (30 females and 30 males) participated. Overall, 240 smears were prepared, of which 120 smears (60 processed with Methylene blue and 60 with saffranine stains) were made from contact lens solution and 120 smears (60 processed with Methylene blue and 60 with saffranine stain) using buccal scrapings.

All the prepared microscopic slides were taken for the cytological assessment using a binocular compound microscope tested at 40X magnification. Each sample is tested by two examiners (PV and MK) separately and scored their findings as per table 1 given below.

Among multiple cells screened, cells with clear visible details and easily distinguishable Barr bodies under either stain were considered for grading (Figure 1 a-d).

Both the examiners, PV and MK, were blinded and not aware of the exact predetermined sex of the sample. This procedure

Sr. no	Description	Score
A	No stain/Absence of cell staining	0
B	Cell stained but nucleus is not seen	1
C	Cell stained properly, but Barr body is not seen	2
D	Shrunken cell with the presence of Barr body	3
E	Barr Body was seen but did not distinctly appear	4
F	Distinct presence of Barr bodies seen	5

Table 1: Grading strategy followed for cytological assessment of Barr bodies.

Figure 1: Cytological smears with cells positive for Barr bodies (X40). a Methylene Blue stain Buccal cells with Barr Body. b Saffranine stained Buccal cells with Barr body. c Methylene Blue stained ocular surface cells from contact lens with Barr body. d Saffranine stained ocular surface cell from contact lens evidencing Barr body. The insets show the nuclei with the Barr body of the respective cells.

is followed to prevent bias in scoring. Samples scored ≤ 2 are considered males whereas, scoring between 3 to 5 with > 2 Barr bodies seen in a sample were recorded as females [18,19]. The verdict of the cytological assessment is then compared with the actual known sex of the sample to find the efficacy of sample usage and stain used in the current study.

Statistical analysis

Statistical analysis was performed in Statistical Program for Social sciences (IBM SPSS Statistics 22 Version). The normality of the data is assessed through Kolmogorov-Smirnov and Shapiro-Wilks tests. Due to a lack of normal distribution, non-parametric tests were used for further analysis. Descriptive statistics were computed, including sex classification based on Barr body grading and its Median staining scores. Inter and Intra observer reliability is assessed using Kendall's Tau correlation. Kruskal-Wallis is performed to assess the superiority of staining scores among Contact lens and Buccal smears. A Post Hoc analysis with Dunn-Bonferroni correction is assessed for pair-wise comparisons among genders and stains within contact lenses and Buccal smears.

Results

One hundred twenty samples were collected (60 from females and 60 from males), of which half were taken from Contact lenses and the other half from Buccal smears. In the Contact lens and Buccal cells group, 30 females and 30 males were uniformly involved. Overall, 240 smears were prepared, 120 stained with Methylene blue and the rest 120 with Saffranine.

The Barr body grading three scores are higher among the contact lens smear group compared to the Buccal smear group and higher among the female gender in either group (see Table 2).

A Kendall's Tau correlation test was performed to assess whether there is an association between the judgement of Barr bodies cytological assessment made by both examiners, i.e., PV and MS using smears of buccal cells and contact lens with Methylene blue and saffranine preparations. Except for male samples from contact lenses stained with saffranine, most showed a high positive correlation with significant association (see table 3).

Barr Body examination grading	Contact lens Smears				Buccal Smears			
	Saffranine		Methylene Blue		Saffranine		Methylene Blue	
	Female	Male	Female	Male	Female	Male	Female	Male
3	30 (100%)	4 (13.3%)	30 (100%)	4 (13.3%)	29 (96.7%)	2 (6.7%)	29 (96.7%)	3 (10%)
<3	0	26 (86.7%)	0	26 (86.7%)	1 (3.3%)	28 (93.3%)	1 (3.3%)	27 (90%)

Table 2: Sex classification based on grading Barr bodies seen among both samples.

Inter-observer variability between samples tested by PV and MS	Gender from which samples taken	Stains used	r	P Value
Contact lens smears	Female	Methylene Blue	0.91	0.01
Contact lens smears	Female	Saffranine	0.99	0.02
Buccal smears	Female	Methylene Blue	0.91	0.02
Buccal smears	Female	Saffranine	0.95	0.63
Contact lens smears	Male	Methylene Blue	0.98	0.01
Contact lens smears	Male	Saffranine	0.25	0.05
Buccal smears	Male	Methylene Blue	0.99	0.14
Buccal smears	Male	Saffranine	0.98	0.33

Table 3: Inter-observer variability assessed between PV and MS examiners.

Similarly, Kendall's Tau correlation test was performed to assess whether there is an association between the judgment of Barr bodies cytological assessment in Contact lens and Buccal samples under methylene blue and Saffranine stains graded by each examiner. Though there is a high correlation between both samples, no significant association seen could probably be due to the tested samples (i.e., Contact lens and Buccal) (see Table 4).

Gender of the Tested samples	Examiner tested	Stain used	r	P value
Female	1	Methylene blue	0.61	0.17
Female	1	Saffranine	0.97	0.52
Male	1	Methylene blue	0.98	0.14
Male	1	Saffranine	0.96	0.32
Female	2	Methylene blue	0.53	0.24
Female	2	Saffranine	0.99	0.03
Male	2	Methylene blue	0.99	0.05
Male	2	Saffranine	1	0.10

Table 4: Inter-observer variability assessed between PV and MS examiners.

A Kruskal-Wallis test showed a significant difference between the staining scores of samples stained by saffranine and Methylene blue across both genders among Contact lens and Buccal smears with $H(7) = 178.57$, $P < 0.001$. Post Hoc analyses with a Dunn-

Bonferroni correction adjusted P value less than 0.05 showed P < 0.001. The median scores were higher for females than males across both the stains and samples (i.e., Contact lens and Buccal smears). On relative comparison, except for the female gender stained with saffranine, all the mean ranks are higher in the contact lens smear group. The Saffranine female Buccal smear has the highest mean rank of 189.19 (Median Md = 5) compared to the female contact lens smear with a mean rank of 187.95 and Md = 5 (see tables 5 and 6).

	Contact lens Smears				Buccal Smears			
	Saffranine		Methylene Blue		Saffranine		Methylene Blue	
	Female	Male	Female	Male	Female	Male	Female	Male
Median staining score	5	2	4	2	5	2	4	2
Inter Quartile Range (Q3-Q1)	5-4	2-2	5-4	2-2	5-4	2-2	4-4	2-2

Table 5: Saffranine and Methylene Blue staining scores among Contact lens and Buccal smears.

	Contact lens smears				Buccal smears			
	Saffranine		Methylene blue		Saffranine		Methylene blue	
	Female	Male	Female	Male	Female	Male	Female	Male
Mean Rank	187.95	69.38	167.68	58.47	189.18	65.38	160.48	65.47

Table 6: Saffranine and Methylene Blue Means Ranks among Contact lens and Buccal smears.

The sensitivity is higher, i.e., 100%, among Contact lens smears with both the stains and relatively; the specificity is higher among Buccal smears having 93.3% with Saffranine and 90% with Methylene blue. The Overall accuracy is higher with Saffranine among Buccal smear samples (see table 7).

	Contact lens Smears		Buccal Smears	
	Saffranine	Methylene Blue	Saffranine	Methylene Blue
Sensitivity	100%	100%	100%	96.7%
Specificity	86.7%	86.7%	93.3%	90%
Accuracy	93.3%	93.3%	96%	93.3%

Table 7: Sensitivity, specificity and Accuracy of Saffranine and Methylene blue in sex determination.

Discussion

The last decades of literature evident that Barr bodies are formed by the inactivation of X isochromosomes. These are specifically evident during the interphase of the cell cycle and evidencing q-arm-to-q-arm fusing [20]. Barr bodies are seen explicitly in the female gender and people with disorders of sex development (DSD) [4,5,11]. Diagnostic value is added by assessing the presence of Barr bodies in cases of infertility among Klinefelter's men [5]. Previous studies also used to identify the presence of Y bodies in identifying the male gender, where Y bodies are predominantly seen in males. However, Klinefelter's men have both Barr and Y bodies [21]. The current study has determined the gender using samples of both contact lenses and Buccal smears purely based on Barr bodies seen during the cytological assessment. The male gender does present with Barr bodies in the case of Klinefelter's. In contrast, females decrease Barr's body count due to chromosomal abnormalities such as Turner's syndrome. A 24-year-old woman diagnosed with Aortic dissection found dead on her bed showed decreased Barr bodies in the tissues [22].

In earlier studies, the cell grading and choosing criteria differ for sex determination. Few studies have considered 50 cells in a slide and then graded them with various criteria by excluding cells with less chromatin length, bacterial contamination etc. [23]. Few studies considered the number of Barr bodies seen among 50 cells. More than or equal to 5% of Barr bodies seen is considered female, and <5% are considered male [24]. In another study, ≤2 Barr bodies seen are male, and >2 Barr bodies are female [19]. In the current study, we considered a specific grading scale and tried to visualize the best cell having less contamination among multiple cells. We graded accordingly on the best cell seen in a slide.

Kendall's Tau correlation showed very well inter-observer reliability with stronger correlation and significant association across the samples. In addition to masking the gender of the sample to prevent bias, both examiners were made to assess samples individually without following the order of samples tested by the first examiner to avoid the second examiner's prejudiced conclusion based on the conclusion given by the first examiner. All the female contact lens smears were stained with either saffranine or Methylene blue graded >= 3 and classified as female. On the other hand, 3% of females stained with either stain of Buccal smears scored <3 and classified as male. In our study, 96 to 100% of females had Barr bodies, whereas, in a previous study, 39.29% of females had Barr bodies [25]. The earlier study on female cat neurons by Murray L Bar and Ewart G Bertam revealed that 30-40% of female cells bear Barr bodies, and 36% of male Jail inmates showed Barr bodies in Buccal smears [2,9]. In another study, samples stained with Giemsa and Methylene blue showed 92-96% have Barr bodies [19]. Such high discrepancies in the percentage of females seen with Barr bodies could be due to the differences in methodologies adopted in each study.

There were no evident studies performed on Contact lens samples in gender determination. In a comparative study, peripheral blood smears and Epithelial buccal cells were stained with Giemsa and Methylene blue. Both stains showed higher accuracy, between 94-98% [19]. Similarly, peripheral blood smears showed 100% positivity for Barr bodies, and Methylene blue proved to be a superior stain [26]. In our earlier study on Buccal smears, a comparison was made between saffranine and methylene blue for the first time, and we found Saffranine with superior stain efficacy [27].

So far, comparisons have been made between blood smears and Buccal or dental pulp. The current study compared the novel contact lens sample and Buccal smears. Kruskal-Wallis mean ranks were higher with saffranine among females in buccal smears, followed by females in contact lens samples stained with saffranine. The accuracy of Saffranine is also higher among Buccal smears (96%) compared to contact lens smears (93%). In earlier studies, Haematoxylin and Eosin (H&E hereafter) showed 98.9% and 54% accuracy by Papanicolaou stain [28,29]. In another study on buccal smears, methylene blue showed 88% accuracy, and H&E showed 80% accuracy [30]. In the current study, saffranine remained a superior stain with higher mean ranks and median staining scores than methylene blue across both the buccal and contact lens smears. Qualitatively the size of the nucleus of the epithelial cells from buccal smears appears relatively larger than the ocular surface cells drawn from the contact lens sample. Possibly the reason for greater mean ranks among females in buccal smear using saffranine is due to greater visibility of the nucleus, thereby achieving higher grading scores.

The sensitivity and specificity of the PAP stain were 98%, and the Aceto-Orcein stain with 90 and 100% among buccal smears [23]. In the current study among Contact lens smears, both Saffranine and Methylene blue showed 100% sensitivity, and Buccal smears evidenced 100% sensitivity for Saffranine and 96.7% for Methylene. On the other hand, specificity is higher among Buccal smears with both stains. Saffranine showed 93.3% specificity and 90% using Methylene blue in Buccal smears. The sensitivity and specificity are the same across the stains in contact lens smear with 100% and 86.7%, respectively.

Changes in sensitivity and specificity are evident with changes in samples assessed and stains used across the literature. In our previous study, also among buccal smears, saffranine showed 100% sensitivity and 93.3% specificity. On the other hand, Methylene blue evidenced 93.3% sensitivity and specificity [27]. Overall, it is evident that Saffranine is considered a superior stain among both samples compared to methylene blue. Contact lenses can be used as evidence in finding the gender of an individual.

Limitations

Gender determination is possible using a disposed contact lens. However, to what extent of disposal time it is possible to determine

the gender is unknown. In the current study, we tried to establish the Contact lens as evidence in gender determination by assessing cytological smears immediately after lens disposal. Future studies can work on the efficacy of gender determination with a change in time of lens disposed to understand the feasibility of gender determination.

Conclusion

It is very conspicuous that Contact lenses can be considered as evidence at the crime scene to identify the gender of the suspect using soft contact lenses found in the crime scene. Saffranine seemed to be a superior stain with greater sensitivity, specificity and accuracy in gender determination.

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Statement of Ethics

This study is performed in accordance with the declarations of Helsinki. Informed written consent is provided to all the subjects. No invasive procedures were included in the study methodology.

Conflict of Interest Statement

There are no professional, political, personal, or other forms of conflict of interest.

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Author Contributions

Manisai Koduri: Conceptualization and study framework, data collection, data analysis, analysis interpretation, manuscript writing and critical revision.

Pravallika Vataparthi: Conceptualization and study framework, data collection, manuscript writing and critical revision.

Nikhila Bolleda: Conceptualization and Data collection

Vijaya Laxmi Golla: Conceptualization and Data collection

Sravani Mereddy: Critical revision of the manuscript and final approval

Sony Gunaganti: Critical revision of the manuscript and final approval.

Data Availability Statement

Data sets are available with the corresponding author and have no restrictions to sharing with editors and reviewers.

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