

Buccal Mucosal X-chromatin Frequency in Breast and Cervix Cancer

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ABSTRACT Buccal mucosal X-chromatin frequency was studied in 50 breast and cervix cancer patients to ascertain reactivation of X-chromosome in buccal mucosa. Two groups of menstruating and menopausal women acted as controls. Frequency of X-chromatin was significantly higher in menopausal cancer patients as compared to menopausal normal controls, but the difference between the menstruating normal women and breast cancer patients was not statistically significant.

INTRODUCTION

A mammalian somatic cell from a normal (XX) female typically has one fully active X-chromosome. The other X- is transcriptionally inert, heterochromatic, late replicating, and termed X-chromatin or Barr body. According to Lyon (1961) hypothesis, X-chromosome inactivation occurring early in embryogenesis is random, irreversible and clonally transmitted.

X-chromatin was initially believed to be of a stable nature and persisting throughout life. But various studies showing alteration of X-chromatin frequency during age changes, different phases of menstrual cycle and pregnancy (Chakravaty et al., 1978) and Neoplasia (Atkin, 1958) suggested reactivation of the inactivated X-chromosome whenever body was under physiological stress.

Contradictory results of variations in frequency of Barr bodies have been reported in Neoplasia. Low frequency of X-chromatin indicating reactivation of the inactive X-chromosome has been correlated with malignancy (Camargo and Wong, 1980; Gros, 1973; Shats and Shvili, 1970; Smethurst et al., 1981; Straub et al., 1969 and Therman et al., 1985). But in some studies (Lyon, 1961 and Zus, 1971), non-significant differences in X-chromatin frequency were observed between cancer patients and normal individuals. Higher incidence of X-chromatin frequency in breast cancer was reported in a single study (Gros, 1973).

The present study aims to find the X-chromatin frequency changes, if any, in breast and cervix cancer patients as compared to frequency in normal women.

MATERIAL AND METHODS

For the present study a total of 100 female subjects were taken. Keeping in view the changes in X-chromatin frequency during different phases of menstrual cycle (Zus, 1971), two control groups comprising of menstruating normal and menopausal normal women were included in the study. The four groups studied were:

Group I comprising of 25 normal healthy menstruating females in age range of 18-24 years.

Group II comprising of 25 normal healthy menopausal females in age range 45-60 years.

Groups I and II subjects were selected randomly from campus of Punjabi University, Patiala, depending on their willingness to allow buccal scraping.

Group-III comprised of 25 patients of carcinoma breast in age range 20-70 years.

Group-IV comprised of 25 patients of cervix cancer in age range 30-70 years.

Groups III and IV subjects were selected from Mohan Dai Oswal Cancer Hospital, Ludhiana.

Prior to sampling, all subjects were made to rinse their mouth with water, following which skin scrapings were taken with a metal spatula from inner side of cheek. First scraping was discarded to avoid bacteria. The second scraping obtained under firm pressure from deeper epithelial layers was spread on a clean dust free glass slide with the help of another slide. Scrapings were taken from both the cheeks. The slides were numbered serially and immersed for 30 min. atleast in the fixative (96% alcohol) to prevent the slides from drying. Slides were air dried and transported in a slide box to the laboratory where staining and analysis was carried out. Slides were stained with

Table 1: X-chromatin frequency in different groups studied

Group	No. of subjects	Age range in years	X-chromatin mean frequency	Standard deviation	Standard error of mean
Menstruating normal female	25	18-24	21.00%	±4.12	0.823
Menopausal normal female	25	46-60	19.14%	±3.64	0.728
Menstruating breast cancer patients	12	28-49	22.78%	±2.749	0.791
Menopausal breast cancer patients	13	38-60	22.20%	±2.744	0.794
Menstruating cervix cancer patients	4	30-35	18.50%	±1.50	0.753
Menopausal cervix cancer patients	21	36-70	22.00%	±3.61	0.788

Table 2: Comparisons between X-chromatin frequency of different groups.

Group	t-value	Probability (p)	Statistical significance
Menstruating normal vs menstruating cervix cancer patients	2.16	P < 0.05	Significant
Menstruating normal vs menstruating breast cancer patients	1.126	P > 0.10	Non-significant
Menopausal normal vs menopausal cervix cancer patients	2.80	P < 0.01	Significant
Menopausal normal vs menopausal breast cancer patients	2.979	P < 0.01	Significant

carbol fuchsin according to the technique of Barr (1965). Slides were put in stain for 15 minutes and subsequently passed through 96% ethyl alcohol for 1-2 minutes, followed by a dip for 1 minute in absolute alcohol. They were then rinsed in xylene and mounted in DPX.

Slides were scanned through the Zeiss microscope. For each individual 100 cells were counted. Only well preserved, mediumly stained nuclei clearly showing X-chromatin were labeled as X-chromatin positive. Cells with shrunken nuclei too darkly stained cells or those dotted with bacteria were not considered. All other nuclei lacking X-chromatin were counted as negative.

RESULTS AND DISCUSSION

The X-chromatin frequency was observed to be significantly lower in menstruating cervix cancer patients as compared to normal menstruating women. The X-chromatin frequency was only slightly higher in menstruating breast cancer patients. In menopausal cancer patients, X-chromatin frequency was significantly higher than menopausal normal women (Table 1 and 2).

Reactivation of inactive X-chromatin in cancer tissue has been confirmed on the basis of enhanced G-6PD activity and lowered X-chromatin frequency in cells of cancer tissue (Straub et al., 1969).

In the present study no significant differences were obtained between X-chromatin frequency in menstruating breast cancer patients and menstruating normal women. The results obtained

were similar to those of Sheshadri (1970) and Zus (1971). Significantly higher X-chromatin counts were found in menopausal breast and cervix cancer patients as compared to normal menopausal subjects. Similar results had been obtained by Gros (1973).

Significantly lower X-chromatin counts were obtained only in menstruating cervix cancer patients. This result was not conclusive due to the smaller number of patients studied as compared to controls.

The overall observations in this preliminary study indicate no effect of breast or cervix cancer on inactive X-chromosome of buccal tissue. In the previous studies reported, low X-chromatin frequency has been observed only in studies conducted on cancer tissue. On studies from buccal smears, no significant change in X-chromatin frequency has been observed. Possibly the reactivation of X-chromatin due to physiological stress occurs only in the tissue directly involved in the change.

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