Original Article

Indian Journal of Anatomy
Volume 9 Number 4, October – December 2020
DOI: http://dx.doi.org/10.21088/ija.2320.0022.9420.1

Study of Sex Chromatin in Primary Amenorrhoea Patients and their First Degree Relatives

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How to cite this article:
Isha Jaiswal, A P Kasote, M P Fulpatil, et al. Study of Sex Chromatin in Primary Amenorrhoea Patients and their First Degree Relatives. Indian J Anat. 2020;9(4):237–242.

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Abstract

Introduction: The mean age of menarche has become younger this century. Primary amenorrhoea is defined as the absence of menses by 13 years of age when there is no visible secondary sexual characteristic development or by 15 years of age in the presence of normal secondary sexual characteristics. In all cases of primary amenorrhoea, there is a need to determine the sex chromatin pattern in the nuclei of epithelial cells obtained by buccal scrapings.

Aims and objectives: To study the sex chromatin among patients of primary amenorrhoea and their first degree relatives.

Materials and Methods: 32 patients having primary amenorrhoea along with their first degree relatives were studied (total 64). Buccal scrapings were taken from both the patient and relative and stained with Giemsa stain for study of various parameters like presence or absence, number, size, shape and staining intensity of Barr body.

Results: Total numbers of patients studied were 32. 56.25% of the patients were negative for sex chromatin. 37.5% of the patients had absent Barr bodies in their cells. The shape of Barr body was found to be planoconvex in all patients and relatives. 1 patient was found to have a smaller sized Barr body and 1 had a larger sized one. The staining intensity of Barr body was equally dark in both patients and their first degree relatives. Mean % of Barr bodies among patients was 13.41 ± 14.30% (p value <0.0001, HS).

Conclusion: Primary amenorrhoea is an extremely stressful problem for a young girl and her parents. The clinician should handle the case with great sensitivity. Patient awareness and proper counselling of parents is of great importance regarding the treatment options available and the need of follow up.

Keywords: Primary Amenorrhoea; Sex Chromatin; Barr Body; First Degree Relatives.

Introduction

Adolescence is the milestone of womanhood. Because a woman is not born as woman, she becomes woman with the attainment of reproductive maturity which starts with puberty or the beginning of adolescence. Of all the changes of puberty, initiation of menstruation (menarche) is the most important.[1] Amenorrhea or absence of menstruation is a symptom and not a disease.

As the term denotes, it is one of the prime causes for female infertility and can be either primary or secondary in nature.[2]

The mean age of menarche has become younger this century. Primary amenorrhoea is defined as the absence of menses by 13 years of age when there is no visible secondary sexual characteristic development or by 15 years of age in the presence of normal secondary sexual characteristics.[3]

What should be the clinical approach to a case of amenorrhea? Fundamentally it is to determine the cause. When the complaint is primary amenorrhea, cryptomenorrhea has to be excluded. Thereafter the clinical features of the case deserve the closest study.

In all cases of primary amenorrhoea, there is a need to determine the sex chromatin pattern in the nuclei of epithelial cells obtained by buccal scrapings. On obtaining the buccal smears for Barr bodies, the diagnostic possibilities can be divided into two groups:

1. those that are sex chromatin positive
2. those that are sex chromatin negative

Also, when facilities are available, the chromosome complement should be studied by the appropriate examination of cultures of leucocytes and other tissues.[4]

The present study will also be carried out on first degree relatives of cases, on which not much literature is available. So, a detailed study on them will be quite helpful for the family members and the physician to predict any abnormalities in them and accordingly, proper counselling and management can be done.

Aims and Objectives

To study the sex chromatin in primary amenorrhoea patients and their first degree relatives.

Materials and Methods

32 patients having primary amenorrhoea were studied along with their first degree relatives (total 64). After asking the patients and relatives to rinse their mouths with water, buccal scrapings were taken from both of them and smeared on different glass slides and fixed in 95% alcohol overnight. Next day, the slides were stained with Giemsa stain for about 20 minutes, washed off to get rid of excess stain and were observed under oil immersion objective.[5] Various parameters of Barr body were studied like presence or absence, number, size, shape and staining intensity. 100 cells were counted each for the patient and relative and the sample was considered as 'sex chromatin positive' if ≥20% of the cells showed the presence of Barr body and 'sex chromatin negative' if cells showing Barr body were <20%.[6] The size was determined by observing the Barr body under ocular micrometer having a pitch length of 1μ under oil immersion objective.[7] The staining intensity of Barr bodies was compared by observational method and experience.

Results

Number of patients and their first degree relatives studied were 32 each (total 64). The Barr body was studied among cases and controls for the % of sex chromatin (positivity or negativity), number of Barr bodies per cell, size, shape, staining intensity and statistical analysis of Barr bodies was done.

56.25% of the patients had a sex chromatin percentage of below 20 (i.e. negative sex chromatin), whereas 43.75% showed positive sex chromatin (≥20%). Among the controls (relatives), all were positive for sex chromatin (Table 1 and Diagram 1). We found 1 case (3.12%) in which 4 out of the 20 cells which were positive for Barr body, contained 2 Barr bodies (Fig. 1 and 2). Among the controls, all showed only 1 Barr body in a cell (Table 2 and Diagram 2). Table 3 and Diagram 3 reveal that 30 patients (93.75%) had a normal sized Barr body, 1 patient each had a size of >1μ (3.12%) (Fig. 4) and <1μ (3.12%) (Fig. 5). Among the controls, all showed a normal sized Barr body (100%). Fig. 3 shows a normal sized Barr body. In all 32 cases and the controls, the shape of Barr body was found to be planoconvex (Table 4). None of the cases or controls had lighter or paler staining Barr bodies. All showed darkly stained Barr bodies (Table 5). The mean % of Barr bodies among the patients was 13.41 ± 14.30%, while among the first degree relatives, it was 30.63 ± 4.47%. On applying the Mann Whitney test, the percentage of Barr bodies in cases was found to be significantly lower as compared to the controls, the p value being <0.0001, which is highly significant (Table 6).

Table 1: Distribution according to % of sex chromatin.

Sex chromatin	NP	%	NR	%
Positive (≥20%)	14	43.75	32	100
Negative (<20%)	18	56.25	0	0

(NP – No. of patients; NR – No. of relatives)

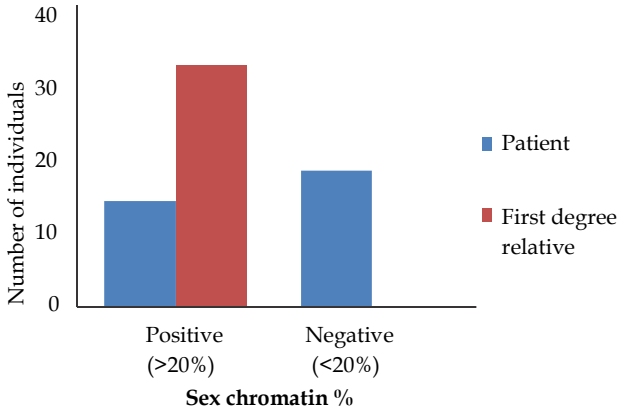

Diagram 1: Comparison of positivity or negativity of sex chromatin.

Table 2: Distribution according to number of Barr bodies per cell.

No. of Barr bodies per cell	NP	%	NR	%
0	12	37.5	0	0
1	19	59.37	32	100
>1	1	3.12	0	0

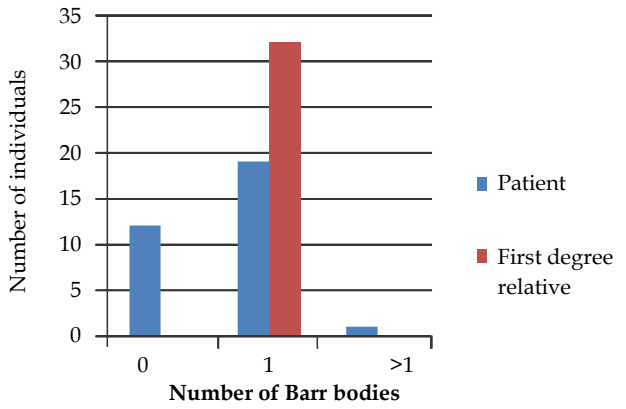

Diagram 2: Comparison of no. of Barr bodies per cell.

Table 3: Distribution according to size of Barr body.

Size of Barr body	NP	%	NR	%
<1 μ	1	3.12	0	0
1 μ	30	93.75	32	100
>1 μ	1	3.12	0	0

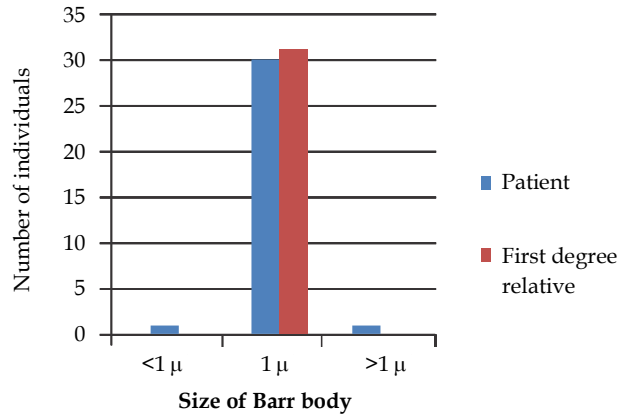

Diagram 3: Comparison of sizes of Barr body.

Table 4: Distribution according to shape of Barr body.

Shape of Barr body	NP	%	NR	%
Planoconvex	32	100	32	100
Triangular	0	0	0	0

Table 5: Distribution according to staining intensity of Barr body.

Staining intensity of Barr body	NP	%	NR	%
Light	0	0	0	0
Dark	32	100	32	100

Table 6: Statistical analysis of % Barr bodies.

Parameter	Cases	Controls
Mean	13.41%	30.63%
SD	14.30%	4.47%
Range	0-35	20-36
Z – value	4.593	
p value	<0.0001, HS	

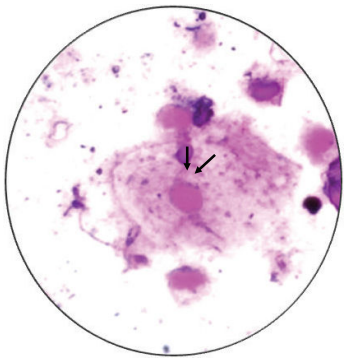

Fig. 1: A cell showing 2 Barr bodies at 12 and 1o'clock positions in the nucleus.

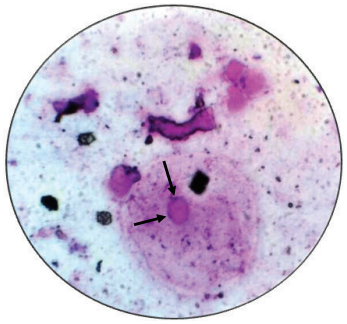

Fig. 2: A cell showing 2 Barr bodies at 8 and 12o'clock positions in the nucleus.

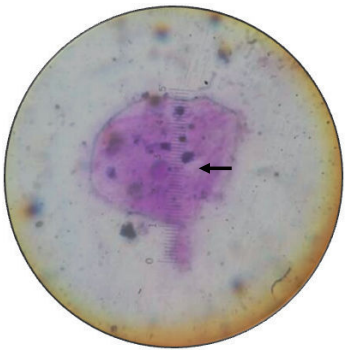

Fig. 3: A cell with Barr body at 3 o'clock observed with the ocular micrometer. The Barr body corresponds to a single pitch length (1 μ) of the ocular micrometer scale as depicted by the arrow.

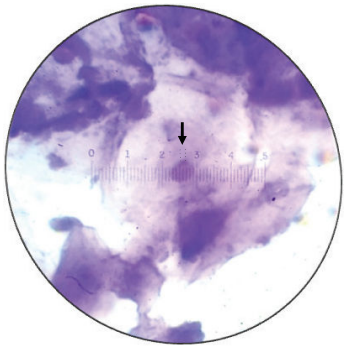

Fig. 4: A cell showing Barr body at 12o'clock position of larger size (approximately 2 μ).

The arrow is pointing towards the Barr body and the dotted lines are corresponding to the 2.5 and 2.7 mark of the ocular micrometer, which is equal to two pitch lengths, which is equal to 2 μ when observed under the 100X objective lens.

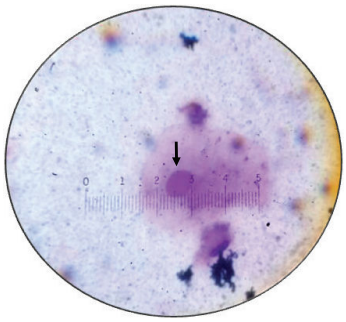

Fig. 5: A cell showing smaller sized Barr body (<1 μ) at 12 o'clock position seen as a small dot.

Discussion

The patients in our study positive for sex chromatin were 43.75% and those negative for it were 56.25%, whereas among controls, all were positive for it. The present study is similar to the study done by Coco R and Bergada C[8] in which 45.60% of the patients were positive and 54.40% were negative for sex chromatin. The present study is contrasting to a similar study conducted by Chhabra V. et al.[9], a correlation study of sex chromatin and primary amenorrhea in which they compared the percentage of cells showing Barr bodies in primary amenorrhoea patients and normal fertile Indian women of different age groups. In their study, 80% of the patients were chromatin positive while 20% were chromatin negative. In a study done by Lacramioara Butnariu et al.[10], the sex chromatin was abnormal in 40.8% and normal in 59.2% of the cases. Our study is also in contrast to the study done by Chryssikopoulos A. and Grigoriou O.11 and V. Lakshmi Kalpana et al.[12] in which 76.62% and 91.43% of the patients were positive and 23.37% and 8.57% of them were negative for it, respectively. Our study is also contrasting to the study done by Naushaba Rizwan and Razia Mustafa Abbasi[13] in whose study, buccal smears of 73.68% patients were positive and 26.31% were negative for Barr bodies (Table 7). These contrasting results could be due to the underlying hormonal imbalances in the patients. In general, PA is associated with hormonal disturbances. Stoyan I. Dokumov and Spas A. Spasov[14] performed a study on the influence of sex hormone administration on the incidence of nuclear sex chromatin in women. According to their study, testosterone uniformly led to a marked reduction in the incidence of sex chromatin body while progesterone produced a reduction in the majority of cases, whereas diethylstilbestrol (oestrogen) administration resulted in a significant increase in the nuclear sex chromatin material in all the cases. In our study, we found only 1 patient with 2 Barr bodies, whereas Lacramioara Butnariu et al.[10] found 16 patients with 2 Barr bodies (Table 8). This difference can be attributed to the great difference in the sample size taken for study. We studied only 32 patients whereas they studied 531 patients with primary amenorrhea. Only 2 patients in the present study showed abnormalities in size of Barr bodies. One patient each, had a Barr body of size >1μ and <1μ. In the study done by Lacramioara Butnariu et al.[10], 10 patients were found to have Barr body with a size >1μ (Table 8). These differences in the size of Barr bodies may be due to numerical and stuructural abnormalities of X chromosome (<1μ suggesting deletion of a part of X chromosome, and >1μ suggesting an isochrosome). The shape of Barr bodies of all the patients and first degree relatives in the present study was found to be planoconvex. We found no studies in which the different shapes of Barr bodies were taken as a parameter under study. Barr bodies have several distinct shapes. Many appear to be planoconvex or wedge shaped, with the plane side resting against the nuclear membrane and the convex part pointing towards the cytoplasm. Barr bodies in the centre of the nucleus appear to be rectangular, and some rectangular Barr bodies may also be observed at the periphery of the nucleus.[15] In an X-chromatin survey done by Janet K. Lyman,[16] the cell was considered positive for X-chromatin body only if the nucleus contained a condensation of chromatin material that was planoconvex to triangular in shape and closely applied to the nuclear membrane. However, we did not find any triangular or rectangular shaped Barr bodies in our study. In the present study, the staining intensity of sex chromatin was equally dark in all cases and controls. We did not find lighter stained sex chromatin in any of the individuals. Reitalu J.[17] conducted a sex chromatin study

Table 7: Comparison of % of Barr bodies of present study with other studies.

	% of Barr bodies (Positivity or negativity of sex chromatin)						
	Present study	**Coco R. and Bergada C[8]**	**Chhabra V et al.[9]**	**Lacramioara Butnariu et al.[10]**	**Chryssikopoulos A. and Grigoriou O.[11]**	**V Lakshmi Kalpana et al.[12]**	**Naushaba Rizwan and Razia Mustafa Abbasi[13]**
+ve	43.75%	45.60%	80%	59.2%	76.62%	91.43%	73.68%
–ve	56.25%	54.40%	20%	40.8%	23.37%	8.57%	26.31%

Table 8: Comparison of number of Barr bodies per cell and size of Barr body of present study with other study.

Parameter	**No. of patients**	
	Present study	**Lacramioara Butnariu et al.[10]**
> 1 Barr body per cell	1	16
Size of Barr body (>1μ)	1	10

Table 9: Comparison of mean % of sex chromatin of present study with other studies.

	Mean % of sex chromatin		
	Present study	**Chhabra V. et al.[9]**	**Priseila G. Otto[18]**
Patients	13.41 ± 14.30%	21.7%	18.63%
Controls	30.63 ± 4.47%	35.82%	–

Table 10: Comparison of range of Barr bodies of present study with other studies.

Range of Barr bodies	
Present study	0–35%
Chhabra V et al.[9]	0–40%
Jacobs et al.[19]	0–60%
Lakshmy et al.[12]	15–45%
Moore K L[20]	20–70%
Beaver D L and Douglas L E[21]	2–23%
Pansegrau D G and Peterson R E[22]	97.6%

on liver cells of female rats in which he noted a variation in the staining ability of the sex chromatin with age. It was rather pale in the nuclei of young female rats and more intensely and darkly stained in the nuclei of older animals. We tried to find out a similar correlation, if any, exists in the staining ability of sex chromatin in human also. However, we could not find any such variation. The mean % of sex chromatin in our study among the patients was 13.41 ± 14.30%, whereas among the first degree relatives (control group of fertile females), it was 30.63 ± 4.47%, which is highly significant ($p < 0.0001$). This is similar to the study by Chhabra V. et al.,[9] in which a significant difference was found among the mean percentage of sex chromatin in patients (21.7%) and control group (35.82%), p value being <0.01 (highly significant). Our study is also quite similar to the study done by Priseila G. Otto et al.[18] in which the average frequency of Barr bodies among the patients was 18.63% (Table 9). Regarding the range of Barr bodies, in the present study it ranged between 0 – 35% in the patients and in the range of 20 – 36% in the control group. Our study is similar to the study by Chhabra V. et al.[9] in which the range was 0 – 40%. Our study is in contrast to the study done by Jacobs et al.[19] and Lakshmy et al.[12] whose studies showed the ranges of 0 – 60% and 15 – 45% respectively. Moore K. L.20 estimated the percentage of Barr bodies in normal XX chromosome complement females to be between 20 – 70%; however, values above 60% are rare. Beaver D L and Douglass L E[21] examined 257 buccal smears and estimated the percentage of positive cells in normal females to be 2 – 23%. Their lower X chromatin values resulted from the inflexible use of more rigid criteria than previously used by others. Pansegrau D G and Peterson R E[22] reported an average X chromatin frequency of 97.6% in female buccal cells (Table 10). They, unlike the previous studies mentioned above, included both peripheral and central chromatin bodies in their counts; this would explain the elevated frequency of Barr bodies.

Conclusion

Primary amenorrhoea is an extremely stressful problem for a young girl and her parents. The clinician should handle the case with great sensitivity. Detection of sex chromatin by buccal smear in the present study proved to be an important tool for the clinicians in making right decisions for further evaluation and management of primary amenorrhoea patients. Patient awareness and proper counselling of parents is of great importance regarding the treatment options available and the need of follow up. Though different treatment modalities are available, outcome regarding regular menses and fertility potential are not so satisfactory. With continuing advancement of technologies of artificial reproduction, there is still hope for some patients with primary amenorrhoea to have their genetic offspring.

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