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Original Research Article

STUDY OF SEX DETERMINATION BY PRESENCE OF SEX CHROMATIN IN ORAL MUCOSAL CELLS

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ABSTRACT

Introduction: In oral smear, a small condensed mass of sex chromatin usually located just inside the nuclear membrane of the nucleus is called Barr body. The study of Barr bodies is advantageous for sex determination by presence or absent. Oral mucosal smear of 150 students (79 female students and 71 male students in the age group of 17 to 32 years) from P.D.U. Govt. medical college, Rajkot were selected with aim to study oral mucosal cells for presence of sex chromatin in oral mucosal smear and to measure efficacy of oral smear in determination of sex by presence of sex chromatin during 2012 to 2014.

Method: Oral smear was prepared for sex determination by scrapping buccal mucosa with wooden spatula and obtained turbid suspension, these were smeared on a glass slide, fixed by mixture of Methanol and Glacial acetic acid in the ratio of 3:1 for 10 min and stained by Carbol Fuschin for 15-20 min at room temperature, after taking informed written consent from students. Smears were examined with Compound light uniocular microscope under 100x magnification (Oil immersion), cells and nuclei were easily identified.

Observations and Results: One hundred cells were counted in each slide. Mean value of Barr body positive cells in male was 0.647 and Mean value of Barr body positive cells in female was 35.215 and range of percentage of Barr body positive cells in male was 0-5% and range of percentage of Barr body positive cells in female was 0-55%. Presence of sex chromatin in oral mucosal cell of female was statistically significant. (P value < 0.05).

Conclusion: Mean value of Barr body positive cells in male was 0.647 ± 1.148, Mean value of Barr body positive cells in female was 35.215 ± 10.28, range of percentage of Barr body positive cells in female was 0-55% and range of percentage of Barr body positive cells in male was 0-5%. Presence of sex chromatin in oral mucosal cell of female was statistically significant. (P value<0.05) Mean values of sex chromatin positive oral mucosal cells of male was lower than mean value of sex chromatin positive oral mucosal cells in female, that is the supporting fact that sex chromatin is present in female in higher frequency. Sex chromatin can be used as simple and easily available method for sex determination.

KEY WORDS: Buccal Mucosa, Microscope, Barr Body.

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INTRODUCTION

The term sex refers to sexual phenotype. The mechanism by which sex is established is termed as sex determination. The sex of an individual may be defined in three different ways. In oral smear, a small condensed mass of s e x

chromatin usually located just inside the nuclear membrane of the nucleus is called Barr body.

The study of Barr bodies is advantageous in that it can be studied under an ordinary compound microscope with simple staining techniques. The easily available material for Barr body studies is the buccal mucosa, which can be obtained without inflicting trauma on the subject. Since Barr bodies are present within nuclear material, special stains for nucleus such as Papanicolaou stain, Feulgen, orcein, Haematoxylin & Eosin, Cresyl Violet, Carbol Fuschin and fluorescent staining are used to visualise them.

The present study was conducted on 150 medical students in Department of Anatomy, P.D.U. Govt Medical College Rajkot, Gujarat with aim to study oral mucosal cells for presence of sex chromatin in oral mucosal smear and to measure efficacy of oral smear in determination of sex by presence of sex chromatin.

MATERIALS AND METHODS

A Cross sectional study was conducted at Department Of Anatomy, Pandit Dindayal Upadyay (P.D.U.) Government Medical college Rajkot, Gujarat during a year 2012 to 2014. The subject was asked to rinse his/her mouth with drinking water. The slide was cleaned with distilled water to remove dust. The serial number was written on one end of the slide with the diamond headed pencil.

A mucosal smear (cellular material) was obtained with flat wooden spatula. Wooden spatula was scrapped on the buccal surface of the cheek of the subject. Cellular material was quickly taken over slide and diluted with distilled water to avoid drying. Cellular material was spreaded with help of another slide. The other slide was dragged with an angle of 45 degree on surface of first slide and smear was prepared. Mixture of Methanol and Glacial acetic acid in the ratio of 3:1 was used as a fixative. The cellular material was fixed by fixative for 10 min at room temperature. After fixation of slide staining was done by Carbol Fuschin stain. Stain was dropped over slide with the help of dropper and kept for 15-20 min at room temperature. After that slide was thoroughly washed with running tap water and dried at room temperature. Slide was mounted by using Dibutyl Phthalate Xylene (D.P.X.), covered with microscopic cover glass and dried for 2 min in Hot air oven. Smears were examined initially under 10x and 40x magnification with Compound light uniocular microscope. Then slides were examined under 100x magnification (Oil immersion) and cells and nuclei were easily identified. One hundred cells were counted in each slide. Out of 100 cells, the total number of Barr body positive cells (cells which showed presence of a Barr body) were counted. As 100 cells were observed, number of Barr body positive cells indicated the percentage of Barr body positive cells per smear. All data were analysed statistically with Epi info software version 7.0 & Microsoft excel 2010 to find out the frequency, mean and standard deviation for each of the above parameters.

OBSERVATIONS AND RESULTS

The present study was conducted on 150 medical students in Department of Anatomy, P.D.U. Govt. Medical College Rajkot, Gujarat.

Table 1: Distribution of students according to gender.

Sex of students	Male	Female
Number (No.) of students	71 out of 150 (47.33%)	79 out of 150 (52.67%)

Table -1 show distribution of students according to gender which suggest that 71 out of 150 (47.33%) students were males and 79 out of 150 (52.67%) were females.

Table 2: Distribution of students according to presence or absence of sex chromatin in oral mucosal cells.

	Total number of subjects	Number of subject showed presence of Sex chromatin	Number of subjects not showed presence of Sex chromatin
Female	79	77(97.47%)	2(2.53%)
Male	71	25(35.21%)	46(64.78%)
chi square value		83.76	
P value		0.00(<0.05)	

Table 2 show that out of 150 students, 71 were males and 79 were females, in this 97.47% (77 out of 79) females showed presence of sex chromatin in oral mucosal cells and sex chromatin was absent in 2.53% (2 out of 79) students and 35.21% (25 out of 71) male showed presence of sex chromatin in oral mucosa cells and Sex chromatin was absent in 64.78%(46 out of 71) students, Presence of sex chromatin in oral mucosal cell of female was statistically significant. (P value<0.05).

Table 3: Frequency distribution of female students.

No. of cells with sex chromatin per 100 cells	Female students
0-5	2.53% (2)
6-10	1.26% (1)
11-15	1.26%(1)
16-20	3.80% (3)
21-25	3.80% (3)
26-30	12.65%(10)
31-35	17.72%(14)
36-40	27.84%(22)
41-45	13.92%(11)
46-50	12.65%(10)
51-55	2.53%(2)

Table 3 show frequency distribution of female students according to no. of cells with sex chromatin per 100 cells in oral mucosal smear show that sex chromatin was absent in 2.53%(2 out of 79) of female students in oral mucosal cell, 1.26 %(1 out of 79) of female students showed presence of sex chromatin positive cells in range of 6-10,1.26 %(1 out of 79) of female students showed presence of sex chromatin positive cells in range of 11-15, 3.80 %(3 out of 79) of female students showed presence of sex chromatin positive cells in range of 16-20, 3.80%(3 out of 79) of female students showed presence of sex chromatin positive cells in range of 21-25,12.65 %(10 out of 79) of female students showed presence of sex chromatin positive cell in range of 26-30,17.72 %(14 out of 79) of female students showed presence of sex chromatin positive cells in range of 31-35,27.84 %(22 out of 79) of female students showed presence of sex chromatin positive cells in range of 36-40,13.92 %(11 out of 79)of female students showed presence of sex chromatin positive cells in range of 41-45,12.65 %(10 out of 79) of female students showed presence of sex chromatin positive cells in range of 46-50 and 2.53 %(2 out of 79) of female students showed presence of sex chromatin positive cells in range of 51-55.

Table 4 Show distribution of Male students according to no. of cells with sex chromatin per 100 cells show that sex chromatin was absent in 67.60 %(48 out of 71) male students, 25.35 %(18 out of 71) of male students showed presence of sex chromatin in range of 1-2 cells per 100 cells,4.22 %(3 out of 79) of male students showed presence of sex chromatin in range of 3- 4 cells per 100 cells and 2.81 %(2 out of 7) of male students showed presence of sex chromatin in range of 5-6 cells per 100 cells.

Table 4: Distribution of male students.

No. of cells with sex chromatin per 100 cells	Male students
0	64.78% (46)
1	19.71% (14)
2	8.45% (6)
3	2.81% (2)
4	1.41% (1)
5	2.81% (2)

Table 5: Detection of barr body positive cells in male and female

Sex	Range	Mean	Standard deviation
Male	0-5%	0.647	±1.148
Female	0-55%	35.215	±10.28
P value	0.000(<0.001)		

Table 5 shows Range of Barr body positive cells in smear, mean and standard deviation in male were 0-5%, 0.647 ±1.148 and range of Barr body positive cells in smear, mean and standard deviation in female were 0-55%, 35.215 ±10.28.Present study not found any cell with e”1 Barr body per cell.

Unpaired student t test was applied to test significance of mean value of Barr body positive cells in male and female students. Presence of sex chromatin in oral mucosal cell of female was statistically significant. (P value<0.001).

Comparison of range of sex chromatin positive cells in oral mucosal cells in females of present study with females of other studies which used papanicolaou stain: Range of sex chromatin positive cells reported by Herrman w et al [1] was 10-32% and 50 out of 50 females showed presence of sex chromatin in oral mucosal smear. Mittal T et al [2] reported range of sex chromatin positive cells in female was 20-78% and 100 out of 100 females showed presence of sex chromatin in oral mucosal smear. Datar Uma et al [3] reported range of sex chromatin positive cells in female was 4-20% and 60 out of 60 females showed presence of sex chromatin in oral smear.

In present study, Carbol Fuschin stain was used and reported that range of sex chromatin positive cells was 0-55% and 77 out of 79 females showed presence of sex chromatin.

So, the present study reported higher range of sex chromatin positive cells than Herrman w et al [1] and Datar Uma et al [3]. The present study reported lower range of sex chromatin positive cells than Mittal T et al [2].

Comparison of range of sex chromatin positive cells in oral mucosal cells in females of present study with females of other studies which used acridine orange stain: Reddy S P et al [4] reported range of sex chromatin positive cells was 18-72% and 20 out of 20 showed presence of sex chromatin in oral smear. Datar Uma et al [3] reported range of sex chromatin positive cells was 18-72% and 60 out of 60 showed presence of sex chromatin in oral smear. In present study, Carbol Fuschin stain was used and range of sex chromatin positive cells was 10-55% and 77 out of 79 females showed presence of sex chromatin. So, the present study reported higher incidence of sex chromatin positive cells than Datar Uma et al [3] and lower incidence of sex chromatin positive cells than Reddy S P et al [4].

Comparison of range of sex chromatin positive cells in oral mucosal cells in females of present study with females of other studies which used cresyl violet stain: Dixon A D et al [5] reported range of sex chromatin positive cells was 30-50% and 95 out of 98 females showed presence of sex chromatin in oral smear. In present study, Carbol Fuschin stain was used, range of sex chromatin positive cells was 0-55% and 77 out of 79 females students showed presence of sex chromatin.

Comparison of range of sex chromatin positive cells in oral mucosal cells in males of present study with males of the other studies which used papanicolaou stain: Herrman w et al [1] reported range of sex chromatin positive cells was 0-2% and 42 out of 50 males showed presence of sex chromatin in oral smear. Mittal et al [2] reported range of sex chromatin positive cells in male was 0-4% and 67 out of 100 female showed presence of sex chromatin in oral smear. Datar U et al [3] reported range of sex chromatin positive cells in female was 0-8%.

In present study, Carbol Fuschin stain was used, range of sex chromatin positive cells was 0-5% and 23 out of 71 males showed presence of sex chromatin.

So the present study reported higher range of sex chromatin positive cells than Herrman w et al [1] and Mittal T et al [2]. The present study reported similar range of sex chromatin positive cells as showed by Datar Uma et al [3].

Comparison of range of sex chromatin positive cells in oral mucosal cells in males of present study with males other studies which used acridine orange stain: Reddy S P et al [4] reported range of sex chromatin positive cells were 0-3% and 18 out of 20 males showed presence of sex chromatin in oral smear. Datar Uma et al [3] reported range of sex chromatin positive cells was 0-5%.

In present study, Carbol Fuschin stain was used, range of sex chromatin positive cells was 0-5% and 23 out of 71 males showed presence of sex chromatin. So, the present study reported higher range of sex chromatin positive cells than.

CONCLUSION

Mean value of Barr body positive cells in male was 0.647±1.148, Mean value of Barr body positive cells in female was 35.215±10.28, range of percentage of Barr body positive cells in female was 0-55% and range of percentage of Barr body positive cells in male was 0-5%. Presence of sex chromatin in oral mucosal cell of female was statistically significant. (P value<0.05) Mean values of sex chromatin positive oral mucosal cells of male was lower than mean value of sex chromatin positive oral mucosal cells in female, that is the supporting fact that sex chromatin is present in female in higher frequency. Sex chromatin can be used as simple and easily available method for sex determination. Thus, presence of sex chromatin in oral mucosal cells can be used as a primary method for sex determination clinically.

Conflicts of Interests: **None**

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