PREVALENCE OF SEX CHROMATIN IN BENGALEE CASTE HINDU FEMALES: STUDY ON INTERRELATIONSHIP OF HORMONES

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ABSTRACT:

The development of genetic sex determination and cytologically distinct sex chromosomes leads to the potential problem of gene dosage imbalances between autosomes and sex chromosomes and also between males and females. To circumvent these imbalances, mammals have developed an elaborate system of dosage compensation that includes both up regulation and repression of the X chromosome. Random X-chromosome inactivation is the transcriptional silencing of one X chromosome in female mammalian cells that equalizes dosage of gene products from the X chromosome between XX females and XY males. The process of inactivation is controlled by the X-inactivation center (*Xic*) which includes *Xist* and its antisense transcription unit *Tsix/Xite*, somehow senses the number of X chromosomes and triggers *Xist* up-regulation from one of the two X chromosomes in females. The differential behaviour of the two X chromosome of female cells undoubtedly has biological implications. An effect of hormonal factors on the frequency of sex chromatin has been subject matter of importance. The present study was conducted to evaluate the prevalence of sex chromatin among Bengalee Caste Hindu females of different age groups. Material for the present study consisted of the samples of buccal smear of 150 females

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categorized into five different physiological phases each of 30 samples in accordance with their hormonal effect. These physiological phases were pre-menarche, menarche, pregnant, lactating and menopause. Altogether 15000 (fifteen thousand) buccal epithelial cells has been studied. Results indicated differential prevalence of sex chromatin among the different maturity and developmental status of Bengalee caste Hindu females. Present study demonstrated that the prevalence of sex chromatin was significantly lowest (P <0.001) among the pre-menarcheal females, while significantly highest (P <0.001) among the lactating mothers. On the other hand, the menopausal women revealed significantly lower prevalence (P <0.001) of sex chromatin compared to all status of females. These results suggested possible association between the presence of steroid hormone receptors and the prevalence of sex chromatin.

KEYWORDS:

Sex Chromatin, Bengalee Hindu Females (BHF), Menarche, Menopause

Introduction

The human X chromosome has a unique biology that was shaped by its evolution as the sex chromosome shared by males and females (Ross *et.al*., 2005). During evolution, the mammalian X chromosome has generated and recruited a disproportionately high number of functional retroposed genes whereas the autosomes experienced lower gene turnover (Emerson *et al.*, 2004). Recent inquiries have revealed a surprisingly large number of naturally occurring antisense transcripts, but their function remains largely undiscovered. A well-documented case occurs in X inactivation, the mechanism by which X-linked gene expression is equalized between XX females and XY males. The antisense gene Tsix determines X chromosome choice and represses the noncoding silencer, Xist (Shibata and Lee, 2004). This process of X chromosome inactivation (XCI) is a remarkable example of long range, mono-allelic gene silencing and facultative heterochromatin formation. (Smith *et al.*, 2004, Heard and Disteche, 2006, Lyon 2003).

The sex chromatin of interphase nuclei has proved a useful marker in several practical and theoretical problems of biology and medicine. Sex chromatin study in the buccal mucosa is a method widely applied for rapid identification with anomalies of sex development. Apart from chromosomal aberrations, research studies have demonstrated quantitative variation in prevalence of sex chromatin among normal female individuals (Bataineh and Al-Azab,2004,Yen et.al.,1981, Voitenko 1980, Purandare and Chakravarti1978,1980). It has been observed by many researchers that the frequency of sex chromatin fluctuates in women with cardiovascular diseases, especially with arterial hypertension and acute disturbance of coronary of cerebral circulation (Voitenko, 1977). The frequency of X-chromatin was determined in pregnant women during the second trimester of gestation in order to establish possible correlations with the changes in steroid hormone levels occurring during this period and compare the curve with that for normal non pregnant women and the mean frequency of X-chromatin revealed significantly higher value than that obtained for non-pregnant women (De Sampaio *et al.,* 1992) due to steroid hormone receptor (Smethurst *et al.,* 1980). Biological and epidemiological data suggest that progesterone has an important role in mammary tumorigenesis (De Vivo *et al.,* 2003). Quantitative features of chromatin structure in the prognosis of breast and other neoplasia (Smethurst *et al* .1981) have indicated its importance (Komitowski and Janson 1990). In Indian context, (Seshadri *et al.,* 1970, Purandare and Chakravarty 1980, Sastri and Rao, 1985) studies regarding the prevalence of sex chromatin in relation age as and effect of natural and synthetic sex steroids (Chakravarty *et al.,* 1978, Roohi *et al.,* 1979) have been studied.

In the view of the above, the present study investigated the frequency of sex chromatin in different physiological phases among the Bengalee Hindu caste females.

Materials and Methods

Material for the present study consisted of the samples of buccal smear of 150 females collected from the Bengalee Hindu Caste Population (BHCP). The total sample was collected from five groups as follows:

Group	Status	Criteria for selection
A (n = 30)	Pre menarcheal (8 - 10 years) (Non-ovulation)	Under the influence of minimal sex steroids
B (n = 30)	Menarcheal (22 - 24 years) (Ovulation)	Under the influence of sex steroids
C (n = 30)	Pregnant (22 - 29 years) (Non-ovulation)	Under the influence of high level natural sex steroids
D (n = 30)	Lactation (22 - 29 years)	Under the influence of sex steroids and prolactin hormone
E (n = 30)	Menopausal (50 – 60 years) (Non-ovulation)	Under the physiological withdrawal of sex steroids

In each group, the samples were collected from 30 females. Apparently healthy and non-contraceptive user individuals have been taken under study. After taking verbal consent from the individuals, biodemographic information regarding age, age at menarche, menarcheal status, socio-economic status, contraceptive efforts etc. has been collected schedule. Each subject was requested to rinse out the mouth and was asked to open the mouth fully to obtain the buccal smear with the help of sterile 6" foam-tip buccal cell collection swab (Epicenter, Tebu-bio) from the inside of the cheek by scrapping firmly against the finger held outside the cheek. The scrapped material was spread quickly over the glass slide. Fixation and staining (Carbol Fuchsin) was done following standard technique (Weiner and Lourie 1981). The results were uniform, and no objectionable staining of bacteria due to Carbolic acid and Formaldehyde used for working solution. For microscopy (resolution 10 x 40) the slides were DPX mounted by cover glass.

Result:

The prevalence of sex chromatin among the different physiological phases of Bengalee females has been shown in the table1.The result demonstrates the differential prevalence of sex chromatin among the different maturity and developmental status of Bengalee female. The sex chromatin analysis of the present study demonstrated that the frequency of the sex chromatin is significantly ($P < 0.001$) lowest (16.9±2.29) among the pre menarcheal females and significantly

(P < 0.001) highest (61.06±4.32) among lactating mother. On the other hand, the menopausal women revealed significantly (P < 0.001) lower prevalence of sex chromatin (29.46±3.19) compared to all status of women.

Discussion –

It is clearly known that female sex hormones in the circulation vary in their levels during different physiological phases such as pre menarche, menarche, pregnancy, lactation and menopause (Takahashi *et al.*, 2004). Their hormonal system consists of three different factors *viz*. hypothalamic releasing factor, the anterior pituitary hormone and ovarian hormones. Two gonadotropic hormones from the anterior pituitary regulate the hormone and gamete producing functions of the gonads: follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones combine with intra nuclear cytosolic receptor proteins and form a complex, which interacts with DNA itself to stimulate transcription. Thus it can give a physiological stimulation to cell differentiation and growth. This cell differentiation can influence the fluctuation in sex chromatin frequencies.

The sex chromatin analysis may be made from the different tissues of women such as, hair follicle epithelial, foetal tissues, cells from reproductive system, peripheral blood smear etc. Sex chromatin analysis demonstrated that the incidence of available of sex chromatin varies with different tissues within an individual such as the number of chromatin positive nuclei is often higher in vaginal smear than buccal smear. Although it is expected the existence of 100% sex chromatin in females but the prevalence is less due to the intervention of hormonal effect. Furthermore the variation of sex chromatin during the phases of menstruation also revealed differential prevalence in menstruation cycle might be due to the developmental endrometrium changes that are regulated by the ovarian hormones (Campo and Ramirez 1965). Therefore, from the present study it was evident that since, hormones of the endocrine system are important regulatory agents in the processes of growth and maturations, therefore, an association of hormones in growth and maturation of females in variable physiological phases and indicated differential variability of growth and maturation in time and space could be envisaged. Thus the

present study being the first attempt from Bengalee females regarding the prevalence of sex chromatin could be utilized for understanding the different physiological phases of females in terms of the prevalence of sex chromatin among other population to find out the variation.

Table 1.Prevalence of Sex chromatin among Bengalee females in different Physiological Phases according to age

Status	No.of individual	No of cells studied	Range in years	Mean +- SD
Pre-menarche	30	3000	8-10	16.9±2.29*
Menstrual	30	3000	22-25	44.8±3.80*
Pregnant	30	3000	18-29	50.93±4.16*
Lactating	30	3000	18-35	61.06±4.32*
Menopausal	30	3000	48-62	29.46±3.19*

*Significance at P<0.001

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