

What is My Risk of Cancer ?

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KEY WORDS: Cervix Cancer, Breast Cancer, Risk assessment.

ABSTRACT There are various approaches towards risk assessment in cancer, eg, identification of mutations/deletions in oncogenes, anti-oncogenes, mutator genes and epigenetic changes. These procedures are time consuming and expensive. Therefore, a search was made for a procedure, which would be time saving and inexpensive. Net result of accumulation of the above mentioned mutations/deletions/changes leads to a gradual increase in genomic instability. Therefore, it was considered logical to use a test to measure DNA damage in suspected cases for the identification of high-risk individuals. For this purpose, single cell gel electrophoresis assay (commonly known as comet assay) was selected because (1) it is the most sensitive assay known to estimate DNA damage, (2) it gives information on single cell basis, and (3) it requires a small sample size and is efficient in time. In our laboratory, we have used this method to identify individuals at high risk of cancer of the cervix and breast. Our results in general are comparable with the figures reported in the literature. Since cancer has a long gestation period, high risk individuals need to be followed-up for confirmation of the predicted outcome as well as for counseling.

INTRODUCTION

An essential feature of malignancy is that, the malignant phenotype is passed on from the parent cell to its daughter cell. This suggests that the fundamental lesion of malignancy resides in the DNA. Although mammalian cells are richly endowed with both efficient DNA repair and free radical scavenging systems, it is probable that some DNA lesions generated via intrinsic and extrinsic mutagenesis would escape these DNA "surveillance" processes and constitute an early step in human carcinogenesis. Strong supporting evidence for this theory comes from DNA instability syndrome, like ataxia telangiectasia (Gatti et al. 1988) in which DNA repair

mechanisms are defective with an associated risk of malignant disease. As most of the human cancers are associated with genomic instability, its assessment is an important aspect. The three most commonly used methods for ascertaining DNA damage and repair are (a) Cytogenetic techniques, which involve scoring of chromatid exchanges, require proliferating cell populations, (b) Unscheduled DNA synthesis (UDS), which involves detection of DNA repair in pooled cell population. It requires exposure of damaged cells to radioactivity, and is limited in sensitivity (Tice and Selton 1985), and (c) Alkali elution test which involves detection of single strand breaks and alkali labile sites, is technically cumbersome, and again provides information in pooled cell population.

Cancer being monoclonal, it would be desirable to investigate it at the single cell level. Hence, there is a need for a simple test that gives information at the single cell level. Single cell gel electrophoresis assay, commonly known as Comet assay, is a recent method for detecting the presence of DNA single strand breaks and alkali labile damage (Singh et al. 1988; Ahuja and Saran 1999). The assay can be conducted on relatively small number of cells (~10,000) and the results can be obtained within a short time after sampling (ie, within a few hours). Single strand breaks in the nucleoid produce fragments of varying length which migrate towards anode in the form of a tail, giving the appearance of a comet (Saran et al. 1999). The tail length is directly related to the magnitude of DNA damage. This assay offers a high degree of sensitivity, data is obtained at the level of individual cell, and virtually any eukaryotic cell population is amenable to analysis (Singh 1996). Micronucleus test (MNT) is another simple and reliable indicator of chromosomal aberrations at the numerical as well as structural level, and provides information at the single cell level.

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CERVIX CANCER

Cervix cancer is the most common cancer amongst women in India. Its incidence in the Indian population is about 34 per 1,00,000. It accounts for 20-50% of all cancers in women and 80-85% of female genital tract cancers (Annual report ICMR 1992).

Cervix cancer is preceded by precancerous lesions, clinically diagnosed as dysplasia, which may be broadly classified into mild dysplasia (MD), and severe dysplasia (SD). The biological behaviour of these lesions is totally unpredictable (Mitra et al. 1995). Some lesions remain in the same state, many regress back to normal condition and a small proportion of these lesions progress towards carcinoma. In a 5-year survey carried out at Rochester Medical Centre, it was found that about 0.5% of MD and 9.5% of SD progressed to cervix cancer (Patten 1978). As of today, there is no biological parameter available to predict the outcome of dysplasia. A study was undertaken in our laboratory to make an effort to explore such a parameter.

The study included 407 female subjects. Based on the pap smear test (Koss 1979), these subjects were classified into the following four groups: Normal cervix, ie, controls (103), MD (100), SD (100), carcinoma of the cervix (104). 100 μ ls of heparinised blood sample and a swab of cervical epithelial cells were collected from each of the above subjects and were used to study basal DNA damage, damage after treatment with a mutagen and a carcinogen, MNNG, and residual damage after repair. Comet assay was performed on each of these (blood and cervical epithelial cells) samples to study the DNA damage in cells which were untreated, treated with MNNG, as well as those cells which were allowed 2 hours of repair time after treatment.

Besides cervical epithelial cells, DNA damage was seen in the leucocytes, which are not the target tissue of the precancerous and cancerous conditions of the cervix. Although the exact reason for such damage is unknown, it could be due to some mutagenic substance released by cancerous as well as precancerous tissues into the blood stream. This mutagenic substance may be responsible for damaging the leucocyte DNA (Werkmeister et al. 1980).

There was a significant stepwise increase in the mean basal DNA damage in leucocytes as well as cervical epithelial cells from controls to

patients with mild dysplasia, severe dysplasia and cancer of the cervix. When the cells were treated with a mutagen (MNNG) there was a significant increase in DNA damage within each category. Again the trend in terms of DNA damage between the categories remained the same as in the basal DNA damage. After 2 hours of repair, the DNA damage came back to the basal level in each category. A regressive trend in repair capacity was observed from precancerous to cancerous lesions of the cervix compared to controls.

Most of the repair defective syndromes, like ataxia telangiectasia and Bloom syndrome, are associated with a risk of malignant disease (Gatti et al 1988). Also cancer patients not belonging to any of these syndromes have been reported to have lower DNA repair capacity (Kovacs et al. 1986). In the present study too DNA damage increased in a significant stepwise manner from controls to MD, SD and cervix cancer patients indicating a repair deficiency in the same order. The individuals who exhibited, high mean (top 5 percentile) DNA damage based on the 3 aspects such as (a) high means basal DNA damage (b) high mean DNA damage after treatment with MNNG and (c) low repair capacity, were considered as high risk individuals at 1st level of screening. These suspected high-risk individuals were considered for the 2nd level of screening. Among the suspected high risk individuals, who showed a larger percentage (top 5 percentile) of cells with high DNA damage without treatment, with treatment with MNNG, and post repair were considered to be at added risk of developing cervical carcinoma.

The main aim of the study was to biomonitor high-risk individuals among those with precancerous lesions (MD, SD) of the cervix. From the present study high-risk individuals were 1.3% of the MD and 5.5% of the SD patients, as compared to 0.5% of the MD and 9.5% of the SD reported by the Rochester study (Patten 1978). The discrepancy in figures in these two studies could be due to the populations surveyed, ie, Indian vs American. In addition, our criterion of using top 5 percentile as the cut off point may not be statistically satisfactory.

Recently, molecular epidemiology has gained a lot of importance, particularly in early detection and prediction of cancer risk factors. The observations of the present study suggest that comet assay may serve as a novel tool to predict

the relative risk of developing cancer of the cervix in women having cervical dysplasia. Detailed follow-up studies are required to confirm this contention. (Jaiswal et al. 1994; Udmudi et al. 1988).

BREAST CANCER

Breast cancer, one of the solid tumors affecting women, is a major and increasing health problem in the world. In India, it is the second most common cancer affecting women, and its incidence has been reported to be 22 per 1,00,000 (Sanghvi et al. 1998). Human breast cancer is a multi-factorial disease with risk factors relating to hormonal exposure, diet, environmental agents, heredity and family history of the disease (Mant and Vessey 1991). Familial clustering may be *due* to shared genetic factors and/or common environmental factors or an interaction between the two.

A number of numerical and structural chromosomal abnormalities (Mars and Saunders 1990), and amplification of oncogenes and loss of certain anti-oncogenes have been reported to be associated with breast cancer (Mackay et al. 1990; Ahuja and Araga 1997). Two important predisposing genes, BRCA1 on chromosome 17q21 and BRCA2 on chromosome 13q12-13, which account for about two-thirds of familial breast cancer cases (Ponder 1994), have been mapped and cloned (Wooster et al. 1994).

Of all the factors, a well-established risk factor is the family history of disease. A 2-3-fold increase in risk has been reported for the first-degree female relatives in breast cancer families. Families vary in their susceptibility to breast cancer, and within a family also, among the first-degree female relatives (FDFRs) of patients, there remains a substantial heterogeneity in the risk. Inter-individual differences can be inherited or acquired. However, no definite genetic, endocrine, and/or immunological markers are available that distinguish the high risk from the low risk women.

The female subjects for the current analysis included 121 controls with no family history of any type of cancer, 188 FDFRs of breast cancer patients, and 88 breast cancer patients. Comet assay was carried out on these subjects to evaluate DNA damage and repair as described by Singh et al. (1988) and Ahuja and Saran (1999). MNT on the buccal epithelial cells of the subjects

was also carried out in addition to comet assay. (Rajeswari et al. 2000).

There was a stepwise increase in DNA damage and micronucleus frequency from controls to FDFRs, and from FDFRs to breast cancer patients. AU (1993) reported increased mutagen sensitivity and impaired DNA repair ability in cancer patients as compared to the controls. In this study also, within each group, DNA damage was enhanced after MNNG treatment and regressed after 2 hours of incubation allowing repair. Similar trend was observed in all the groups but the stepwise increase from controls to FDFRs, and then to breast cancer patients still remained.

It has been reported in other studies that the inter-individual variability in terms of chromosomal aberrations was minimum in normal subjects and maximum among cancer patients (Hsu et al. 1985; Saritha and Ahuja 1990; Knight et al. 1993). In the present study also, the range of inter-individual variability increased from controls to FDFRs, and from FDFRs to breast cancer patients. This increase is probably due to stepwise increase in genetic instability in these three groups.

The main objective of the present study was to pick out the 'high risk' individuals or 'outliers' (crossing 3 SD) among the first-degree female relatives of the breast cancer patients. Individuals with excessively (a) high basal DNA damage, (b) increased susceptibility to mutagen treatment, and (c) reduced repair efficiency were considered as the 'high risk' individuals. Out of the 188 FDFRs studied, 4 (2.13%) individuals who fulfilled these prerequisites were identified as the high-risk individuals. Our results are comparable with those of Parkin et al. (1997) who reported that average lifetime cumulative breast cancer incidence in India was 2.11%

ANIMAL MODEL

To test the above mentioned hypothesis, that individuals with excessively increased genetic instability and poor repair efficiency were to be considered as the high-risk individuals, a follow-up study would have been necessary. Since a follow-up study for the occurrence of cancer in identified high risk human subjects was not practical due to a long period of cancer latency, it was planned to include an animal (murine) model.

In the animal model, spontaneous and

induced susceptibility to mammary tumors was studied by evaluating DNA damage and repair using comet assay on the peripheral blood leucocytes of mice and rats. These animals were followed-up from birth to death. The incidence of tumor formation, DNA damage and repair were evaluated in all these animals at regular intervals.

According to the proposed hypothesis in the present study, animals with high DNA damage and reduced repair efficiency initially in their life, as evaluated by comet assay, were predicted to have increased risk of mammary tumor. Our prediction was confirmed on follow-up. Only the high-risk animals identified in early age developed mammary tumors later on in life.

CONCLUSIONS

Cancer poses an unquestioned health hazard, but even more troublesome challenge is to identify the individuals prone to it. The present study on biomonitoring individual susceptibility to cancer in cervical dysplasia patients and FDFRs suggests that comet assay seems to have the potential to serve as a biomarker. Inclusion of naimal model has supported the hypothesis that individuals with high DNA damage and low repair efficiency are at an increased risk to cancer. However, long-range follow-up studies in the high-risk human subjects are required to confirm the conclusions reached in this study. Furthermore, other types of cancer need to be investigated to test the validity of the hypothesis proposed here.

Since the latent period for cancer is long, it is difficult to see the outcome in such studies. However, wherever possible, efforts are being made to follow-up the high-risk individuals. For example, the Nordic study-group has verified the association of the magnitude of chromosome damage with cancer risk (Hall et al. 1990).

The entire concept of cancer prevention would be revolutionised if we could identify high-risk individuals.

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