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# A comparison study of carbol fuchsin and papanicolaou staining methods for the demonstration and enumeration of barr bodies in buccal smear

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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. In practice stains such as Papanicolaou, and carbol fuchsin, have proved suitable because they produced stronger contrast and are much simple to manipulate.
**Aim:** Our study aimed to assess the efficacy of gender determination by a comparison between Carbol fuchsin and Papanicolaou stain in buccal mucosal scrapings.
**Material and Methods:** For the study to be conducted, 500 samples were taken in total, with 250 smears for carbol fuchsin stain and 250 smears for papanicolaou stain obtained from students. The smears collected were subsequently stained with carbol fuchsin and papanicolaou stains.
**Results and Discussion:** In considering descending order of merit, papanicolaou stain yielded 46.14% of barr body positive cells, against 8.68% of barr body positive cells harvested by carbol fuchsin stain. Papanicolaou stain excelled in both the efficacy and accuracy, from the carbol fuchsin stain.
**Conclusion:** In comparison the papanicolaou stain came out better and low time consuming as compare to the carbol fuchsin stain.

**Keywords:** Barr body, Papanicolaou stain, Carbol fuchsin stain.

## 1. Introduction
Barr body [1]. 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. 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.
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 [2]. It has normal size of about 1µ with average of 0.7-1.2µ in section of human, is preferentially located at the periphery of the cell nucleus and is considered heteropyknotic X chromosome [3].
In the segmented nuclei of granulocytes, it may form a characteristic appendage the so called drum-stick. Barr body (X-chromatin) can be seen well on the nuclear membrane of squamous epithelial cells of the epidermis and buccal mucosal cells as round, oblong, triangular, plano-concave, or flattened body lying adjacent to the nuclear membrane internally [4]. The distribution of Barr body present in an individual cell per se when there is more than one X-chromosome in the chromosomal structure can be understood by the knowledge of Lyon inactivation hypothesis [5].
The investigation of Barr bodies in cell nuclei allows provisional designation of the sex chromosome status of individuals hermaphroditism, gonadal, and some complicated sex chromosome anomalies from easily accessible tissues as buccal mucosa, hair root and blood cells, whereas the use of amniotic fluid enables a prenatal sex diagnosis [7]. Identification and evaluation of Barr bodies can be carried out in living cells with the use of phase contrast microscope under favourable conditions [8], Fluorescence microscopy using fluorochrome such as acridine orange or quinacrine is possible [9].

As regards the cytological staining techniques for the demonstration of Barr bodies, the haematoxylin in the routine haematoxylin and eosin (H&E) stain (in fact all of the basic dyes) will stain Barr body, but many have a preference for what they consider to be more selective-stain i.e. Feulgen stain, cresyl fast violet, thionin, Papanicolaou technique, acetoorecin, or Guard's stain. In principle, all methods which stain the chromatin of the entire cell nucleus and differentiate it from the nucleus are suitable. But methods which stain the chromatin deeply and trace the cytoplasm and the nucleoli are considered superior.

In practice however, other stains such as Papanicolaou [11], and carbol fuchsin, have proved suitable because they produced stronger contrast and are much simple to manipulate. Staining with haematoxylin and eosin usually yields rather low contrast picture of X chromatin in comparison to the above mentioned stains. Our study aimed to assess the efficacy of gender determination by a comparison between Carbol fuchsin and Papanicolaou stain observed under an oil immersion microscope for the detection of Barr bodies in buccal mucosal scrapings.

## 2. Aims and Objectives

The aim of the present study was to assess the accuracy and efficacy of nuclear stains; Carbol fuchsin and Papanicolaou methods in staining Barr bodies.

## 3. Material and Methods

250 healthy Students volunteered for collection of samples from normal buccal mucosa from the department of anatomy, Sardar Patel Medical College, Bikaner. These students were above 18 years old.

### 3.1 Inclusion and exclusion criteria

Healthy subjects were included in the study; subjects with harmful habits such as smoking, gutkha consumption etc were excluded from study.

### 3.2 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 micro-organisms 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 two grease-free, graphite-labelled slides for two different staining methods. 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.

### 3.3 Method for processing of samples

For the study to be conducted, 500 samples were taken in total, with 250 smears for carbol fuchsin stain and 250 smears for papanicolaou stain obtained from students. The smears collected were subsequently stained with carbol fuchsin and papanicolaou stains.

For papanicolaou staining, the smears were fixed in 95% ethyl alcohol for 15–30 minutes, rinsed in distilled water, and stained in Harris's hematoxylin for 4 min. The slides were washed under tap water for 1–2 min, differentiated in acid alcohol, blued in tap water or 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 OG 6 for 1–2 min, these were rinsed in three changes of 95% alcohol for a few seconds each and then stained in EA 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 and xylene.

For Carbol fuchsin staining, smears were spread over albumenized slides. They were fixed by keeping them in a fixative (absolute alcohol-95 cc and Distilled Water-5 cc) and fixation time would be ½-24 hours. Then smears were hydrated using 80%, 70%, 50% alcohol in that descending order and water. The slides were kept for 2-5 minutes in each concentration of alcohol and were stained by Carbol Fuchsin. Then differentiate them in 95% ethyl alcohol. After that slides were put in absolute alcohol for varying, from few dips to 1 minute. Then slide were cleared them in xylene. Lastly the slides were mounted in dibutyl phthalate and xylene.

The smears stained were observed under an oil immersion microscope at 100 X magnification. 100 cells were observed in each slide. Out of these 100 cells the total number of barr body positive cells were counted. Also, a comparison was made between the factors as, number of barr body positive cells, nuclear staining, nuclear membrane integrity, cytoplasmic staining and cytoplasmic transparency in the smears stained with Carbol fuchsin and Papanicolaou stains.

### 3.4 Statistical methods

The statistical methods of standard deviation, t- test, arithmetic mean were employed to assess the accuracy of the staining method. Further the percentage was applied to assess the efficacy difference between both the stains compared.

## 4. Results

Buccal scrapings were collected from 250 healthy students between the age ranging from 17 year to 21 years (143 males and 107 females) 100 cells in each sample were analyzed for identification of barr bodies and other staining properties of the chosen both the stains. Those with Barr bodies were therefore expressed as a percentage of the total. In the male samples, there was no barr-body-positive cells were observed by both the stains.

In the female samples, the percentage of barr-body-positive cells ranged from 4-14% for Carbol fuchsin stain and 20-74% for the papanicolaou stain respectively (Table 1), 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 and 20% for the Papanicolaou stain. The mean percentage of barr body positive cells was observed 8.68±2.97% for the carbol fuchsin stain and 46.14±15.76% for the papanicolaou stain respectively. When the students t test was applied for the percentage of barr body cells for both the stains, the difference between mean percentage values was found to be highly significant p= <0.000.

**Table 1:** Barr body percentage observed by Carbol fuchsin stain and Papanicolaou stain in female samples

| Barr Body Percentage by | Number of female samples | Min. | Max. | Mean | SD |
|---|---|---|---|---|---|
| Carbol fuchsin stain | 107 | 4 | 14 | 8.68 | 2.970 |
| Papanicolaou stain | 107 | 20 | 74 | 46.14 | 15.762 |
| t=30.172, df =106, P-value | | | | <0.000 | |

By both the stains only three type of shapes of the barr body was seen. The shapes observed were oblong, round and planoconcave. We did not record any other shape of the barr body (Table No. 2).

**Table 2:** Descriptive statics of different type of barr body shape observed by both the stains (Female Samples)

| | Shape of Barr body observed by Papanicolaou Stain | | | Shae of Barr body observed by Carbol Fuchsin Stain | | |
|---|---|---|---|---|---|---|
| | **Oblong** | **Round** | **Plano-concave** | **Oblong** | **Round** | **Plano-concave** |
| Min.% | 11 | 7 | 2 | 2 | 1 | 0 |
| Max. % | 39 | 26 | 9 | 7 | 5 | 2 |
| Mean % | 24.49 | 16.13 | 5.51 | 4.64 | 2.90 | 1.14 |
| S.D | 8.334 | 5.534 | 1.939 | 1.487 | 1.046 | .621 |

Table No. 3 shows the characteristics of staining for various parameters observed by Carbol fuchsin stain and Papanicolaou stain. Both the stain gave violet staining to the nucleus but in different intensity. The corbol fuchsin stain gave deep violet staining in higher percentage than Papanicolaou stain. In our observation we have seen that the light nuclear staing is better for barr body detection, because in light nuclear background the deeply stained barr body can be seen prominently.

The corbol fuchsin stain preserved nuclear membrane integrity in low percentage than Papanicolaou stain. Preserved nuclear membrane was seen smooth and less preserve nuclear membrane seen rough, but the smooth nuclear membrane integrity is better for barr body detection.

Carbol fuchsin stain gave violet staining to the cytoplasm and Papanicolaou stain gave eosinophilic colour to the cytoplasm. The corbol fuchsin stain gave deep staining in higher percentage than Papanicolaou stain. In our observation we have seen that the light cytoplasm staining is better for barr body detection.

Carbol fuchsin stain gave low cytoplasm transparency to the cell cytoplasm and Papanicolaou stain gave high cytoplasm transparency to the cell cytoplasm. The corbol fuchsin stain gave low cytoplasm transparency in higher percentage than Papanicolaou stain. In our observation we have seen that the high cytoplasm transparency staining is better for nuclear and barr body detection, because in high cytoplasm transparency the deeply stained barr body can be seen more promptly.

We achieved selective staining by using a short period of 8-10 minutes as against 12-15 minutes from papanicolaou stain and carbol fuchsin stain respectively. In comparison the papanicolaou stain came out better and low time consuming as compare to the carbol fuchsin stain.

The counts speak themselves. In considering descending order of merit, papanicolaou stain yielded 46.14% of barr body positive cells, against 8.68% of barr body positive cells harvested by carbol fuchsin stain. This result was considered accurate because of the age, number of donors, and samples specificity, and reproducibility of staining reaction of papanicolaou stain and carbol fuchsin stain.

**Table 3:** Distribution of nuclear Staining as light and deep between both the stains.

| **Parameters Observed** | **Nuclear Staining Observed** | | **Nuclear Membrane Integrity Observed** | | **Cytoplasmic Staining Observed** | | **Cytoplasmic Transparency Observed** | |
|---|---|---|---|---|---|---|---|---|
| Staining Characteristics | Light Stained | Deep Stained | Smooth | Rough | Light Stained | Deep Stained | High | Low |
| Papanicolaou Stain | 34.8% | 65.2% | 90.0% | 10.0% | 85.6% | 14.4% | 81.6% | 18.4% |
| Carbol Fuchsin Stain | 13.2% | 86.8% | 20.8% | 79.2% | 18.4% | 81.6% | 14.8% | 85.2% |

## 5. 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 4-14% and 20-74% barr bodies in observed buccal mucosal cells by carbol fuchsin stain and papanicolaou stain respectively.

The Barr body was analyzed in a human oral cavity in 1955 when Hermann and Davis [10] 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.

Manjula Bhai *et al.* [11] Also did not report any Barr-body-positive cells in male. A few studies [12] reported a higher range (20-78%) of barr body positive cells. Reddy *et al.* [13] in 2012 examined mucosal samples stained with Acito orecin to assess confocal microscopy for the detection of Barr body positive cells and found out that female sample showed 18-72% cells showing barr body positive cells whereas male samples showed 1-3% which is almost equal to the present study.

In the study by Datar U *et al.* [14] 2013 in which the papanicolaou stain was used the range of barr body positive cells was observed as 4-20 in females and 0-5 in males, The difference in the range as compare to present study of barr body positive cells may be due to less number of samples observed by the Datar U *et al.* as compared to the present study (250 Samples).

No previous study came in our notice which has reported the percentage of different barr body shapes. In the present study we attempt to calculate the percentage of different types of shapes of barr body. In our observation we found that the oblong shape barr body was more in count than the round and planoconcave shapes. The planoconcave shape was lest observed in the present study.

However no previous study has compared the papanicolaou stain with the carbol fuchsin stain. In the study conducted by Verma U *et al.* [15], the corbol 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%, which is similar to present study coarbol fuchsin stain samples.

When we compared the accuracy of both the stains, the papanicolaou stain scored better in barr body positive cells (Mean percentage 46.14%) as compared to the carbol fuchsin stain (Mean percentage 8.68%). So we can conclude that the papanicolaou stain is much accurate for barr body demonstration.

We compared the efficacy of both the stains to show effectively the barr body in given samples, for that four

parameters (nuclear staining, nuclear membrane integrity, cytoplasmic staining, cytoplasmic transparency) were observed between both the stains. Considering above compared parameters between both the stains the papanicolaou stain excelled in both the efficacy and accuracy, from the carbol fuchsin stain.

## 6. Conclusion

In conclusion the papanicolaou stain for Barr body is better than the usual carbol fuchsin stain because it is more reliable and gives a highest count. Seen in that light, papanicolaou stain is suggested as a routine replacement for carbol fuchsin stain for demonstration and enumeration of barr body in cytology.

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