International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online)
An Online International Journal Available at http://www.cibtech.org/jms.htm
2012 Vol. 2 (1) January-April, pp132-137/ Didla et al.
Research Article

EVALUATION OF POTENTIAL ROLE OF SISTER CHROMATID EXCHANGES IN EARLY CANCER DETECTION AND GENETIC INSTABILITY DISORDERS: A PRELIMINARY STUDY FROM COASTAL ANDHRA PRADESH

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ABSTRACT
Sister Chromatic Assay (SCE) is very sensitive technique which has got important role in Genetic toxicology studies and clinical diagnostic setups. In order to utilize the technique efficiently in diagnostic setups, the referral ranges of SCEs in the given population is to be established. In the light of this, we have attempted to establish referral range of SCE frequency of general population, and also that of clastogen induced from coastal Andhra Pradesh. We also studied the frequency in cancer patients before treatment is initiated. Our results indicated the baseline SCE frequency is 7.52 ± 0.27, from general population and 23.35 ± 0.38 in clastogen induced samples. The increased level of SCE frequency (13.4 ± 0.43) is observed in all cancer patients irrespective of type of cancer. The SCE assay could be used as surrogate marker for early cancer detection, provided more studies involving various cancers proved the same. The baseline frequency of our study, with and without Mytomycin - C induction, which fall in range of other studies, could serve as a referral range for genetic disorders and genotoxic assays.

Key Words*: SCE, Xeroderma Pigmentosum, Brdurd- Labelling, Mytomycin-C, Metaphase*

INTRODUCTION
Sister Chromatid exchange (SCE) is the exchange of genetic material between two identical sister chromatids of chromosomes. This phenomenon was originally described by Taylor *et al.* (1957) with an autoradiography technique Latt (1973) introduced the current BrdUrd- labelling method which allows more precise and reliable analysis of this phenomenon. Even though the mechanism leading to SCE is not yet fully understood, the highly increased incidence of SCE after treatment of cells with DNA-damaging agents such as chemicals, ionizing radiation, and UV cause an increased incidence of SCE (Kato,1977) suggests that SCE is a reflection of DNA damage and subsequent repair processes (Kato,1977) . The analysis of the SCE incidence after treatment (Kato,1977) with various types of mutagen/carcinogens also provides evidence for possible relevance of SCE to mutagenesis-carcinogenesis (Carrano *et al*., 1978*;* Perry and Evans, 1975; *and* Popescu *et al.*, 1977*)* SCE had been analyzed in cells from patients with various genetic disorders highly characterized by a susceptibility to genetic damage and prone to develop cancer. These studies include patients with Bloom's syndrome, Fanconi's anemia, ataxia telangiectasia, and xeroderma pigmentosum (Arieti and Lehman, 1978). A greatly increased incidence of SCEs has been demonstrated in cells from patients with Bloom's syndrome (Chaganti *et al.*, 1974), but cells from patients with Fanconi's anemia, ataxia telangiectasia, and xeroderma pigmentosum have displayed normal levels of SCE. When certain strains of xeroderma pigmentosum cells were treated with chemical agents, irradiation, or UV, an increased SCE frequency was observed (Perry *et al.*, 1974*)*. In other words, in above mentioned genetic disorders, as such baseline frequency falls in normal range, but when they are exposed to mutagens, SCE's frequency increases. That underlines the importance of, assessing clastogen induced SCE base line frequency, apart from general SCE base line frequency, in diagnostic set up. Normally 5-10 SCE's per metaphase cells are present, but are greatly increased in the patients with disorders

like Bloom Syndrome, Xeroderma Pigmentosum, Falconic Anemia as well as malignancy. The background or baseline frequency of SCE can be increased dramatically when cultured cells were exposed to a wide variety of DNA damaging agents **(** Littlefield and Takehisa, *1982*). Various pre malignant and malignant studies revealed the increase in SCE's frequency indicating the potential role of SCE's measurement in early cancer detection. Studies from various populations showed variation in back ground frequencies across the populations. Hence, there is a dire need to establish own normal reference ranges of SCEs in the population. . Such studies are not reported in coastal Andhra population, where various tribal population and ethnic groups reside. Keeping this in view, we have initiated this study to access baseline frequency of SCE's from volunteers of general population also with Mitomycin-C which is known SCE inducer and anticancer drug. We also aimed to stud y the SCE's frequency in cancer population before treatment started, to explore the potential role of this test in early cancer detection.

MATERIALS AND METHODS

A total of 10 blood samples from 5 adult males and 5 adult females (ages ranging between 20 and 60 years) from general population, were collected after taking oral consent. An eleven blood samples from cancer patients (3 carcinoma lung and 3 carcinoma of colon and 1 mandible sarcoma and 1 carcinoma tonsil) were collected. Metaphase chromosome preparations from blood were carried out by routine phytohemaagglutinin (PHA) stimulated cultures as described elsewhere (*Perry.P and Wolff (1974), Latt (1974)* Cells were cultured in Roswell Park Memorial Institute Medium (RPMI) 1640 with 10% fetal calf serum in the presence of BrdUrd (*Sigma Chemical Co., St. Louis Mo)* at a final concentration of 14.4ug/ml, after 24hr of culture was initiated. The fluorescence plus Giemsa (FPG) technique was used for SCE. Duplicate cultures were initiated from samples of gen eral population with and without Mitomycin C (MMC). The MMC (MMC (20 ng/mL) dose in the present study is known dose inducing SCEs. All slides were coded and scored by a single individual. Metaphases were classified as first (M1), second (M2), and third (M3) generation after exposure to BrdUrd, according to the pattern of sister chromatid differentiation (Latt, 1973). The M2 cells were used for scoring SCEs. Between 15 and 25 metaphases were evaluated per sample. The results were analyzed by Analysis of variance (ANOVA).

RESULTS

The fig 1 represents the metaphase prepared from general population showing the frequencies of sister chromatid exchanges (SCE). The fig 2 shows high frequency of SCES in Mytomycin- C *fig. 3* shows increased SCE frequency from cancer patients. The frequencies of sister chromatid exchanges (SCE), from current study are shown in **Tables 1**and **2. Table 1** shows SCE frequency of control, MMC-induced SCEs, and that of cancer samples. A significant increase (***P<0.0001***) in frequencies of SCE was observed in Mitomycin-C induced culture. The SCE values of cancer cases deviate significantly from that of controls. The **Table 2** shows the SCE frequency of males and females from three groups. There is no significant difference in SCE frequency among genders.

DISCUSSION

This study was initiated to establish the baseline frequency of SCE in Coastal Andhra Population. The studies done in various populations revealed SCE ranging from 4 to 10/ cell. No studies were done earlier in coastal Andhra Pradesh. In our current study, we observed 7.52 ± 0.27 SCE frequencies. Whereas, MNC induced samples, revealed 23.62 ± 1.248 SCE frequencies per metaphase. These values are significantly different from that of normal referral range. The SCE's frequencies in cancer patients in our study are significantly higher when compared to general population. Earlier studies in pre-cancerous lesion and cancers elsewhere, also demonstrated the same **(**Sou *et al*., 1986). In our study, there is no sex difference in the mean frequencies of SCE's across gender from the above three groups.

Our results are in agreement with few studies in which there is no correlation found between SCE's and gender of the subjects (Nagaraj, 1986; and Carrma,1982).

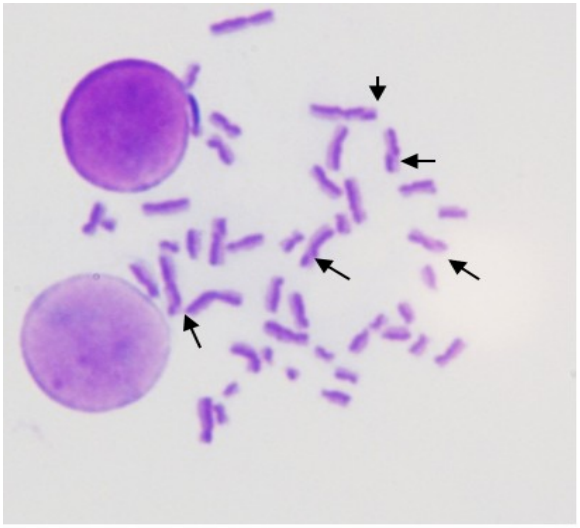

Fig 1: Metaphase showing SCEs from Control group (general population)

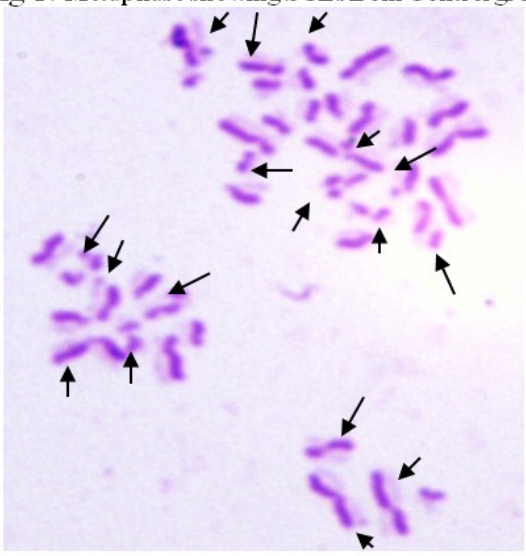

Fig 2: Metaphase showing SCE's from MNC induced group

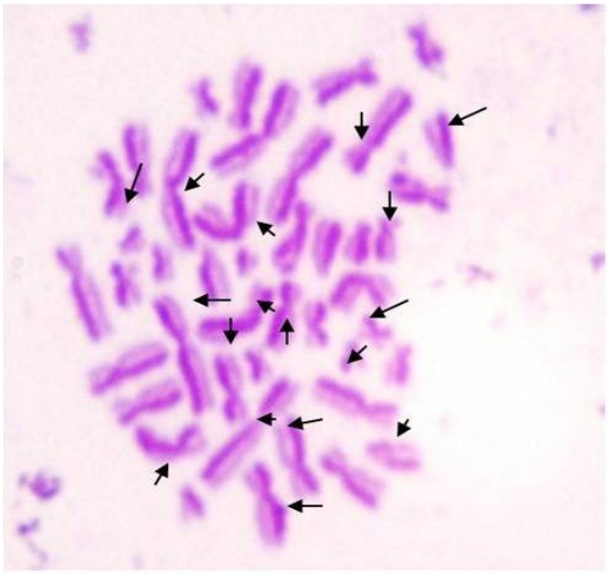

Fig 3: Metaphase showing SCEs from Cancer group

Table 1: Sister Chromatid Exchange Frequency In Various Groups General Population, MNC Induced & Cancer Patients).

CATEGORY	NUMBER OF SAMPLES	NUMBER OF MEATPHASES SCORED/SAMPLE	SCE/CELL Mean ± S.E
CONTROL	10	150	7.52 ± 0.27
MNC INDUCED	10	323	23.35 ± 0.38*
CANCER GROUP	8	147	13.4 ± 0.43*

**Significantly different (P < 0.0001) from control*

Table 2: Variation Of SCE Frequency In Males And Females Of All The Study Groups

GROUP	MALE	FEMALE
CONTROL (NORMAL POPULATION)	7.1 78± .628	8.075 ± .676
MNC INDUCED	24 .2± 1.97	23.62 ± 1.248
CANCER PATIENTS	13.94±.6657	13.06 ± .4745

CONCLUSION

When SCE test is exploited in diagnostic setup, this range could be used as referral range for certain genetic disorders such as Xeroderma pigmentosum. The dose of Mitomycin-C, we have selected for our study is the dose which is routinely used in Diagnostic setups. With more number of samples being studied, these established ranges, could be exploited in pre-clinical studies of pharmaceuticals and genetic disorder diagnostics. This preliminary study indicates the possibility of using SCE as a

preclinical marker for early cancer detection. Systematic study of various cancers with large population will throw more light to use this test as surrogate marker in early cancer detection.

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Sou.S, Takabayashi.T, Ozawa.N et al., (1986) found that there was a significantly higher 52 spontaneous SCE frequency in cancer group than in control group (8.21+/-1.42) against (5.62+/-0.55).

Perry.P and Wolff.S (1974), Latt.S.A (1974) showed that studies of SCE on mammalian cells were greatly facilitated by differential staining techniques based on incorporation Of the base analogue 5-Bromodeoxyuridine.