Original Article

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Oncology Section

Correlation between Sex Chromatin and Female Breast Tumour in Paraffin Sections, Buccal Smears and Peripheral Blood Films

BODAL VIJAY KUMAR[1], KALRA RAVNEET[2], BAL MANJIT SINGH[3], BHAGAT RANJEEV[4]
KALYAN GURDEEP SINGH[5], GUPTA NISHIT[6], SURI ANIL[7], RICHIKA[8]

ABSTRACT

Introduction: Sex chromatin is a plano-convex to triangular DNA mass measuring approximately 1µm in size and lying adjacent to the inner side of nuclear membrane in the somatic cells of the females. There is consistent loss in the sex chromatin percentage in the carcinoma cases in comparison to benign lesions and normal individuals.

Aim: To know the correlation between the sex chromatin status in female breast tumors on paraffin sections, buccal smears and peripheral blood films.

Materials and Methods: The study was conducted on the paraffin sections prepared from carcinoma breast patients from their lumpectomy and mastectomy specimens. Buccal smears and a peripheral blood films were also prepared from each patient.

Discussion: The control group had shown a mean sex chromatin of 54.6±6.73% which was found to be similar to the mean sex chromatin percentage in the fibroadenoma breast cases i.e. 54.91±6.06%. However, the mean sex chromatin in the carcinoma breast cases was markedly reduced i.e. 8.22±6.03%. Maximum no. of fibroadenoma cases (67%) were in the younger age group i.e. 15 to 25 year, while maximum number of carcinoma breast cases (42%) occurred in the 4th and 5th decade.

Conclusion: There is a loss of sex chromatin in cases of carcinoma breast and is associated with poor histological markers. A statistically significant correlation was also found between sex chromatin status and microscopic grading in carcinoma breast. The tumors with higher microscopic grade had lower sex chromatin as compared to those with lower microscopic grading.

Keywords: Sex chromatin, Fibroadenoma, Carcinoma breast

INTRODUCTION

Tumors of the breast are the most important and common cause of morbidity and mortality among women. There are number of factors which may influence the prognosis of carcinoma breast i.e. size of primary tumour, lymph node involvement, histological type, grade of tumour and presence or absence of estrogens and progesterone receptors [1]. Various investigators have tried to determine additional parameters, which would correlate better with prognosis in a case of carcinoma breast. The intrinsic character of tumour cells in a given case like the presence or absence of sex chromatin (Barr body) in tumour cells, superficial epithelial cells and leucocytes (drumsticks) has been shown to have some correlation with prognosis.

The sex chromatin is a feulgen positive intranuclear structure of female mammalian cells, measuring 0.8µ x 1.1µ in size. Most frequently, it is situated adjacent to the nuclear membrane as a plano-convex body. Depending on the tissue examined and technical factors, 20-90% of nuclei of female tissues contain sex chromatin [2]. Sex chromatin is derived from one entire X-chromosome. Early in the embryonic development of the normal female foetus, one member of the X-chromosome pair within each cell is inactivated and gets attached to the nuclear membrane where it forms the sex chromatin.

The single X-chromosome of normal male cell does not get inactivated [3]. In tumours of various locations, a clear-cut inverse proportional dependence between a decrease in the number of nuclei with sex chromatin bodies and an increase in the mitotic index has been demonstrated [4]. Breast carcinomas have consistently shown loss of sex chromatin in significant proportion of tumours. A positive correlation between sex chromatin frequency and metastatic potential of tumour was suggested [5].

Thus this study was undertaken to know the sex chromatin status of patients and its correlation in benign and malignant breast tumours on paraffin sections, buccal smears and peripheral blood film.

MATERIALS AND METHODS

The study was done on 100 cases (45 benign, 45 malignant and 10 control cases) of female breast specimens received in our institution. The control cases were taken from cadavers. The buccal smears and peripheral blood films were prepared preoperatively from all these cases. Sex chromatin status was studied in relation to various histological parameters. The specimen was grossly examined for tumour size, involvement of overlying skin and for any lymph node involvement. The tissue was adequately processed and the histological sections were stained with H&E and with Feulgen reaction. The benign tumours of breast were classified into four categories depending upon percentage of sex chromatin positive nuclei:

1. 44-50%
2. 51-57%
3. 58-63%
4. >63%

The malignant tumours were classified into four categories depending upon percentage of sex chromatin positive nuclei:

1. 0-6%
2. 7-14%
3. 15-20%
4. >20%

Histopathological study was done on tissue sections stained with H&E. The following points were observed in histopathology of carcinoma breast:

A. Histological type – Carcinoma breast was classified into various histological types according to WHO classification of breast tumours.

B. Microscopic grading – The microscopic grading was done

according to Nottingham modification of the Bloom and Richardson system as mentioned in review of literature.

C. Involvement of skin, nipple and areola.

D. Lymph node metastasis.

The data was collected as in the attached proforma. Results were tabulated and analyzed statistically using chi-square test and student t-test.

RESULTS

Sex chromatin counts were compared in the controls, fibroadenoma and carcinoma of breast cases. Also the sex chromatin counts in the buccal smears and peripheral blood films prepared from the patients of fibroadenoma breast and carcinoma breast were compared. The mean percentage of sex chromatin in cases of fibroadenoma 54.91±6.06% was found to be similar to control cases 54.6±6.73% while mean sex chromatin percentage was markedly decreased in cases of carcinoma i.e. 8.22±6.03 % [Table/Fig-1].

Thirty-one cases (69%) of fibroadenoma had tumour size in the range of 2-5 cm, 7 cases (15.5%) had tumour size less than 2 cm and 7 (15.5%) cases had tumour size more than 5 cm. Tumour size was compared with the sex chromatin status. No significant relation was observed between sex chromatin status and tumour size in fibroadenoma breast. The calculated value of x2(2.50) is less than the table value (12.59). Hence there is no correlation between the sex chromatin status and size of tumour in fibroadenoma breast.

Cases of carcinoma breast were similarly divided into three groups depending on the tumour size; less than 2 cm, between 2-5 cm and more than 5 cm in diameter. The size of the tumour was compared with the sex chromatin status.

Twenty-nine (64%) patients had tumor size in the range of 2-5 cm in diameter. Out of these 16 cases,13 had sex chromatin counts in the range of 0-6%, 9 cases had sex chromatin count in the range of 7-14%, 5 cases had sex chromatin counts in the range of 15-20% and 2 cases had sex chromatin count in the range of more than 20%. No significant relationship was observed between the tumor size of carcinoma breast and sex chromatin status.The calculated value of x2(2.42) is less than the table value (12.59). Hence there is no correlation between the sex chromatin status and size of tumour in carcinoma breast.

Breast carcinomas were classified according to WHO classification of breast tumours. Incidentally, all of the 45 (100%) malignant tumours were of infiltrating duct carcinomas. Breast carcinomas were divided into three grades depending on tubule formation, nuclear pleomorphism and mitotic count according to Nottingham modification of the Bloom and Richardson system. 18 tumours (40%) were classified as Grade I, 11 (24%) were Grade II and the remaining 16 (36%) were classified as Grade III. The grade of the tumour was compared with the sex chromatin status. Among the 18 grade I tumours, two cases had sex chromatin counts in the range of 0-6%, 10 cases had sex chromatin count in the range of 7-14%. Four cases had sex chromatin counts in the range of 15-20% and two cases had sex chromatin count of more than 20%. Among the 6 grade II tumours, four cases had sex chromatin counts in the range of 0-6% and 1 case each had sex chromatin count in the range of 7-14% and 15-20%. Among the 9 Grade III tumours, eight cases had sex chromatin counts in the range of 0-6% and one case had sex chromatin count in the range of 15-20%.The calculated value of x2(23.76) is more than the table value (12.59). Hence there is positive relation between the sex chromatin status and microscopic grade. A statistically significant relationship was found between sex chromatin status and microscopic grade i.e. those tumours with a higher microscopic grade had lower sex chromatin as compared to those with lower microscopic grade [Table/Fig-2].

Out of 16 cases with skin involvement, 14 cases had sex chromatin count between 0-6% and 2 cases had sex chromatin count between 15-20%. Out of 29 cases without skin involvement, Nine cases had sex chromatin count between 0-6%, 12 cases had sex chromatin count between 7-14%, six cases had sex chromatin count between 15-20% and two cases had sex chromatin count more than 20%. A statistically significant relation was found between sex chromatin status and skin involvement in carcinoma breast. The calculated value of x2(14.54) is more than the table value (7.82). Hence there is a relation between the sex chromatin status and skin involvement in carcinoma breast [Table/Fig-3].

The mean sex chromatin count in the buccal smears prepared from 45 patients of benign tumours i.e. fibroadenoma was found to be within normal limits i.e. 20.82±1.62% while the mean sex chromatin count in the buccal smears prepared from 45 patients of carcinoma breast was 21.08±1.51%. By conventional criteria, this difference is considered to be not statistically significant. Henceforth, no statistically significant relation was found between the sex chromatin frequency in the carcinoma breast and fibroadenoma cases on buccal smears [Table/Fig-4].

The mean sex chromatin percentage (drumstick) in the neutrophils of the peripheral blood film prepared from the fibroadenoma cases is within the normal limits i.e. 2.48± 0.54% while the mean sex chromatin percentage (drumstick) in the neutrophils of the peripheral blood film prepared from the carcinoma breast cases is 2.33± 0.67%. By conventional criteria, this difference is not statistically significant. Henceforth, no statistically significant relation was found

Group	Number of Cases	Mean±S.D. of Sex Chromatin (%)
Control	10	54.6±6.73
Fibroadenoma	45	54.91±6.06
Carcinoma	45	8.22±6.03
Total	100	--

[Table/Fig-1]: Comparison of sex chromatin status of control, fibroadenoma and carcinoma breast

Sex Chromat in Status (%)	Number of Patients	Microscopic Grade		
		I	II	III
0-6	23	02	07	14
7-14	12	10	02	--
15-20	08	04	02	02
>20	02	02	--	--
Total	45	18	11	16

[Table/Fig-2]: Correlation between sex chromatin status and microscopic grade of carcinoma
x^2=23.76, D.F.=6 p<0.05 (significant) for D.F.=6, x20.05=12.59

Sex Chromatin Status (%)	Number of Patients	Skin Involvement	
		Present	Absent
0-6	23	14	09
7-14	12	--	12
15-20	08	02	06
>20	02	--	02
Total	45	16	29

[Table/Fig-3]: Correlation between sex chromatin status and involvement of overlying skin in carcinoma breast
x2=14.54, D.F. =3 p<0.05 (significant) for D.F. =3, x20.05=7.82

Diagnosis	No. of Cases	Mean±S.D. Sex Chromatin (%)
Fibroadenoma Breast	45	20.82±1.62
Carcinoma breast	45	21.08±1.51
Total	90	---

[Table/Fig-4]: Comparison of sex chromatin counts in buccal smears in fibroadenoma cases and carcinoma breast cases
t-value= 0.7876 D.F. = 88 standard error of difference = 0.3300, p>0.10 (not significant). The two-tailed p-value equals 0.4331

between the sex chromatin frequency in the carcinoma breast and fibroadenoma cases [Table/Fig-5].

Diagnosis	No. of Cases	Mean±S.D. Sex Chromatin (%)
Fibroadenoma Breast	45	2.48±0.54
Carcinoma breast	45	2.33±0.67
Total	90	---

[Table/Fig-5]: Comparison of sex chromatin counts (drumsticks) in the neutrophils of the peripheral blood film in fibroadenoma and carcinoma breast cases
t = 1.1693 D.F. = 88 standard error of difference = 0.128 p>0.10 (not significant). The two-tailed p-value equals 0.2454

DISCUSSION

Various investigators have tried to emphasize on a range of prognostic indicators including the clinical stage, histopathological findings and the count of sex chromatin. The biological behaviour of the tumour and immune response of the host tissue may be the only deciding factor to assess the prognosis in a given case of carcinoma breast [6].

Dawson et al., [7] observed that not all patients with clinically and histologically favourable tumour pathology behave in a similar way. This has stressed the need to establish a more reliable parameter.

The status of sex chromatin in tumours of breast has been shown to have some prognostic value by various investigators like Wacker & Miles [8], Shirley [9], Seshadri et al., [10], Murthy and Verma [11] and Arora et al., [12].

Sex chromatin count has been determined both in tissue sections as well as on imprint smears by various workers. In the present study, tissue sections were used for the study of sex chromatin. Shirley [9], Wacker & Miles [8], Perry [13] and Sharma & Moghe [14] used tissue sections for sex chromatin count. Seshadri et al., [10], Murthy & Verma [11] and Arora et al., [12] used impression smears for studying sex chromatin.

Various workers have used different stains for sex chromatin study. In the present study, H&E stain and feulgen reaction has been used to study the sex chromatin on tissue sections, Papanicolaou stain for buccal smears and Leishman stain for peripheral blood films. Wacker & Miles [8] and Perry [13] used H&E for sex chromatin study. Shirley 32 used H&E and feulgen reaction for sex chromatin staining. Sharma and Moghe [14] used biebrich scarlet red and fast green stain for sex chromatin staining. Seshadri et al., [10] used 1% cresyl violet and Murthy & Verma [11] used thionine stain for sex chromatin staining. Arora et al., [12] used H&E, thionine, aceto-orcein and Papanicolaou stain for demonstration of sex chromatin. Davidson and Smith [15] used Leishman stain to demonstrate the sex chromatin (drumstick) attached to the nuclear lobe of neutrophils.

In the present study, the average incidence of sex chromatin is 54.91±6.06%, in cases of fibroadenoma breast and the range was 44%-66% [Table/Fig-1].

Moore and Barr [16] observed an average incidence of sex chromatin in cases of fibroadenoma to be 71%. Sharma and Moghe [14] observed that majority (67.1%) of benign tumours had sex chromatin in the range of 21-30%. Arora et al., [12] observed average incidence of sex chromatin to be 57.48±7.30% in cases of fibroadenoma breast.

The sex chromatin status in fibroadenoma breast in the present study was consistent with the findings of Arora et al., [12]. Thus, the incidence of sex chromatin in cases of fibroadenoma breast is high [Table/Fig-4].

In the present study, the mean percentage of sex chromatin in carcinoma breast is 8.22±6.03% and its incidence varied from 1-22% [Table/Fig-6].

Name of Author	Year of Study	Sex Chromatin Status
Moore & Barr	1955	Average sex chromatin=71%
Sharma & Moghe	1981	21-30% sex chromatin in 67.1% cases
Arora et al.,	1989	Average sex chromatin=57.48±7.30%
Present study	2012	Average sex chromatin=54.91±6.06%

[Table/Fig-6]: Sex chromatin status in fibroadenoma breast – a comparison between different authors

Shirley [9] observed sex chromatin count of 1-22% in 52% of cases, 24-37% in 23% and 45-77% in 25% of cases of carcinoma breast. Seshadri et al., [10] observed that 72% of carcinoma breast were negative for sex chromatin i.e. sex chromatin count <20%. Sharma and Moghe [14] observed that all the cases of carcinoma breast had shown sex chromatin count between 1-10%. Murthy and Verma [11] found that 54% of the cases of carcinoma breast had sex chromatin in the range of 0-9% which was followed by 10-14% in 26% cases. Arora et al., [12] observed that the mean percentage of sex chromatin in carcinoma breast is 8.96±7.17%. 56% of the cases had sex chromatin in the range of 0-6% followed by 15-20% in 20% of cases, 7-14% in 16% of cases and more than 20% in 8% of cases [Table/Fig-7].

Name of Author	Year of Study	Sex Chromatin Status
Shirley	1967	<22% sex chromatin in 52% cases
Seshadri et al.,	1977	<20% sex chromatin in 72% cases
Sharma & Moghe	1981	<10% sex chromatin in 100% cases
Murthy & Verma	1986	<10% sex chromatin in 54% cases 10-14% sex chromatin in 26% cases
Arora et al.,	1989	<20% sex chromatin in 92% cases
Present study	2012	<20% sex chromatin in 96% cases

[Table/Fig-7]: sex chromatin status in carcinoma breast – a comparison between different authors

In all the studies, the sex chromatin incidence in majority of cases of breast carcinoma is less than 20%. In the present study, 96% of the cases had sex chromatin count of less than 20%, which was consistent with the other studies. It can be concluded that the sex chromatin incidence decreases in cases of carcinoma breast.

Murthy & Verma [11] and Arora et al., [12] used histologic grading as proposed by Bloom & Richardson and nuclear grading as proposed by Fisher. In the present study, microscopic grading was done according to Nottingham modification of the Bloom and Richardson system.

In the present study, a significant correlation was found between sex chromatin status and microscopic grade. The findings were consistent with the observations of Arora et al., [12]. On the basis of these findings, it can be concluded that the sex chromatin status correlates with the differentiation of the tumour i.e. incidence of sex chromatin in higher grade tumours was less than the lower grade tumours.

A significant correlation was observed between sex chromatin status and skin involvement by breast carcinoma. The findings of the present study were similar to those of other investigators. The tumors involving the overlying skin had shown a lower incidence of sex chromatin as compared to the tumors without skin involvement.

No significant association (lymph node metastasis in 27 out of 45 cases, $x2=2.302$ and $p>0.05$) was seen between lymph node metastasis and sex chromatin status in the present study. The findings in the present study were consistent with those of other authors. Therefore, the sex chromatin status in carcinoma breast is independent of lymph node metastasis.

No significant association was found between the sex chromatin counts in the buccal smears prepared from the fibroadenoma and carcinoma breast cases. The findings were consistent with those

of other authors Yule et al., [17], Spiers et al., [18] Satbir et al., [19], Natekar et al., [20]. Therefore, the sex chromatin counts in buccal smears in fibroadenoma and carcinoma breast cases have no correlation and are independent of each other.

CONCLUSION

It is concluded that there is a loss of sex chromatin in cases of carcinoma breast. The data emphasizes that the tumours having low counts of sex chromatin were more likely to be associated with poor histological markers such as higher microscopic grade and skin involvement and thus are likely to have a poor prognosis. On the other hand, tumours with a high sex chromatin count are associated with good prognosis. Thus sex chromatin evaluation can help to determine prognosis in a given case of carcinoma breast. However a large study group with follow-up of patients for at least 5 years is needed to establish the role of sex chromatin in prognosis of carcinoma breast.

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PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Pathology, Government Medical College Patiala Punjab, India.
2. Junior Resident, Department of Pathology, Government Medical College Patiala Punjab, India.
3. Professor and Head, Department of Pathology, Government Medical College Patiala Punjab, India.
4. Assistant Professor, Department of Pathology, Government Medical College Patiala Punjab, India.
5. Professor and Head, Department of Pathology, Government Medical College Patiala Punjab, India.
6. Junior Resident, Department of Pathology, Government Medical College Patiala Punjab, India.
7. Associate Professor, Department of Pathology, Government Medical College Patiala Punjab, India.
8. Junior Resident, Department of Pathology, Government Medical College Patiala Punjab, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Bodal Vijay Kumar,
Associate Professor, Department of Pathology, Government Medical College Patiala Punjab, India.
Phone: 09814714946, E-mail: vijay_bodal@yahoo.com

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