

Sister Chromatid Exchange in Oral Submucous  
Fibrosis Patients and Pan Chewers

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## CERTIFICATE

This is to certify that this dissertation titled “**SISTER CHROMATID EXCHANGE IN ORAL SUBMUCOUS FIBROSIS PATIENTS AND PAN CHEWERS**” is a bonafide record of work done by **Dr. D. JEYA PRADHA** under my guidance during his post graduate study period between 2002-2005.

This Dissertation is submitted to THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, in Partial fulfillment for the Degree of **MASTER OF ORAL AND MAXILLOFACIAL PATHOLOGY, BRANCH IV**. It has not been submitted (partial or full) for the award of any other degree or diploma.

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## INTRODUCTION

Mutation refers to genetic damage or change brought about by environmental or chemical factors. Vast majority of the recognized human carcinogens are genotoxic. Sister Chromatid Exchange (SCE) right from the time when Taylor.J.H (1958) discovered an unexpected exchange of labeled DNA between sister chromatids<sup>50</sup>, has remained a good biomarker possibly even a biosimulator for exposure to potential carcinogens<sup>28</sup>. It is interesting to know that every mutation is being carried on to forth coming generations and there are also attempts to evade the deleterious effects of mutation and one such process is SCE, which acts in its own way right from the process of mitosis.

Mitosis is a process of somatic cell division during which each chromosome divides into two sister chromatids. Each of these sister chromatids is incorporated into each of the daughter cell. As a result of this the number of the chromosomes per nucleus remains unchanged. The process of mitosis thus involves two identical sister chromatids being formed as a result of DNA replication having taken place in the S phase of the cell cycle and gets separated and dispersed into two daughter cells.

But mutation occurring either in a hereditary fashion or due to environmental factors like high-energy radiation, certain chemicals, viruses etc., have caused disturbance in this normal morphological patterns of mitosis. Recently the different chemical constituents of pan namely nicotine, arecholine, arecaidine, anatarbine and dimethyl sulphoxide have gained acceleration for the fact of causing genotoxic effects. These genotoxic agents are capable of causing nicks in the DNA strands and the nick in a single DNA strand frees the severed end, which then invades the

exposed complementary helix to create a short pairing region. This initiates a general recombination event and DNA renaturation. DNA nicking agents induce reparative exchanges between sister chromatids during cell cycle when replicate sisters are on hand. So this causes an exchange or cross over of genetic material between the two chromatids of a chromosome during mitosis. So SCE is a mechanism to safeguard and repair the damaged DNA. The results of genotoxic effects- chromosomal damage (fragility, aberration etc.,) and repair as SCE can be detected by a sensitive cytological procedure. SCE is defined as a symmetrical exchange at one locus between sister chromatids and appear to involve DNA breakage and repair mechanism during S phase of the cell cycle, which does not alter the overall chromosomal morphology.

Normally 5-8 SCEs per cell are present but is greatly increased in the patients with hereditary disorders like Bloom's syndrome, Xeroderma pigmentosum, Fanconi's anemia as well as malignancy and premalignancy. Oral sub mucous fibrosis (OSF) is a chronic, insidious, fibrotic change affecting any part of the oral mucosa when analyzed is found to have a possible correlation with tobacco and betel nut chewing habits which have been proved to induce genotoxic effects in initiation of OSF<sup>1</sup>. However very little information is available regarding the use of pan chewers and OSF patients. The present study is aimed to evaluate chromosomal/DNA instability using SCE as a marker.

## REVIEW OF LITERATURE

### HISTORY OF SCE:

**Taylor.J.H, Woods.P.S and Hughes.W.L** (1957) performed experiments that were designed to test the Watson and Crick hypothesis. The results of these experiments not only showed that Watson and Crick model of DNA replication was operative at the level of chromosome, but also showed that there was an unexpected exchange of labeled DNA between sister chromatids.<sup>7</sup>

**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.<sup>7</sup>

**Ikushima.T and Wolff.S** (1974) initially suggested that the number of exchanges is proportional to the length of the chromosomes except for chromosome number 1 because of its excess length.<sup>58</sup>

**Stetka.D.G and Carrano.A.V** (1977) stated that the intercalating dye Hoechst 33258 interact synergistically with BrdU – substituted DNA to induce SCE.<sup>7</sup>

**Carano. A.V, Thomson.L.H, Lindl.P.A. et al.,** (1978) stated that SCE is a reciprocal interchange of DNA between chromatids, is easily visualized in metaphase has been applied to study chromosome structure, chromosome damage, instability and DNA repair deficiency syndromes. Since SCE can be induced by sub toxic doses of carcinogens and mutagens their analysis offers the possibility of a rapid sensitive quantitative assay for genetic damage.<sup>6</sup>

**Ohtsuru.M, Ishii.Y, Takai.Y *et al.***, (1980) showed that the peripheral blood lymphocytes from patients exposed to cytostatic drugs show elevated SCE frequencies.<sup>7</sup>

**Speit.G, Wolf.M and Vogel.W** (1980) stated that cystamine interact synergistically with BrdU – substituted DNA to induce SCE.<sup>7</sup>

**Morgan.W.F and Crossen.P.E** (1980) considered that X-rays were poor inducers of SCE, but increased SCE frequencies were observed when BrdU substituted DNA is irradiated or when BrdU was added immediately after radiation.<sup>7</sup>

**Natarajan.A.T, Csukas.I and Zeeland.A.A.V** (1981) stated that poly (ADP-ribose) interact synergistically with BrdU – substituted DNA to induce SCE.<sup>7</sup>

**Lambert.B, Lindblad.A, Holmberg.K *et al.***, (1982) showed the high degree of correlation and interpreted that SCE could serve as a sensitive index for the mutagenic, carcinogenic effects or environmental chemicals.<sup>7</sup>

**Littlefield.L.G** (1982), **Takehisa.S** (1982) put forth the fact that the background or baseline frequency of SCE can be increased dramatically when cultured cells were exposed to a wide variety of DNA damaging agents.<sup>7</sup>

**Morgan.W.F and Wolff.S** (1984) stated that 3-aminobenzamide interact synergistically with BrdU – substituted DNA to induce SCE.<sup>7</sup>

**Pinkel.D, Thompson.L.H, Gray.J.W *et al.***, (1985) reported a protocol for detecting SCE using monoclonal antibodies to BrdU-substituted DNA and propidium iodide.<sup>7</sup>

**Ghosh.P.K, Madhavi.R, Guntur.R *et al.***, (1990) reported that halotypes A10, B7 and DR3 showed a significant elevation and halotype DRT was substantially decreased in these patients.<sup>16</sup>

**Park.E.H, Kim.Y.J and Byun.D.H**, (1992) put forth that the frequency of SCE in human lymphocytes is a sensitive indicator in monitoring human populations for exposure to environmental mutagens and carcinogens.<sup>42</sup>

**Labbauf.A, Klopman.G and Rosenkranz.H.S**, (1997) quoted that the vast majority of recognised human carcinogens are genotoxic and the present finding indicates that in

vivo induction of SCE is a good biomarker, possibly even a biodosimeter for exposure of potential carcinogen.<sup>28</sup>

### **STAINING OF SCEs**

**Perry.P and Wolff.S** (1974), **Kim** (1974) first described Giemsa methods for SCE, variations and modifications were reported by **Wolff.S and Perry.P** (1974), **Korenberg.J.R** and **Freedlender. E.R** (1975), **Kihlman.B.A** and **Kronborg.D** (1975), **Goto.K, Akematsu.T, Shimazu.Het al** (1975), **Miller.R.C, Aronson.M.M and Nichols.W.W** (1976), **Latt. S.A, Willard.H.F and Gerald.P.S** (1976), **Scheid.W** (1976), **Sasaki.M** (1976), **Schvartzman.J.B and Cortes.F** (1977), **Takayama.S and Sakanishi.S** (1977). This indicates that the technical problems have not been solved.<sup>18</sup>

**Goto.K, Maeda.S, Kano.Y, et al.**, (1978) stated that photolysis of BrdU substituted DNA was considered as the basic mechanism of the Giemsa methods where the photosensitive Hoechst 33258 played a role as sensitizer.<sup>18</sup>

### **AGE /SEX CORRELATION WITH SCE**

**Galloway.S.M and Evans.H.J (1975), Morgan.W.F and Crossenn.P.E (1977), Hollander.D.H, Tockman.M.S, Liang.Y.W et al., (1978), Schneider.E.L, Kram.D, Nakanishi.Y et al., (1979) and Hender.K, Hogstedt.B, Kolnig.A.M et al., (1982)** have reported that donor's age did not affect the baseline frequency of SCE.<sup>36</sup>

**Carrano.A.V and Moore.D.H (1982)** showed no sex difference in the mean frequency of SCEs.<sup>37</sup>

**Sarto.F, Faccioli.M.C, Cominato.I et al., (1985) showed that the frequency of SCE increases linearly with age and that smoking enhances the frequency of SCE independent of age and sex.**<sup>47</sup>

**Tice and Setlow (1985)** stated that theory of aging involving DNA integrity is that the cells from older members of a species should exhibit increased levels of damaged DNA, possibly accompanied by a decreased efficiency in the repair of induced damage.<sup>11</sup>

**Margolin.B.H and Shelby.M.D (1985) have reported the frequency of SCEs to be significantly higher in females than in males.**<sup>3</sup>

**Murthy.V.V.V.S, Mitra A.B, Luthra.U.K et al., (1986)** stated that the relationship between age and SCE did not show any correlation in controls and oral dysplasia cases.<sup>36</sup>

**Nagaya.T and Toriumi.H (1986)** showed that there is no significant correlation found between spontaneous SCE and age of the patient.<sup>37</sup>

**Dewdney .R.S, Lovell.D.P, Jenkinson.P.C et al., (1986) have reported the frequency of SCEs to be significantly higher in females than in males.**<sup>42</sup>

**Bender.M.A, Preston.R.J, Leonard.R.C, et al., (1988)** showed that the frequency was statistically higher in the females than in the males regardless of the smoking habit. And also showed that age of the subject did not significantly influence SCE frequencies.<sup>3</sup>

**Wulf.H.C and Niebuhr.E** (1985), **Wulf.H.C and Kousgaard.N** (1986) also showed that a mean frequency is statistically higher in females than in males.<sup>42</sup>

**Park.E.H, Kim.Y.J, Byan D.H et al.,** (1992) showed that a mean frequency is statistically higher in females than in males in Korean population.<sup>42</sup>

**Lazutka J.R and Dedonyte.V** (1994) observed a higher SCE frequencies in females and also increased frequencies with increasing age and smoking intensity.<sup>30</sup>

**GangulyB.B** (1995) stated that his study showed a small, nonsignificant increase in SCE frequency in females than in males.<sup>11</sup>

### **SCE AND HABITS(TOBACCO,PAN,BETEL CHEWING, SMOKING AND ALCOHOLICS)**

**Obe.G, Gobel.D, Engeln.H, et al.,** (1980) stated that alcoholics as well as heavy cigarette smokers have an elevated frequency of exchange aberrations of chromosome.<sup>39</sup>

**Butler.M.G and SangeW.G and Veomett.G.E** (1981) stated that the years of alcohol abuse were not considered significant in increasing the frequency of SCEs and SCE frequency does not continually increase with the years of alcohol consumption.<sup>5</sup>

**Lambert, Bredberg.A, Mckenzie.W et al.,** (1982) observed increase in frequency of SCE in peripheral lymphocytes of human subjects exposed *invivo* to certain genotoxic agents. E.g. Cigarette smoking and certain anti cancer chemotherapeutic agents.<sup>29</sup>

**Panigrahi.G.B and Rao.A.R** (1982) stated that Arecoline, the major areca nut alkaloid and areca nut extracts increases SCE.<sup>1</sup>

**Livingston.G.K and Fineman. R.M.** (1983) stated that cigarette smokers showed a significantly higher number of SCEs than observed in the non-smokers and also stated that SCE level in smokers is dose related.<sup>31</sup>

**Reibe.M and Westphal.K** (1983) suggested that nicotine, which is a major tobacco alkaloid, is by itself a weak clastogen, which induces SCE in Chinese Hamster ovary cells.<sup>1</sup>

**Ghosh.R and Ghosh.P.K** (1984) stated that higher frequencies of SCE were observed in individuals who chewed more than 10 betel leaves or betel leaves with tobacco, per day, compared to the people who chewed less than 10 betel leaves or betel leaves with tobacco per day respectively.<sup>14</sup>

**Pursiainen.K.H, Sorsa.M, Jarventears.H et al.**, (1984) stated that the influence of SCEs were significantly higher in group of smokers than in group of non-smokers and observed increase in SCE frequency which correlated with years of smoking measured as cumulative pack years.<sup>44</sup>

**Sarto.F, Faccioli.M.C, Cominato.I et al.**, (1985) stated that smoking enhances the frequency of SCE independent of age and sex.<sup>47</sup>

**Adhvaryu S.G, Bhatt.R,G, Dayal .P.K et al.**, (1986) reported that Arecoline , a major betel nut alkaloid has been reported to have genotoxic effects and to induce increased SCE rates.<sup>1</sup>

**Ghosh.R and Ghosh.P.K** (1987) reported that smokers of bidi has more carcinogenic activity compared with the smokers of cigarettes because of the higher content of several toxic agents.<sup>15</sup>

**Pursiainen .K.H** (1987) stated no significant increase was observed in lymphocytes and SCE level in the group of non smokers with a long term passive exposure to tobacco smoke and also stated that mean SCE frequency of cigarette smokers was significantly increased compared with both mean SCE of passive smokers and that of non exposed non smokers.<sup>45</sup>

**Sarto. F, Mustari. L, Mazzott.D *et al*** (1987) showed a decrease in SCE in ex-smokers is rather rapid during first days after stopping smoking and much slower from from 78<sup>th</sup> to 233<sup>th</sup> day.<sup>48</sup>

**Ghosh.R, Sharma.J.K and Ghosh.P.K** (1988) stated that an increase in frequency of SCEs was observed in patients with oral leukoplakia, which may be attributed to their addiction to tobacco habits and they also suggested that combined habits of tobacco smoking and chewing induces more chromosomal instability as compared to the single habit of betel with tobacco chewing or tobacco smoking alone and they also put forth a fact that chewing and smoking of tobacco have a synergistic effect.<sup>12</sup>

**Ghosh.PK. and Ghosh.R (1988) suggested that increased frequency of SCE in betel chewing normal women, pregnant women and women using oral contraceptives indicates that betel induces increased SCE.**<sup>13</sup>

**Kelsey.K.T, Wienke.J.K, Little.F.F *et al.*,** (1988) showed increased SCE frequency as an effect of smoking and also showed that chronic solvent exposure in painters is not associated with an elevation in SCE in peripheral blood lymphocytes.<sup>26</sup>

**Sorsa.M, Pursiainen.K.H and Jarventaus.H** (1989) stated that tobacco smoke is highly genotoxic and produces chromosomal damage in several experimental systems. Active smokers have been shown to have an increased prevalence of somatic chromosome damage in their peripheral blood lymphocytes and this is seen in most cases as an increased SCE frequency.<sup>51</sup>

**Ghosh P.K, Madhavi.R and Guntur.M** (1990) observed an increase in the frequency of SCE in patients with oral sub mucous fibrosis and attributed it to their addiction to tobacco habits.<sup>16</sup>

**Gorgels. W.J.M.J, Poppel. G.V, Jarvel.M.J *et al.***, (1992) stated that results of SCE in their experiments are in accordance with observations that the increased in cancer risk due to passive smoking is small in comparison with increase risk due to active smoking and also stated that the SCE test may be too insensitive to be useful for evaluation of possible cytogenetic effects related to passive smoking.<sup>17</sup>

**Ozkul.Y, Erememisoglu.A, Cucer.N *et al.***, (1995) stated that average SCE per metaphase and total SCEs increased significantly among both smokeless tobacco users and smokers compared to non-smokers. Effect is significantly lower in smokeless tobacco users than in smokers.<sup>40</sup>

### **SCEs IN MALIGNANT AND PREMALIGNANT CONDITIONS**

**Slavutsky.I, Vinuese. M.L, Larripa.I *et al.***, (1984) concluded with their results that the patients with malignant lymphomas present an increased SCE level possibly related to a high chromosomal instability.<sup>49</sup>

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

**Palmer.R.G, Dore.C.J and Denman.A.M** (1986) reported that SCE frequencies were elevated in patients with Bechets syndromes and Systemic lupus erythematosus but normal in other connective tissue disorders.<sup>41</sup>

**Murthy. M.K, Bhargava.M.K and Augustus.M** (1997) reported that oral cancer patients who were habituated to alcohol in addition to the tobacco habit showed a significantly increased frequencies of SCEs compared to the normal controls and a non significant difference when compared to tobacco using patients.<sup>34</sup>

## **POST TREATMENT PATTERNS OF SCE**

**Livingston.G.K and Fineman.R.M** (1983) observed no significant differences between the SCE frequencies of non-smokers and ex-smokers who quit smoking on average of 15 years previously.<sup>31</sup>

**Manoharan.K and Banerjee.M.R** (1985) from their findings stated that  $\beta$ -Carotene by the virtue of its efficient quenching of singlet oxygen and free radical trapping abilities may reduce the cellular accumulation of electrophilic reactants derived from carcinogens and thereby reduce the SCE frequency.<sup>32</sup>

**Duthie.S.J, Auguo.M.A, Marison.A et al.,** (1990) stated a highly significant effect of long term antioxidant supplementation on endogenous and exogenous oxidative DNA damage in lymphocytes which supports the hypothesis that dietary antioxidants may protect against cancer.<sup>9</sup>

**Illeni.M.T, Rovini.D, Grassi.C et al.,** (1991) observed slightly higher SCE levels than their clinically normal relatives in ten melanoma patients.<sup>21</sup>

## **OTHER FACTORS INFLUENCING SCE**

**Kurvink.K, Bloomfield.C.D and Cervenka.J** (1978) reported high SCEs in patients with herpes simplex infections, upper respiratory tract infections and infectious hepatitis.<sup>7</sup>

**Sasaki.M.S (1980) and Sugimoto.Y, Higurashi.M, .Illama.K et al.,** (1982) showed that mutation induced SCE rates to be significantly higher in the cells of the patients with Ataxia Telangiectasia and in Down syndrome.<sup>1</sup>

**Lambert, Bredberg.A, Mckenzie.W, et al.,** (1982) observed a small but significant increase in SCE frequency of the workers in the rubber industry,

laboratory technicians, nurses handling cytostatic drugs, personnel exposed to styrene and vinyl chloride.<sup>29</sup>

**Livingston. G.K and Fineman.R.M.** (1983) showed that women with breast cancer have a significantly higher mean SCE frequency than control women or women having greater than 5 year post treatment for breast cancer.<sup>31</sup>

**Husum. B, Valentin.N, Wulf.H.C *et al.*,** (1985) showed that SCE in lymphocytes of cigarette smokers were unchanged after both general anesthesia and subarachnoid analgesia and that there was no indication of mutagenic action of halothane, isoflurane and nitrous oxide.<sup>20</sup>

**Kyrkanidou.E.B, Garas.J, Angelopoulos.A.P *et al.*,** (1986) found the correlation between the mean number of SCEs in patient's lymphocytes and the greatest diameter of the tumor and stated that the correlation was statistically significant.<sup>27</sup>

**Frentz.G, Wulf.H.C, Peterson,B.M *et al.*,** (1987) were unable to detect correlations of SCE and UV-radiation induced DNA synthesis, UV radiation tolerance or rate of X-ray damage repair. This suggests that the molecular mechanisms involved in SCE induction and in repair of radiation damage were basically independent.<sup>10</sup>

**Ghosh.P.K, and Ghosh.R** (1988) observed an increased frequency of spontaneous SCE in betel chewing pregnant women and women using oral contraceptives.<sup>13</sup>

**Tohida.H and Oikawa.A** (1988) showed that cells of some excision proficient Xeroderma pigmentosum cell lines are highly sensitive to post Ultraviolet caffeine treatment in terms of SCE induction.<sup>54</sup>

**Nohutcu.R.M, Emre.S, Sakizli.M *et al.*,** (1991) stated that elevated SCE in peripheral lymphocytes supports the idea that high SCE frequencies may

indicate cancer susceptibility in some individuals and those patients should be followed carefully.<sup>38</sup>

**Sardas.S, Karahalil.B, Akyo.D *et al.***, (1995) stated that the presence of DNA adducts in fetal tissue is indicative of potential genomic damage which may result in increased risk for the development of serious diseases such as cancer in childhood or in later life.<sup>46</sup>

**Vijaylakshmi, Reiter.R.J, Leal B.Z, *et al.***, (1996) showed that the lymphocytes treated with mitomycin C demonstrated the expected decrease in mitotic and proliferation indices and an increase in SCE.<sup>57</sup>

**Kang.M.H, Genser.D and Elmadfa.I** (1997) suggested that the increased SCE frequency may be related to generation of oxygen derived free radicals by phagocytic cells of patients with Chronn's disease.<sup>25</sup>

**Murthy.M.K, Bargava.M.K and Augustus.M** (1997) stated that an increase in the mean frequency of SCEs for each group of cancer patients was proportional to the clinical stage of the disease and suggested that it could be due to the biological progression of the disease.<sup>34</sup>

**Wong.R.H, Wang.J.D, Hsieh.L.L *et al.***, (1998) stated that exposure of vinyl chloride monomer were significantly associated with increased SCE frequency.<sup>59</sup>

**Sonmez.S, Kaya.M, Aktas.A.*et al.***, (1998) stated that abnormal scores of SCE were found in a group of patients with connective tissue disorders including Bechets disease and also demonstrated increased SCE frequencies in 15 patients with ankylosing spondilities which is an immunogenetically based multifactorial rheumatic disease.<sup>50</sup>

**Dominguez.I, Daza.P, Natarasjan.A.T *et al.***, (1998) reported defects in enzymes involved in DNA replication and repair in both Bloom's syndrome and proposed that lifetime breaks or gaps produced by X-rays or BrdU-

substituted DNA may play a role for production of unstable aberrations, SCEs or translocation.<sup>8</sup>

**Muro.A.D, Sorrentino.C, Piano.L.D *et al.***, (2000) showed results, which suggested that environment, may influence the potential mutagenicity.<sup>33</sup>

**Joseph.S and Gadhia.P.K** (2000) suggested a significant increase in the frequencies of chromosomal aberrations and SCE in one of the village population exposed to a fluoride concentration higher than the permissible limit.<sup>24</sup>

### **SUMMARY AND CONCLUSION:**

- A total number of thirty patients were taken for this study.
- All were males in the age group of 25 –50, comprising of 10 cases of pan chewers without any lesion, 10 cases of OSF patients and 10 cases as controls.
- Details of chewing habit were recorded in all cases.
- Peripheral lymphocytes were cultured for SCE assay.
- 25 metaphase were studied for each case and SCE recorded.
- SCE/cell were increased in OSF patients and pan chewers when compared to normal controls.
- The present study showed increased genomic damage in case of pan chewers and OSF patients (with the habit of chewing pan-parag) as assessed by SCE assay.
- The genomic damage is more in cases of OSF patients than pan chewers as reflected by the increased SCE/cell.

- The quantity, frequency, duration, intensity of the pan chewing habit plays an important role in the initiation and progression of the disease as observed by increased SCE/cell.
- SCE is a sensitive biomarker that could be utilized to evaluate the progression of not only OSF but also other precancerous lesions.

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