Total DNA Content: An Analytical Marker for Secondary Prevention of Breast Cancer

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Abstract

The most important component of the five levels of prevention, particularly regarding non- communicable diseases like cancer stands to be early Diagnosis and Treatment (EDT). Approximate management and policies of EDT are vital to reduce cancer mortality. This has been amply proved in cases of cervical cancer where Pap smear makes it possible. However lack of methodology for EDT, illiteracy and absence of proper health education/ awareness makes the situation much cloudy and sorrowful. Formulation of screening programs, implementation of control programs, knowledge regarding natural history of cancer service delivery, information system registry and program evaluation, thus remain as the different tasks and activities of the Preventive Oncology as a whole. "Down staging" of different cancer screening at the grass root level by health care workers or the patient herself has also been suggested by WHO. However, the next stage of down staging at the diagnostic arena has been the deficiency of a "marker" to brand a mass in breast as malignant or benign– where decision making for curative measures can become possible.

The present article highlights the importance of the total DNA content of breast tissue as a solution for the same.

Keywords: **Total DNA content, Early diagnosis of breast cancer, Marker Diagnostic procedure, Breast Cancer.**

Introduction

In the global scenario, cancer of lungs and breasts stand to be the first ranking cancers in males and females respectively.[1] Breast cancer has a high incidence in United States, and occupies 7.4% of all cancers in India.[2] It is one of the commonest cancer in middle aged women in many developing countries, the mortality of which has increased during the past 60 years in every country,[3] leading to a "yawning gap" for cases and death between "developed and developing countries", in relation to cancer.[4]

Various risk factors like age, family history, parity, age at menarche and menopause, hormonal factors, diet, socio- economic factors and radiations have been postulated.[5] Current knowledge of etiology, however, offers little scope for primary prevention of breast cancer.[6]

Thus secondary prevention, which includes early diagnosis and treatment, stands to be the sheet anchor of cancer control.[7] Thus down staging for detection of mass in breast by health worker or by the patient herself to screen suspects becomes mandatory. Considering the late reporting of cancer breast cases to the hospitals and the fact that no major improvement in survival rates has yet been shown by current treatment modalities, it was decided to undertake the present study, so as to identify a "marker" for early diagnosis of breast cancer at the hospital setup – in order to institute an early treatment or to choose the line of treatment. In general, the early removal of the tumor is more likely to be curative.[8]

Material and Method

The present epidemiological study was carried out with a case control triplet design in the Oncology

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Department of the Sir Sunder Lal Hospital, IMS, BHU, Varanasi. It was an interdepartmental coordinated study with the Department of Community Medicine. The registration of the patients as well as their matched controls were undertaken by a “Snow Ball Sampling Method” from the surgical out patients, where the clinical examination, case followup and tissue collection were undertaken. The diagnoses of cases were done clinically and on observation in the operation theater before taking the tissue bite. The laboratory research on total DNA content of the breast tissues was carried out in the collaboration of Departments of Community Medicine and Zoology of Banaras Hindu University, Varanasi. The study was under taken for a period of 2 years from 1990 to 1993. The individuals where the diagnosis was confirmed for breast cancer by the surgeons were registered as **“Cases”.** Two controls- age and socio economic status, were matched amongst the cases. Since all cases were females, sex matching was not necessary. The first group of “Controls” was chosen from the cases showing benign breast growths like fibro adenoma. The second group of controls was chosen from the relatives of patients who were registered as normals. Thus the 3 groups constituted as (a) Cases, (b) Benign Breast Disease (BBD Controls), and (c) the normal.

Inclusion Criteria

All breast cancer cases admitted to the hospital during the study period were included. Benign breast growth cases and relatives of the patients (normal) agreeing for participation in the research activities were only registered. Where relatives did not agree, normal matched controls were selected from other surgical cases or their relatives.

Exclusion Criteria

Patients or individuals not giving their consent were excluded from the study. The controls who did not allow collection of tissues mass were also excluded. Advanced cases needing only politic, radiotherapy or symptomatic treatment were referred to specific units and therefore dropped from the study. Case was taken to exclude recurrent breast cancer cases.

Sampling

Since there were fixed criteria to register the cases for the study, 85 cases were studied in each group, to make a total of 255 only.

DNA Content Estimation

Breast tissue samples were subjected to total DNA content estimation for both cases and controls. The DNA content of the breast tissue was estimated by Diphenylamine Method. Contrary to the belief, the kit for the test is neither too expensive nor too difficult to carry out even at district hospital level. Concentrated Trichloro Acetic Acid (TCA) and Diphenylamine reagent (DPA) were taken. Standard DNA solution was prepared by dissolving 3 mgm of calf thymus DNA in 5% TCA solution (6 ml). The breast tissue was weighed and minced thoroughly to prepare 10% cold aqueous homogenate of breast tissue. Two ml of the homogenate in 4 fold dilution was added to 4 ml of DPA and was kept in boiling water bath for 10 minutes. After 30 minutes it was centrifuged. The light absorbance of the supernatant solution was compared with water at 600 nm. The DNA content was then calculated as follows:

$$\text{DNA mg/gm of weight tissue} = \frac{\text{Standard value from tables x Dilution x Absorbance x Total volume of homogenete}}{\text{Weight of individual tissue sample}}$$

Observation and Discussion

The basic hypothesis of the present study was based on the fact that the total DNA would point out some information regarding the pattern of cell growth. Thus abnormal growth, as is seen in cancer, would also show an abnormal pattern.

It was observed that the mean of total DNA content (here after referred as DNA content) in mg per gm wt breast tissue in cases or normal. Benign Breast Disease (BBD) and cancer (cases) were 0.04 ± 0.43 mg/gm, 4.76± 2.37 mg/gm and 27.28 ± 20.86 mg/gm respectively. The range for the groups respectively were found to be 0.2 to 1.75 mg/gm, 0.7 to 10 mg/g and 4.63 mg/g to 92.5 mg/g, indicating an absolute increase in the DNA content in gradient from normal to cancerous stage of breast tissue (table-1).

Total DNA mg/g	Cases (N=85)		Control (BBD) (N=55)		Control (Normal) (N=85)	
	No.	%	No.	%	No.	%
≤2	--	--	9	10.6	85	100.0
2.1 to 10	17	20	76	89.4	--	--
10.1 to 50	54	63.5	--	--	--	--
50.1 to 100	14	16.5	--	--	--	--
Range	4.63 to 92.5		0.75 to 10.0		0.2 to 1.75	
Mean ± SD	27.28 ± 20.86		4.76 ± 2.37		0.84 ± 0.43	

Table 1.Total DNA content of breast tissue

The bar chart given below would make the fact more clear. This could be because of the abnormal and irregular tissue growth in cancerous breasts. But however, it supports the hypothesis. The tumour dynamics in terms of rate of growth and DNA content was also studied and it showed that as the rate of growth of concerned breast tissue increased the DNA content also increased to a certain level (table-2) after which it slightly declined. This could be due to shredding of tissue, cachectic stage of the cases at that stage. Further, metastasis might be one of the reasons of mobilizing cancerous cells from the breast and thereby reducing the DNA content.

Rate of growth cm/ month	DNA content (mg/g/ wt tissues)						Total	
	No.	2-10 mg	No.	10-50mg	No.	50-100mg	No.	
≤1	10	6.64±1.82	39	23.13± 9.37	09	69.14±18.34	58	28.25± 21.93
1.1 to 2.0	03	8.75± 0.0	06	27.23± 6.52	--	--	09	21.07± 10.58
2.1 to 3.0	--	--	06	28.33± 6.86	02	60.63± 0.0	05	48.15± 21.22
3.1 to 4.0	--	--	02	25.50± 0.0	03	63.25± 7.36	05	48.15± 21.32
4.1 to 6.0	04	5.47±0.56	01	20.75± 0.0	--		05	8.53 ± 6.85
R= -0.06287; $p<0.05$								

Table 2.Correlation between rate of growth and DNA content in breast cancer

It was observed that when the rate of growth was less than 1 cm per month (towards the maximum diameter) the DNA content was 28.25 ± 21.93 mg/g which increased up to 48.15 ± 21.32 mg/g at the rate of growth of 3.1 to 4.0 cm/ month. It only declined beyond the growth rate of more than 4.1 cm per month. The reduction of fatty content of the breast tissue during this stage has also some role to play in DNA content. However, even stage wise DNA content showed remarkable findings to stand as a marker for early diagnosis.

Stage wise mean DNA content was also studied to understand the importance of DNA as a marker. During the first stage, the mean DNA content for majority of cases out of all stage I cases (66.67%) was between 2 to 10 mg/gm which increased to 10 to 50 mg/gm during intra stage II (96.55%). However, for stages III and IV, the mean DNA content did not show much variation within their group. This also acnes to the observation as above. The distribution was almost similar in the inter stage group also (table-3).

Stage	Percent of cases studied for						Total	
	No.	%	No.	%	No.	%	No.	%
I	2	11.77	--	--	1	33.33	03	3.53
II	1	05.88	28	51.85	--	--	29	34.12
III	8	47.06	06	11.11	7	50.00	21	24.71
IV	6	35.29	20	37.04	6	42.86	32	37.65
Total	**17**	**20.00**	**54**	**63.53**	**14**	**16.47**	**85**	**100.00**

Table 3.Stage wise DNA content in cancer breast cases

Thus the DNA has been observed to have acted as a marker even in different stages of breast cancer; thus maintaining the specificity and sensitivity into consideration. Nevertheless since a gold standard of marker in cancer detection is not available, the test for sensitivity and specificity could not be undertaken. What is required at this phase is public information and education, a patient or the health worker can screen the cases from the community, using the “Down staging” check list given to them.

The screened cases can there after be referred to district hospital/ medical colleges for Total DNA content study. This would create an arena of barrier to cancer mortality (table-3).

Conclusion

From the present study it can be concluded that the DNA content and tumour dynamics for breast cancer are inter related, and DNA can be used as a marker for early detection of breast cancer cases. What are needed are 3Cs: Convergence of services, commitment of health care providers and community participation to at least reduce the breast cancer mortality. Thus if women carry on SBE (Self Breast Examination) and report to health units in situation where they feel any mass; should be subjected to Total DNA Content; and if it amounts to ≤2 mg/g of breast tissue: Most likely the tumor is of Benign Breast Disease. However, further studies should be undertaken in this direction with more funds and more cases studied, and should be followed for a long period. Use of modern facilities and equipments also cannot be ignored in this type of study.

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