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CYTOGENETIC ANALYSIS OF BREAST CANCER FEMALES ALONG WITH FEMALES WITH DIABETES AND THYROID ISSUES TO FIND A POSSIBLE LINK

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ABSTRACT

Breast cancer has been increasing at an alarming rate in females since the last decade and there is a need to look for novel tumor markers as well as establish high risk categories to screen those females early on as it is very much curable if detected early. In the last decade, Diabetes Mellitus and Thyroid issues have increased exponentially as well and in females these diseases are linked to Breast Cancer development. A number of studies have been done to find a possible link but it is still debatable. There is no cause and effect relation between these diseases; however, some possible mechanisms do show a possibility of these conditions increasing the risk of Breast Cancer in females. In case of diabetes, excess insulin and in case of thyroid issues the excess thyroxine can act as proliferator of cells leading to cancer. Several meta-analysis studies to find out the link between these diseases have indeed shown an increased risk of Breast Cancer in diabetic as well as hyper-thyroid disease females. The present study attempted to find a link between these diseased condition and Breast Cancer development by doing cytogenetic analysis in all the subjects afflicted with these conditions to find a common link by studying their chromosomal aberrations that might indicate any common constitutional impairement in these diseases. The study did find higher percentage of chromosomal aberrations in all the diseased groups when compared to healthy control females thus indicating a common link.

KEYWORDS: Breast Cancer, Cytogenetic Analysis, Diabetes Mellitus, High Risk, Thyroid Issues

Breast cancer is on the rising since the last decade and numerous research work is being carried out to find novel tumor markers and to find the inherent cause of this deadly diseases. The role of diagnostic screening for this disease by mammography has also increased aggressively and while it is undoubtedly the cornerstone for combating this deadly disease, this has unfortunately also resulted in a lot of false negative cases as well thus increasing the anxiety of women further. In order to minimize the false negative cases resulting due to these aggressive screening programs one important step is to establish high risk categories of females who must go for screening annually or at least twice a year. While some known cases of high risk factors like age, parity, chemical or radiation exposure and of course genetic factor like BRCA 1 & 2 mutation in female relatives are there, certain diseases are also being suspected to pose risk for developing breast cancer. Among these, diabetes mellitus type 2 and thyroid diseases top the lists. The aim of the present study was therefore to investigate these diseases by cytogenetic analysis to see any constitutional chromosomal aberrations that might be present in these diseases that are common to breast cancer and might play a role in the development of breast cancer in these patients. Therefore, cytogenetic analysis by chromosomal aberration assay as devised by Moorhead et al., (1965) was done from the peripheral blood of breast cancer patients, thyroid disease patients and breast cancer patients and compared to that of healthy control females without any of the said diseases to see any common link.

MATERIALS AND METHOD

Collection of Sample

After inform consent was taken, 50 blood samples were collected from breast cancer patients of Chemotherapy ward of JNCH&RC, Idgah hills, Bhopal in a heparinized tube that was subjected for lymphocyte culture following standard protocol of Moorhead et al., (1965). Blood samples (20 each) were also taken from females having thyroid issues including hyperthyroidism, hypothyroidism and thyroiditis and also from females have diabetes mellitus type 2. Fifty samples were also collected from healthy control females having no breast cancer or thyroid or diabetes issues to compare.

Lymphocyte Culture

The blood samples were then subjected for lymphocyte culture following standard protocol of Moorhead *et al.,* in which they are incubated in 37 celcius for 72 hours in RPMI 1640 Media and PHA and then on 70th hour treated with colchicine (0.02%) for metaphase arrest and stained in Giemsa to study chromosomes. Slides that showed good metaphase count were then banded by GTG

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banding method and analyzed under microscope (Olympus 120) for chromosomal aberration assay. At least 30 metaphase cells were scored to study the chromosomal aberration from each slide.

RESULTS

Cytogenetic analysis was done from the peripheral blood of 50 registered Breast Cancer patients of age group 18-65 from the Chemotherapy ward of Jawaharlal Nehru Cancer Hospital & Research Centre, Idgah Hills, Bhopal after getting their informed consent and other relevant medical history. Blood samples from 50 age-matched healthy control were also taken to compare for chromosomal analysis. The Chromosomal Assay showed higher frequency of abnormal metaphases with numerical as well as structural chromosomal aberrations observed in 50 Breast Cancer patients as compared to equal number of age-matched Healthy Controls. Most of the Breast Cancer patients showed Hypoaneuploidy, Fragments, Breaks, Acrocentric Association, Rings, PCD and ICD etc. (p value 0.0001***, highly significant) as shown in Table 1 and Figure 1 to Figure 4, whereas Healthy Control females showed normal karyotype. However, very few healthy subjects too were presented with minimal chromosomal aberration pattern. The p value was calculated by Two Sample t test using OriginPro 8 Software.

Table 1: Mean Chromosomal Aberration Assay in all the groups studied

Code No.	TC	AM	Chromosomal Aberrations (Mean ±SD)								
			DM	BR	FR	RG	IC	PCD	AA	Hypo	Hyper
HC	30	1.7 ±1.10	2 ±1.73	2 ±0.89	1.54 ±0.93	2 ±0.70	1 ±1	0.6 ±0.51	0.66 ±0.57	3.06 ±1.76	0
BC	30	23.56 ±3.47	15.62 ±7.54	19.1 ±8.21	14.89 ±9.02	16 ±8.13	21 ±7.28	11.53 ±8.66	20.8 ±7.35	17.48 ±7.84	1.62 ±1.08
DM	30	13.3 ±10.1	25.2 ±1.9	26.6 ±3.06	12.7 ±7.14	17.4 ±8.19	11.8 ±10.18	23.3 ±2.2	11.8 ±6.5	0	0
TH	30	12.5 ±7.8	13.2 ±2.4	8.4 ±6.1	8.3 ±6.2	7.1 ±5.7	2.6 ±1.7	8.4 ±6.1	10 ±8.1	0	0

Legends: HC: Healthy Control, TH: Females with Thyroid Disorders, TC: Total cells scored, AM: Abnormal Metaphase, DM: Double Minutes, BR: Break, FR: Fragments, RG: Ring, ICD: Intercalary Deletion , PCD: Premature Centromeric Division, AA: Acrocentric Association

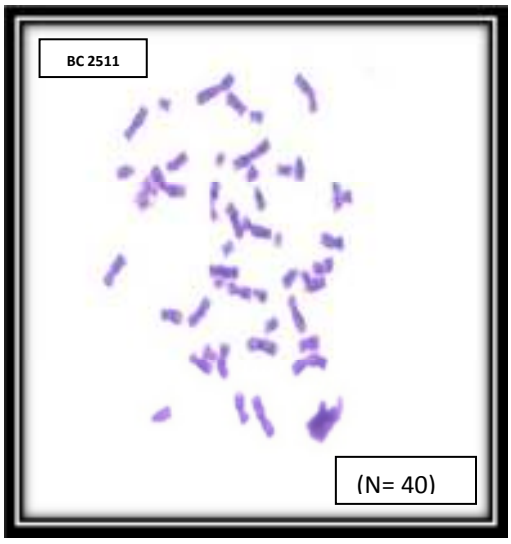


Figure 1: Hypodiploidy in Breast Cancer Females (N=40)

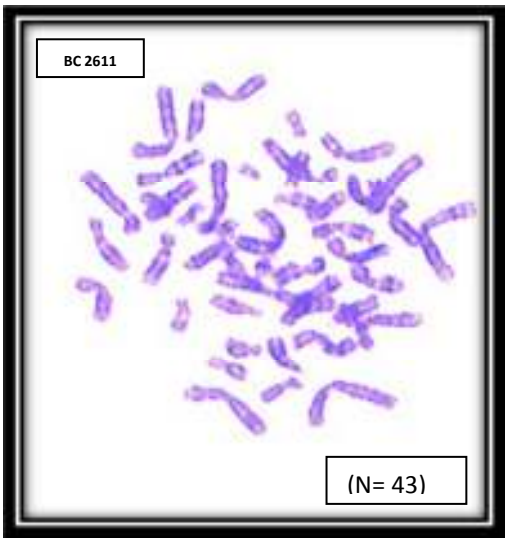


Figure 2: Hypodiploidy in Breast Cancer Females (N=43)

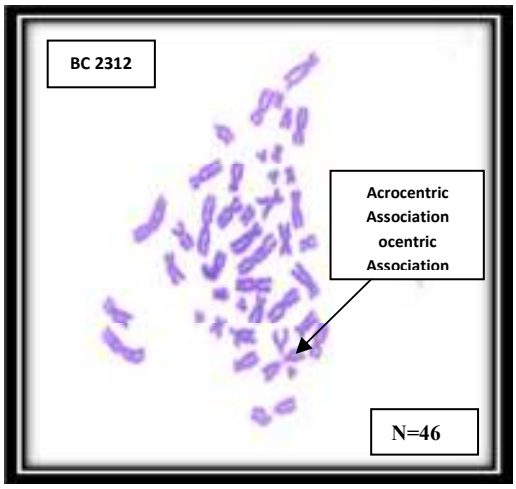


Figure 3: Metaphase showing Acrocentric Association

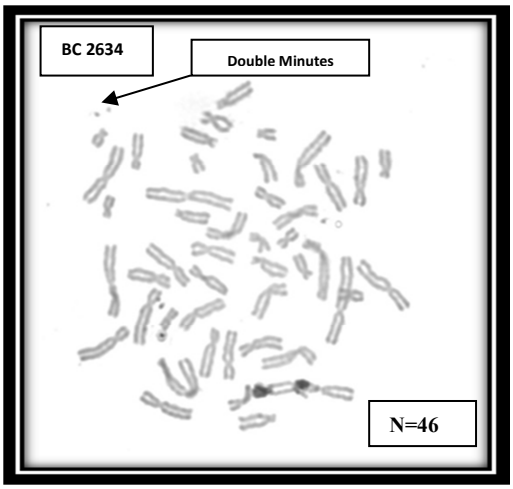
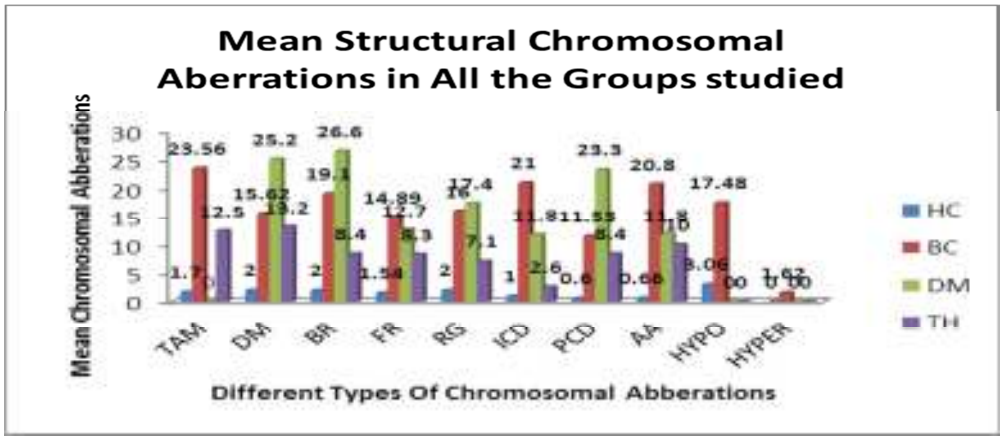


Figure 4: Metaphase showing Double Minutes

Chromosomal aberration assay was also done on 20 Diabetic Mellitus type 2 females as well as 20 females with thyroid problems including hyper and hypothyroidism to find a link between these diseased condition and their risk of developing Breast Cancer by cytogenetic analysis to see chromosomal aberrations in these groups.

Chromosomal aberration was also found in both Diabetic females and females with thyroid disorders. The mean frequency of Abnormal Metaphase in Diabetic Mellitus female was 13.3 while that of Healthy Control was only 1.7, hence it was highly significant (P value= 0.0067**, very significant). The thyroid disorder females also showed chromosomal anomalies and the difference in their means when compared to Healthy Control was found to be significant in this study (P value of 0.0023**, very significant). Mean Frequency of Total Abnormal Metaphase of Thyroid Disorder females was 12.5, which is quite higher than that of Healthy Control females of 1.7. Different types of chromosomal as well as chromatid type of aberrations like Breaks, Acrocentric Associations, Premature Centromeric Divisions, ISD, Rings etc. were found in both the groups studied, i.e., DM and TH females as shown in Table 1 and Graph 1. Different types of chromosomal aberrations found in DM and Thyroid group females are shown in Figure 5 to Figure 12.



Graph 1: Mean Chromosomal Aberration Assay in all groups studied

(Legends: HC: Healthy Control; TH: Females with Thyroid Disorders;TC: Total cells scored; AM: Abnormal Metaphase; DM: Double Minutes; BR: Break; FR: Fragments; RG: Ring; ICD: Intercalary Deletion , PCD: Premature Centromeric Division, AA: Acrocentric Association; HYPO: Hypoaneuploidy, HYPER: Hyperaneuploidy)

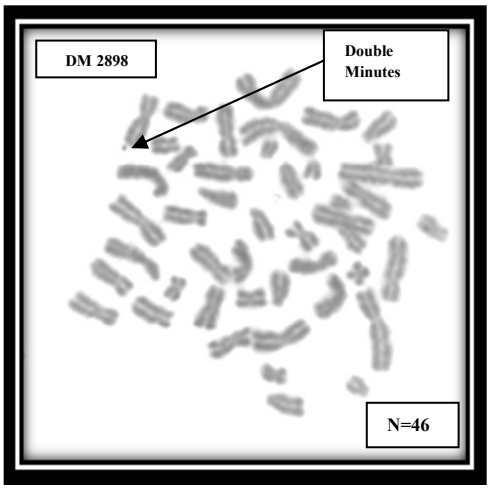


Figure 5: Metaphase showing Double Minutes

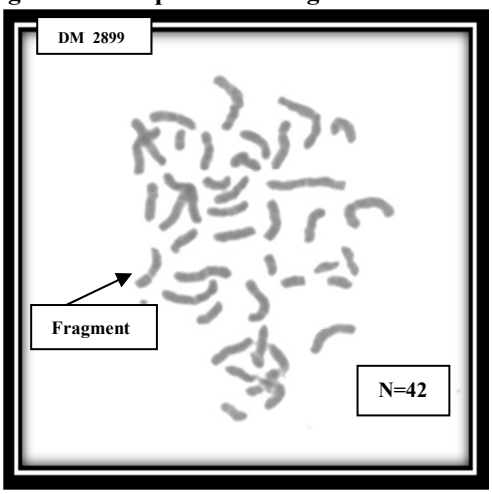


Figure 6: Metaphase showing Fragments

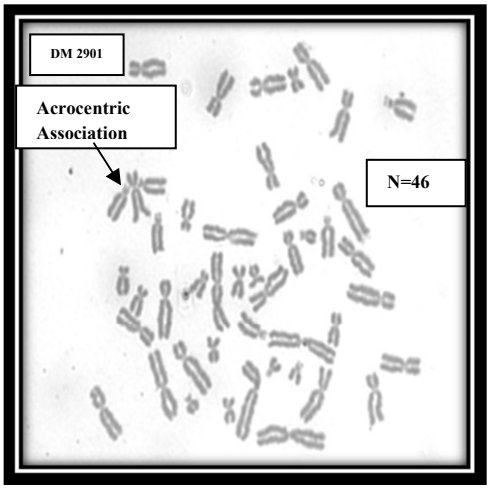


Figure 7: Metaphase showing Acrocentric Association

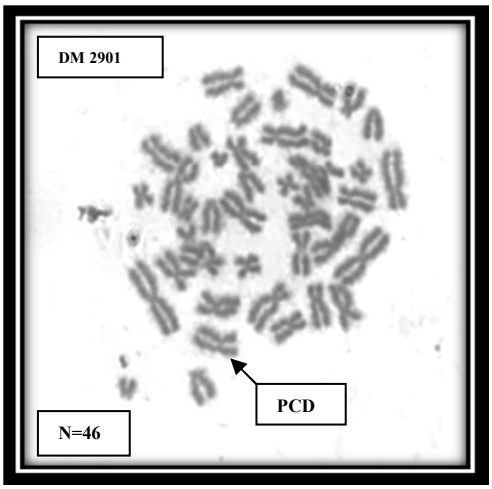


Figure 8: Metaphase showing Premature Centromeric Division

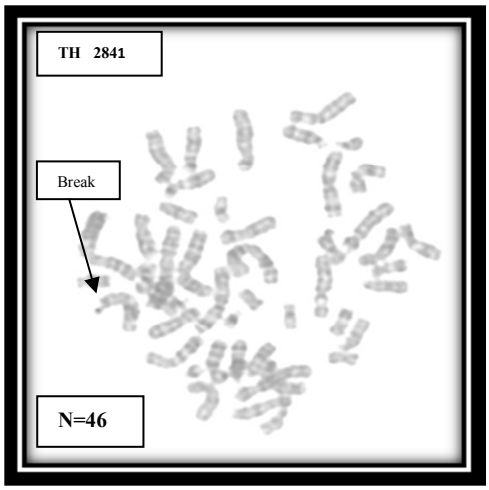


Figure 9: Metaphase showing break

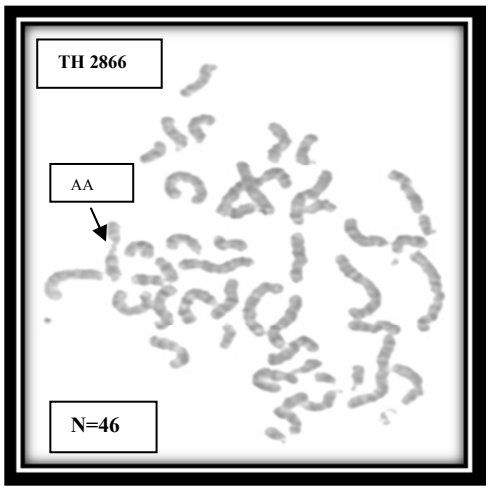


Figure 10: Metaphase showing AA

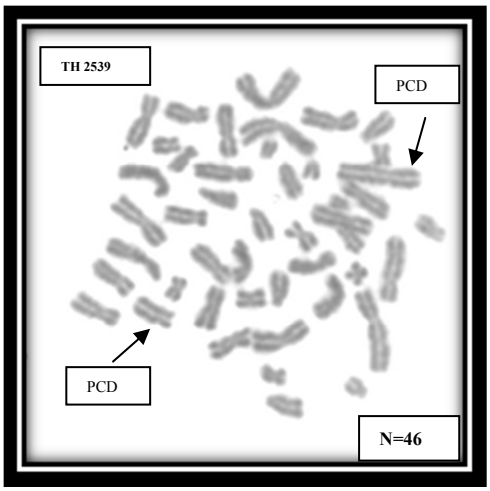


Figure 11: Metaphase showing PCD

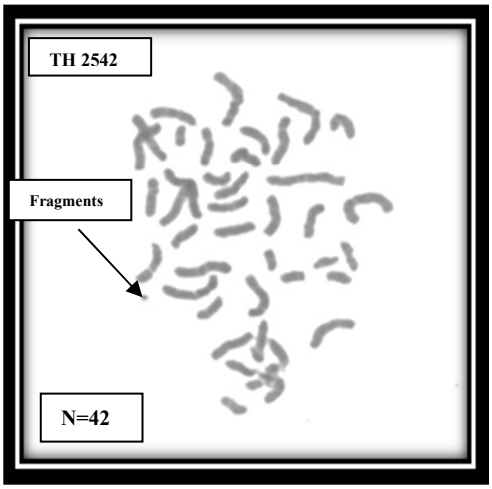


Figure 12: Metaphase showing Fragment

DISCUSSION AND CONCLUSION

The present study was aimed to find a possible link between Breast Cancer risk and the two diseases in question, that is, diabetes mellitus type 2 and thyroid disorder by doing cytogenetic analysis. As it is a well known fact that genetic instability is a signature feature in all types of cancer and thus chromosomal anomalies constitutionally present in breast cancer patients and diabetic and thyroid patients can indicate some link.

In order to screen females for breast cancer, it is very essential to establish high-risk categories, that is, those females who must undergo mammography on a yearly or at least twice a year basis for early detection of breast cancer and hence its timely treatment and cure. Hence, in the present study cytogenetic analysis of diseases like Diabetes Mellitus and Thyroid disorders was done to study any constitutional chromosomal abnormality that might be common in these diseases that make them prone to developing Breast Cancer in future. Our study did find out chromosomal aberrations in all the studied groups which were statistically significant when compared with Healthy Control females.

In the present study, breast cancer patients had a significantly higher percentage of aberrant metaphases as compared with controls. There was a moderate frequency of numerical as well as structural abnormalities in the cultured lymphocytes of patients. Thus, determination of the genomic instability level by chromosomal analysis can be used as a diagnostic tool for identifying females with high risk of developing Breast Cancer and also in monitoring disease progression and prognosis. Our study is supported by a similar study done by Hinglaj Saha *et. al.,* 2013 in which they found a variety of Chromosomal Aberrations including aneuploidy, polyploidy, terminal deletions, Acrocentric Associations, Chromosomal Breaks and Gaps, Cluster of Cells formation were seen in peripheral blood lymphocytes of Breast Cancer patients of West Bengal.

Similarly Diabetic females and females with thyroid issues (hyperthyroidism and hypothyroidism) also showed higher chromosomal anomalies compared to healthy females thus showing a constitutional instability at genomic level present in these diseases that can lead to cancer as well.

Chromosomal aberrations in Diabetic females were also studied by Bernhard O. *et al.,* (2008) in which they found a high load of various chromosomal aberrations in diabetic patients similar to our study as well as aneuploidy in these patients.

The possible explanation for the link between diabetes and breast cancer could be that hyperglycemic state in these individuals leads to oxidative stress which in turn can cause damage to chromosomal integrity and ultimately these chromosomal instabilities accumulated over years can give rise to other diseased conditions including cancer and hence is indicative of a common link between Breast cancer and Diabetes mellitus.

Similarly, some studies have been done to find link between Thyroid Disorders and Breast Cancer. In a study

done by Mette Sogaard *et. al.,* (2016), they examined the association between hypothyroidism, hyperthyroidism and breast cancer risk in a population-based cohort study. They found an increased risk of breast cancer in women with hyperthyroidism and a slightly decreased risk in women with hypothyroidism indicating an association between thyroid function level and breast cancer risk.

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