DNA DAMAGE IN PATIENTS OF CARCINOMA BREAST A CLINICAL STUDY BY USING COMET ASSAY

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ABSTRACT

35 cases of 27-68years old breast cancer individuals and 15 female individuals of FDFRS formed the material for the current study. An equal number of normal healthy females controls were also included to investigate the extent of DNA damaged in cases, FDFRs and controls. 'Comet assay' was done by conventional methods with slight modifications. For Comet metrics, a Trinocular research microscope, Nikkon Optiphot model with automatic photomicrograph attachment was used. Thus quantification of the DNA damage was done by measuring comet tail length in all the three groups (cases, FDFRs and controls).

There was significant increase in the mean comet tail length from controls to FDFRs ($p<0.0001$) and from FDFRs to cases ($p<0.0001$). In other words the DNA damage significantly increased from controls to FDFRs and from FDFRs to cases. It was also observed that among various stages of cases the mean comet tail length increased significantly from stage IIA to stage III B.

Mean comet tail length was found to be increased significantly in the advanced stage of carcinomas, i.e. stage III B followed by stage III A and II A. The FDFRs of breast carcinoma individuals showed significant level of DNA damage. This may be used as a marker/ tool for the identification of the diseased condition which gets manifested in families.

Keywords: Breast Cancer, First Degree Female Relatives (FDFRs), DNA damage, Comet Assay

INTRODUCTION

Malignancies or malignant conditions are frequently sporadic and often rarely familial. Many etiological factors have been attributed for the disease process. The onset of the disease, the expression of it, the severity, with reference to the age and the type of tissue involved are expressed in detail. The heritable malignant conditions are many. The breast cancer is one such condition, where heritable forms are described. The transmission of disease process in families affecting the sibling is expressed in multiple forms. The expression of the condition with reference to age (the onset) is also not consistent. This is either preceded or expressed at advanced age. Irrespective of the tissue affinity, all malignancies or malignant conditions are associated with extreme degree of DNA damages, thereby showing an alteration in the genome of the individual. Malignancies are genomic instability conditions. Current investigation was made to identify the damage occurring in DNA in individuals with breast cancer and the family members, in whom there is a risk of developing the condition (based on the history of having an episode among the family members). The aim of the study is to identifying the extent of DNA getting damaged in cases of different stages of breast cancer and its level in the family members who are suffering from it henceforth be called as first degree female relatives to hypothesize the risk of developing malignancy in them by using normal healthy as controls.

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MATERIALS AND METHODS

35 cases of breast cancer individuals whose age ranging from 27-68yrs and 15 female individuals of First Degree Female Relatives - FDFRs formed the material for the current study. First degree female relative means a close sibling of the affected (the cases with carcinoma breast). An equal number of normal healthy females-controls were also taken to investigate the extent of DNA damaged in cases, FDFRs and controls. The Breast carcinoma cases and FDFRs were from Outpatient Department tumor clinic wing of Department of Surgery, JIPMER hospital. The

comet assay was carried out at Cytogenetics laboratory of Department of Anatomy, JIPMER, and Pondicherry. Regarding the cases with malignancy the age of onset, its duration and medication including surgical interventions if any were noted. The common risk factors were also given significance and were recorded; these were the onset of menarche, family history of malignancy, the history related to breast feeding and the duration of it, the attainment of menopause and lastly the exposure to radiation, and habituation to tobacco etc. For the staging of malignancy TNM classification as mentioned below was followed.

The American Joint Committee on Cancer (AJCC) staging system (AJCC Cancer staging manual, 2002)

In selecting the cases, the following criteria were followed

Inclusion criteria

(1) Newly diagnosed breast cancer case before any treatment.
(2) First degree female relatives of breast cancer patients who are not affected with breast cancer or any other type of malignancy.
(3) Normal controls with no history of any malignancy in the family.

Exclusion criteria

(1) Breast cancer patients who are already on treatment.
(2) First degree female relatives of breast cancer patients who have any other malignancies including breast cancer.

For screening of first degree female relatives FDFRS, the siblings accompanying the cases were interviewed and the relevant data obtained was recorded. The guidelines framed by the human ethics committee Institute for any human studies were strictly followed in these individuals.

The controls for the current study were of a normal healthy individuals/group of staff working in academic and nonacademic sections of the Institute/hospital.

The cases, FDFRs and the normal controls were subjected for DNA damage studies through Comet assay.

Note: The whole procedure was carried out in dim light to minimize artificial DNA damage.

Scoring of comets/comet metrics

For comet metrics, a Trinocular research microscope Nikkon Optiphot model with automatic photomicrograph attachment was used. Comet tail length was measured in each case using an occulomicrometer fitted in the eyepiece. Randomly 50 cells were screened under 20X objective. The measurements pertaining to comets were recorded then. Quantification of the DNA damage was done based on the following.

Comet tail length (μm) = Total length of comet - Head diameter.

The comets were later photographed on soft or glossy grade photographic papers.

OBSERVATION AND RESULTS

All patients (35) were clinically proved to be of invasive / infiltrating ductal type of carcinoma. Cases of carcinoma of breast were of stage IIA to IIIC in the present study. Out of 35 cases of carcinoma breast, 10 were of stage IIB followed by IIIA (08) , IIA (07), IIIB (04) and least in IIIC (01) (All cases were free from either being treated by chemotherapy or radiation). 15 of normal healthy individuals with no history of any type of malignancy in families were chosen as controls. Equal numbers of FDFRs for the current study were of either daughters or sisters of the cases who have been investigated. Among the FDFRs, 14 were of daughters and 1 was a sister; both, cases and controls including FDFRs were from Pondicherry and nearby districts of Tamilnadu. Among 35 cases of breast cancer patients, 7 of them revealed a family history of malignancy and only 3 had a first degree relative suffering from breast cancer. In the present study, 8.5% of breast cancer cases had first degree relatives suffering from the same; while the remaining had the history of having had the malignancy other than of breast cancer.

All cases, controls and FDFRs were subjected for comet assay (Singh et al.1988[2], Ahuja and Saran 1999)[3] Total length of the comets in cases ranged from 21.75 ± 2.21 to 53.19 ± 5.31. Regarding the tail length in different stages of carcinoma i.e. IIA, IIB, IIIA IIIB, IIIC, the tail lengths were found to be longer in IIIC (Table I; Fig. 4). The mean tail length ranged between 15.45 + 3.36 to 30.32 + 3.92 .Of which, 15.45 to 18.37 were confined to stage IIA and IIB while the mean comet tail length of 26.87 to 30.32 were with IIIA and IIIB respectively.

The tail lengths of comets in controls were compared with those in cancer patients to assess the severity of DNA damage taking place in malignancy and also to derive the Basal DNA Damage-BDD The BDD refers to damage taking place in normal

individuals which is considered of permissible level due to some of the risk factors. The assessment/calculation of BDD is essential for application to correlate the damaged DNA of cases with malignancy with that of the controls.

By applying this, the cases of Stage IIA comets tail lengths of 15.45 with controls of 4.58; the tail lengths of 10.87 seem to be statistically significant with a p value being less than 0.0001. Similarly the derived tail lengths of stage IIB (13.79), IIIA (22.29), IIIB (25.74), were also statistically significant with p value less than 0.0001.

While comparing the FDFRs with controls, the following were observed. The mean tail lengths of the FDFRs were significantly increased compared to the mean tail lengths of the controls (p value<0.0001) (Table III; Fig.5). There was significant increase in the mean comet tail length from controls to FDFRs (p<0.0001) and from FDFRs to cases (p<0.0001). In other words the DNA damage significantly increased from controls to FDFRs and from FDFRs to cases (Table II; Fig. 5). When the mean comet tail lengths among various stages of cases were compared it was observed that the mean comet tail length increased significantly from stage IIA to Stage III B (Table I; Fig. 4). It was observed that the mean comet tail length did not differ significantly among various age groups.

Stage of carcinoma	Comet Tail Length in µm (Mean ± SD)
Stage IIA	15.45 ± 3.26
Stage IIB	18.37 ± 1.72
Stage IIIA	26.87 ± 5.08
Stage IIIB	30.32 ± 3.92

Table I: Showing the correlation between mean comet tail length with the various stages of carcinoma

P value >0.0001 statistically significant

	Comet tail length in µm (Mean ± SD)
Breast cancer cases	21.75 ± 7.07
FDFRs	11.73 ± 2.01
Controls	4.58 ± 1.08

Table II: Showing the mean comet tail lengths in breast cancer cases, FDFRs and Controls
P value < 0.0001 statistically significant

	Comet tail length in µm (Mean ± SD)
FDFRs	11.73 ± 2.01
Controls	4.58 ± 1.08
P-Value	<0.0001

Table III: Showing Mean comet tail length correlated with FDFRs and controls
P value < 0.0001 Statistically significant

	comet tail length in µm (Mean ± SD)
Breast cancer patients	21.75 ± 7.07
Controls	4.58 ± 1.08
P-Value	<0.0001

Table no. IV: Showing Mean comet tail length correlated with cases and controls

P value < 0.0001 Statistically significant

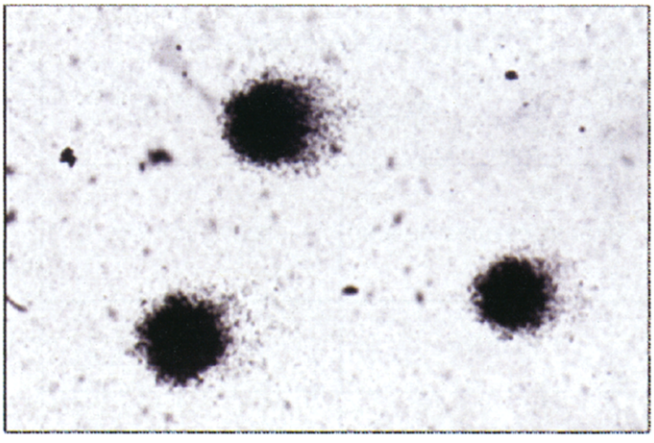

Fig 1: Healthy controls showing minimal DNA damage.

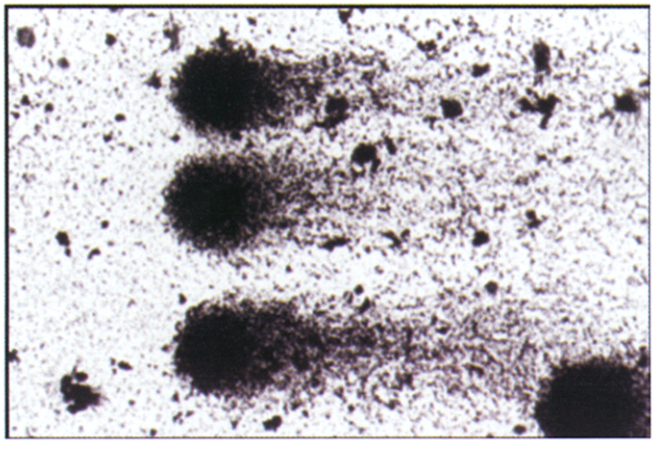

Fig 2: First degree female relative of Breast cancer patient showing moderate DNA damage.

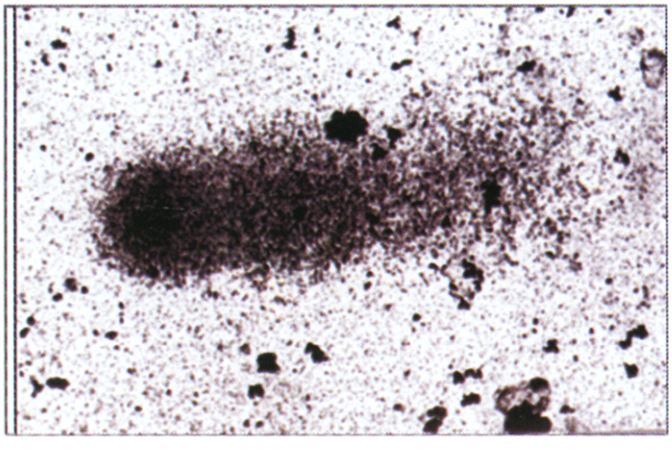

Fig 3: Breast cancer patient showing maximal DNA damage

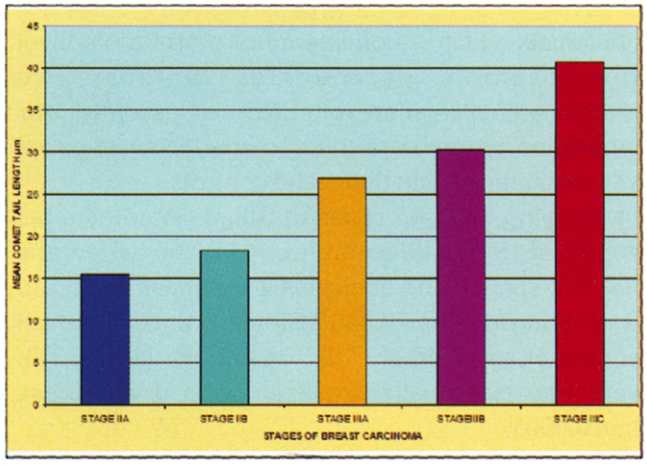

Fig. 4 : Mean Comet tail length (in μm) in Cases With various stages of carcinoma breast

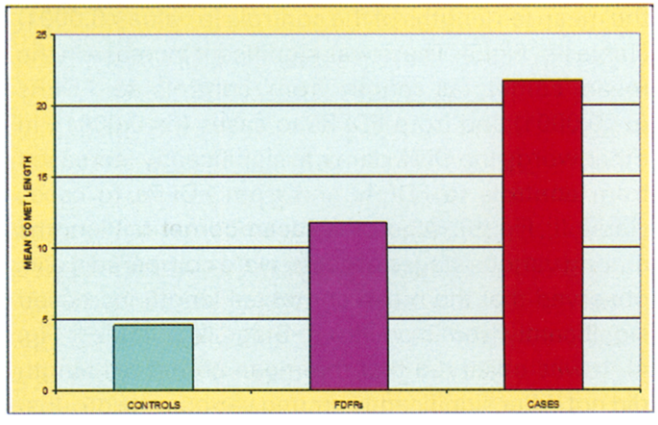

Fig. 5: Mean comet tail length (in μm) In Controls, FDFRs and Cases

DISCUSSION

The loss of genomic stability or increased genomic instability, either acquired or hereditary is accepted as being one of the most important aspects of cancer and in fact considered as a primary event for a neoplastic transformation (Adhvaryu et al. 1988[4], Gupta et al. 1996)[5]. In the current study, the genomic instability or DNA damage was studied in cases with carcinoma breast of various stages, FDFRs, and healthy controls.

The correlation between genomic instability and cancer susceptibility in cases of known genetic syndromes (e.g. ataxia telengectasia, Fanconi anemia) is well established. The extent of DNA damage or genomic instability in established cases of malignancy and a basal DNA damage that exists in normal healthy individuals as a result of exposure to risk factors play a critical role in carcinogenesis as well as in progression of the condition. In current series, the DNA damage increased significantly in

FDFRs and breast cancer patients (Table 2, 3 & 4).The FDFRs showed ~2.75 times higher DNA damage as compared with the controls. It is evident that the FDFRs in the breast cancer families were at an increased risk of breast cancer as compared with control families. In support of this, Rajeshwari et al (Rajeshwari et al. 2000)[6] reported a ~2.5 fold higher DNA damage in FDFRs as compared to controls. Whereas, a higher gradient of damage ranging from 1.8-4.2 in families with breast cancer was observed by Schwartz et a l (Schwartz et a l. 1991)[7].

Maximum DNA damage was observed in the breast cancer patients in the present study. Similar trend of increased levels of DNA damages was observed in individuals with malignancy of bladder and cervix through comet assay (Udumudi et al. 2003[8], McKelvey et al.1997)[9] So also the chromosomal abnormalities, like deletions, breaks and gaps, in breast cancer patients as compared with controls (Mars and Saunders, 1990[10], Ramesh and Bhargava,1992)[11]. In contrast, Kovacs and Almendral (Kovacs and Almendral, 1987)[12] and Jyothish et al (Jyothish et al.1998)[13] observed no difference in the level of spontaneous DNA synthesis and chromosome aberrations between FDFRs and cancer patients .However, in our study we observed a significant difference in the DNA damage between the breast cancer patients and FDFRs: and FDFRs and controls. In the present study there was significant increase in extent of DNA damage from stage IIA to stage IIIB, suggesting that DNA damage was of a progressive nature and it continued as the cancer progressed which is manifested by longer comet tail lengths. The association of longest comet tail length with the cases and relatively longer comets in FDFRs indicate the severity of involvement of the DNA damage.

The present observations with regards to age agree with the finding of the Smith et al (Smith et al. 2003)[14] that the extent of DNA damage remains unaffected.

There were 5 cases where the staging could not be ascertained due to various reasons. The age ranges of these were between 30 and 60yrs. For calculation of the mean comet tail lengths of these individuals they were appropriately placed in various age groups .Hence (mean comet tail lengths of these cases where staging could not be ascertained) formed a group by itself. As there were no corresponding FDFRs with history of involvement, the significance of mean comet tail lengths in these 5 cases and the prediction for the possibility of occurrence could not be hypothesized. While comparing these cases (where the staging could not be ascertained) with the controls, the values of mean comet tail lengths were found to be significantly higher. Coming to cases, where there is no family history of either malignancy of breast or others, the following is suggested:

Because the mean comet tail lengths were significantly increased compared to the matched controls, the possibility of hereditary type of malignancy in these is ruled out. The other possibility of the cumulative effect of basal DNA damage as etiological factors as mentioned by Legerski et al (Legerski et al.1994)[15] may be thought of. This is substantiated by a significant difference in the mean comet tail lengths seen by the application of the derived Basal DNA damaged data over the cases.

As the cases of this group were predominantly of workers of agriculture background, the cause for the manifestation of this condition in these may be due to an involvement with a mutagen specifically like the pesticides, insecticidal sprays including the chemical fertilizers.

To assist with the early detection of breast cancer, reliable risk biomarkers are urgently needed. The comet assay is a simple, quantitative way to determine the level of basal DNA damage. From the present study it is evident that the FDFRs are at an increased risk for breast cancer as compared with the control population. Hence, long-term, large-scale follow-up studies in the high risk FDFRs would be required to confirm the conclusions of this study.

SUMMARY AND CONCLUSION

The current investigation on DNA damages in carcinoma breast cases of different stages and age including the family members of the affected showed significant changes through comet study with reference to the progression of the disease condition and in family members as evidenced by

1. Mean tail length was found to be increased significantly in the advanced stage of carcinomas i.e., Stage IIIB of the current study followed by stage IIIA and IIA.
2. The first degree female relatives-FDFRs of breast carcinoma showed significant level of DNA damage. This may be used as a marker/tool for the identification of the disease condition which gets manifested in families.
3. The levels of DNA damage- Basal DNA damage seen in the normal controls of various age

groups was not consistent but showed variations. This could be due to many risk factors/ environmental.

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