

## CHARACTERIZATION OF VALIDITY AND RELIABILITY OF MAY GRUNWALD-GIEMSA, CARBOL FUCHSIN AND VAN GEISON STAIN IN DETERMINATION OF BARR BODIES IN BUCCAL MUCOSAL SMEARS

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### **ABSTRACT:**

**INTRODUCTION:** Barr body is of the X chromosomes formed from random inactivation and condensation of one of the two female chromosomes in virtually all the somatic cells of female mammals

**AIM:** The study aimed to compare the specificity, sensitivity and reliability of May Grunwald-Giemsa (MGG), Carbol fuchsin (CF) and Van Geison (VG) stain in identifying barr bodies in buccal mucosal smears.

**MATERIALS AND METHODS:** A prospective single blinded cohort study was conducted on buccal mucosal smears obtained from 30 subjects. The study subjects was divided into group I and group II consisting of 15 females and 15 males in each group respectively following obtainment of informed consent. All the buccal mucosal smears were processed accordingly as described by Ayyad S B et al (2012), Meena N et al (2016) and Wadsworth A et al (2012) for May Grunwald-Giemsa stain, Carbol fuchsin stain and Van Geison stain. The barr bodies identification was determined by the presence of a darkly stained condensed area on the nucleoplasm as described by Balderman S et al.

**RESULTS:** The results thus obtained showed that CF had a better BBI compared to MGG and VG and the statistical analysis showed 100% specificity, sensitivity and reliability in all the three stains in identifying barr bodies. Barr body was found to be negative in the male samples and positive for female samples with Carbol fuchsin yielding 9.8% of Bar Body Index (BBI), May Grunwald-Giemsa yielding 5.4% and Van Geison yielding 2.8%.

**CONCLUSION:** On comparison, the efficacy and accuracy for detection of Barr bodies were more with Carbol fuchsin following May Grunwald-Giemsa and Van Geison.

**KEY WORDS:** Barr body, buccal smear, sex determination, May Grunwald-Giemsa, Carbol fuchsin, Van Geison stain.

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### **INTRODUCTION**

The prime concern of Forensic Dentistry is to identify the sex of the individual.<sup>1</sup> Sex determination becomes the first priority in the process of identification of a person by a forensic investigator in the case of mishaps, chemical and

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nuclear bomb explosions, natural disasters, crime investigations and ethnic studies.<sup>2</sup> Specimen like blood, semen, hair, buccal epithelial cells,

fibroblasts of pulp, cervical cells, skin and saliva stains found in various parts of the body or on harmful weapons at a crime prospect as well as at disaster sites can also be used for gender identification.<sup>3</sup> Techniques like Polymerase Chain Reaction (PCR), karyotyping, fluorescent body (Y chromatin), Davidson body in the polymorphonuclear leukocytes, AMEL identification and Barr bodies (X chromatin) examination through cytological procedures can validate the gender.<sup>4</sup> However, PCR and karyotyping are very expensive and are not feasible for use.<sup>2</sup> Thus, Barr body demonstration for gender determination using exfoliative cytology is considered one of the simplest and easiest methods.<sup>5</sup>

Barr body is formed from random inactivation and condensation of one of the two female chromosomes in virtually all the somatic cells of female mammals. Females shut off one of their X chromosomes during embryonic development. The inactivated X chromosome is called a Barr body and is sometimes referred to as sex chromatin.<sup>6</sup> Barr and Bertram in 1949 first described the sex chromatin in mammals.<sup>7</sup> In 1961, Lyon outlined the X-inactivation or what is commonly known as the Lyon hypothesis. It states:

- (1) Only one of the X chromosomes is genetically active.
- (2) The other X of either maternal or paternal origin undergoes heteropyknosis and is rendered inactive.
- (3) Inactivation of either the maternal or paternal X occurs at random among all the cells of the blastocyst on or about the 16th day of embryonic life.
- (4) Inactivation of the same X chromosome persists in all the cells derived from each precursor cell.

Thus, the great preponderance of normal women are in reality mosaics and have two populations of cells, one with an inactivated maternal X and the other with an inactivated paternal X.<sup>8</sup> The inactive X chromosome appears as a facultative heterochromatic body existing visible during interphase as dark-staining, peripheral nuclear structure in a somatic cell nucleus of normal female but absent in male tissue.<sup>6</sup> It has normal size of about  $1\mu$  with average of  $0.7-1.2\mu$  in section of human, is preferentially located at the periphery of the cell nucleus and is considered heteropyknotic X chromosome.<sup>9</sup> Though many stains are used in identifying barr bodies, a comparative study using Carbol fuchsin, May Grunald Giemsa and Van geison stains is not yet reported.

## **AIMS AND OBJECTIVES**

The aim of the present study is to assess the characterization of validity and reliability of May Grunwald-Giemsa, Carbol Fuchsin and Van Geison stain in determination of barr bodies in buccal mucosal smears.

## **MATERIALS AND METHODS**

A total of 30 healthy subjects who visited outpatient department of AECS Maruti College of Dental Sciences and Research Centre were included in the present study. Among 30 subjects, 15 were males and 15 were females above 18 years of age.

**Inclusion Criteria:** Healthy subjects with no lesions and habits, with age group of more than 18 years.

### **Exclusion criteria:**

1. Subjects with detrimental habits such as tobacco and alcohol consumption.
2. Age: Individuals with age group of less than 18 years.

### 3. Subjects with systemic diseases and syndromes.

#### **Method of collection of samples:**

After obtaining informed verbal consent, the students were asked to rinse the mouth with mouthwash and then with water. A sterilized spatula was used to draw along the buccal surface of the cheek. These initial scrapings were discarded as they may be charged with microorganisms and occasional food particles. A fresh spatula was used to collect cells from the cleaned deep epithelial layers. Those collections were spread fairly thinly on three grease-free, graphite-labelled slides. The slides were immediately dropped into a Coplin jar containing 95% ethyl alcohol and were allowed to be air-dried before staining in order to make the cells adhere more firmly to the slide and were then stained with three different special staining techniques, namely CF, MGG and VG

#### **Method of processing of samples:**

A total of three smears were collected from each individual resulting in 90 samples of which 30 smears were stained with CF, 30 with MGG and other 30 with VG.

For Carbol fuchsin staining, smears were spread over albumenized slides. They were fixed for 30 min in 95% ethyl alcohol. Then smears were hydrated using 80%, 70%, 50% alcohol in the descending order for about 2–5 min followed by water. The slides were then stained by CF. Then differentiate them in 95% ethyl alcohol. After that, slides were put in absolute alcohol for 1 minute followed by clearing in xylene. Lastly, the slides were mounted in dibutyl phthalate xylene with a coverslip.

For May Grunwald Giemsa staining, the air dried smears were fixed in methanol for 10–20 mins. The slides were then stained with May-Grunwald working solution (360 ml of May-Grunwald solution in 240ml of buffered water) for 3 mins.

Then the slides were stained with Giemsa working solution (84ml of Giemsa solution in 516ml of buffered water) for 15 mins. The slides were rinsed with clean buffered water for 2, 5 and 2 mins consecutively. The slides were then dried in upright position at room temperature. Lastly, the slides were mounted in dibutyl phthalate xylene with a coverslip. For Van Geison staining, the smears were fixed in 95% ethyl alcohol for 15–30 min, rinsed in distilled water and stained in Harris's haematoxylin for 4 min. The slides were washed under tap water for 1–2 min, differentiated in acid alcohol, blued in 1.5% sodium bicarbonate and rinsed in distilled water. Then, these were transferred to 70% and then 95% alcohol for a few seconds. After staining in orange G 6 for 1–2 min, these were rinsed in three changes of 95% alcohol for a few seconds each and then stained in eosin azure 36 for 1–2 min. These were rinsed again in three changes of 95% alcohol for a few seconds each. Finally, those were dehydrated in absolute alcohol, cleared in xylol and mounted in dibutyl phthalate xylene.

#### **Barr Body Count**

Decision Criteria (DC): The barr bodies identification was determined by the presence of a darkly stained condensed area on the nucleoplasm as described by Balderman S et al. If DC was satisfied then it was tabulated as BB present and if not it was tabulated as absent. The frequency of Barr body was examined by observing 100 nuclei per specimen under binocular light microscope at 100 magnification. The obtained values were tabulated and sent for statistical analysis for Kappa values and Students T test.

#### **RESULTS**

The present study was conducted to evaluate rapidity with reliability of three different special nuclear stains in Barr body demonstration. On evaluation by two examiners, there was no barr-body-positive cells present in the male samples

whereas all the female samples showed barr-body positive cells by all the three stains. The weighted Kappa Value as calculated was 0.84 which indicated a very good agreement between the examiners.

For all BB positive cases Barr Body Index (BBI) was calculated using:

$$\frac{\text{Total number of BB counted cells} \times 100}{\text{Total number of cells (Approx.1000)}}$$

On comparing the accuracy of all the three stains, the Carbol Fuchsin stain scored better BBI with mean BBI value of 9.8% (Fig 1) as compared to May Grunwald-Giemsa with mean BBI value of 5.4% (Fig 2) and Van Geison stain with mean BBI value of 2.8% (Fig 3) (Table 1). Further sensitivity, specificity, positive predictive value and negative predictive value was calculated which showed 100% results for CF, MGG and VG.

	GROUPS	MEAN BBI	P VALUE
CARBOL FUSCHIN	Males (n=15)	0	0.999
	Females (n=15)	9.8	
MAY GRUNWALD GIEMSA	Males (n=15)	0	0.975
	Females (n=15)	5.4	
VAN GEISON	Males (n=15)	0	0.955
	Females (n=15)	2.8	

**Table 1: Mean BBI of Barr positive cells using Student’s t test**

	GROUPS	MEAN BBI	P VALUE
CARBOL FUSCHIN	Males (n=15)	0	1.1
	Females (n=15)	9.8	
MAY GRUNWALD GIEMSA	Males (n=15)	0	
	Females (n=15)	5.4	
VAN GEISON	Males (n=15)	0	
	Females (n=15)	2.8	

**Table 2. P value using Anova test**

## DISCUSSION

The present study was undertaken in search of a technique which combined rapidity with reliability. The males in this study showed 0% Barr bodies and females showed a BBI of 9.8%,

5.4% and 2.8% barr bodies in observed buccal mucosal cells by carbol fuchsin stain, May Grunwald Giemsa and Van Geison stain

respectively. The buccal smear technique to identify Barr bodies was first introduced by Moore and Barr in 1955.<sup>10</sup> The Barr body was

analyzed in human oral cavity in 1955 when Hermann and Davis<sup>11</sup> analyzed oral smears of 100 persons for Barr body and reported 0-2% incidence of barr body positive in males and 10% and 32% in females.

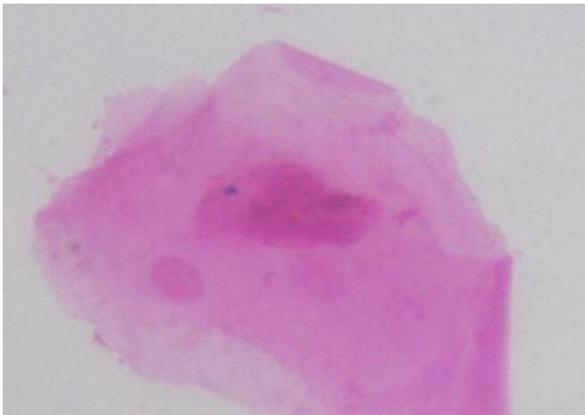


Figure 1: Appearance of barr bodies in buccal scrapes using Carbol Fuschin stain under oil immersion.

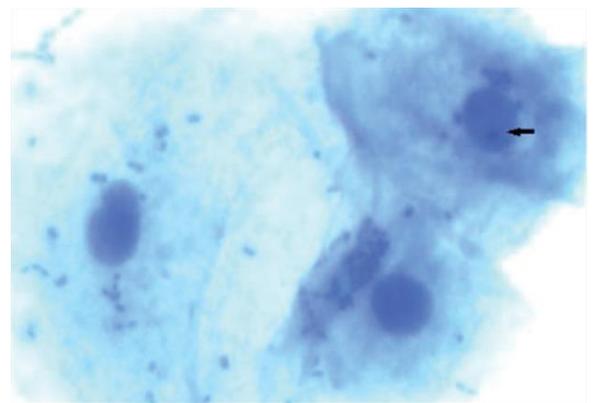


Figure 2: Appearance of barr bodies in buccal scrapes using May Grunwald Giemsa stain under oil immersion.



Figure 3: Appearance of barr bodies in buccal scrapes using Van Geison stain under oil immersion.

In the study conducted by Meena NR et al,<sup>12</sup> the percentage of barr-body-positive cells ranged from 4-14% for Carbol fuchsin stain and all the samples showed the presence of barr bodies

None of the female showed less than 4% Barr-body-positive cells for Carbol fuchsin stain. The mean percentage of barr body positive cells was observed  $8.68 \pm 2.97\%$  for the carbol fuchsin stain which were in accordance with the present

study. Study conducted by Archana T et al<sup>3</sup> did not report any Barr-body-positive cells in males. Barr-body-positive cells were observed with a percentage 5–14% for CF stain which was in contrast to our study. In the study conducted by Verma U et al.<sup>15</sup>, the Carbol Fuchsin stain was used to examine the barr body percentage of normal new born females. They found the barr body present range of 3- 11% and the mean percentage was found as 6.4±0.25%.

When we compared the accuracy of all the three stains, the Carbol Fuschin stain scored better BBI in barr body positive cells (Mean percentage 9.8%) as compared to the May Grunwald Giemsa stain (Mean percentage 5.4%) and Van Geison stain (Mean percentage 2.8%). So we can conclude that the Carbol Fuschin stain is much accurate for barr body demonstration and the statistical analysis showed 100% specificity, sensitivity and reliability in all the three stains in identifying barr bodies. The results showed Van Geison stain to be equally effective as that of Carbol Fuschin and May-Grunwald Giemsa stain identifying barr bodies with 100% specificity and sensitivity. Further Van Geison is cost effective among all the three stains.

However, no previous study has compared the Carbol Fuchsin stain, May Grunwald Giemsa and Van Geison stain to assess the characterization of validity and reliability of these stains in in determination of barr bodies in buccal mucosal smears

## **CONCLUSION**

The present study aimed to compare the specificity, sensitivity and reliability of May Grunwald-Giemsa, Carbol fuchsin and Van Geison stain in identifying barr bodies in buccal mucosal smears. In conclusion, Carbol Fuschin had a better BBI and showed better revelation of

nuclear details compared to MGG and VG and the statistical analysis showed 100% specificity, sensitivity and reliability in all the three stains in identifying barr bodies.

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