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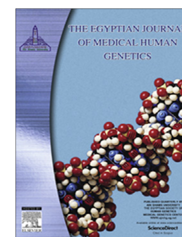

ORIGINAL ARTICLE

Cytogenetical analysis in blood lymphocytes of cigarette smokers in Tiruchirappalli district, Tamil Nadu, India

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KEYWORDS

Chromatid aberration;
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Comet assay;
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Abstract *Background:* Tobacco smoke causes serious health ill effects to human population. Cancer and cardiovascular diseases are more common in smoking subjects.

Aim: The present study is focused about the genetical changes in smoking subjects based on their age and pack years.

Subjects and methods: Based on a survey report, 160 subjects are selected from Tiruchirappalli district, Tamil Nadu, India. Venous blood and buccal smear samples are collected from each subject.

Results: Increased CA is observed in heavy smokers compared to light and non smokers which is 8.90 ± 2.58, 4.58 ± 2.36 and 4.31 ± 1.17, respectively. Both medium and light smokers showed significantly increased CA frequencies than control. Comet assay showed increased percentage of abnormalities in smokers (light, medium and heavy) than non-smokers.

Conclusion: The frequencies of MN in buccal epithelial and blood lymphocytes are high in smokers; particularly heavy smoker group showed significantly increased results. Among them, the lymphocytic cells showed high MN frequency.

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Abbreviations: %TL, percentage of tail length; ANOVA, analysis of variance; CAs, chromosome aberrations; CBMN, cytokinesis-block micronuclei; CTAs, chromatid type aberrations; DNA, deoxyribonucleic acid; DSB, double strand break; EDTA, ethylene diamine tetra acetic acid; PAHs, polycyclic aromatic hydrocarbons; KCl, potassium chloride; MN, micronuclei; MTL, mean tail length; MTM, mean tail movement; NaCl, sodium chloride; PBLs, peripheral blood lymphocytes; SCGE, single cell gel electrophoresis; SPSS, statistical package for social studies; TCA, total chromosome aberrations

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1. Introduction

Smoking is a major cause for cancer, cardiovascular diseases and chronic obstructive pulmonary diseases ([1,2]). Cigarette smoke constitutes approximately 5311 chemical compounds [3] including over 50 known carcinogens such as polycyclic aromatic hydrocarbons (PAHs), *N*-nitrosamines, aromatic amines, and trace metals [4], which act as important mutagenic factors which cause damages to human genetic material [5].

Tobacco smoke induces an array of genetic aberrations including gene mutations, chromosome aberrations (CAs), sister chromatid exchanges and DNA strand breaks [6]. Chromosome aberrations and micronuclei (MN) frequency studies are considered as cytogenetic endpoints and act as sensitive parameters for assessing genotoxic effects of chemical or physical mutagens [7]. Chromosomal aberrations are the important biological consequences of human exposure [8] having a firm place in screening strategies for mutagenic/carcinogenic agents ([9,10]).

Structural chromosomal aberrations in peripheral blood lymphocytes (PBLs) have been applied as a biomarker of early effects of genotoxic carcinogens [11] and usually divided into chromosome-type aberrations (CSAs) and chromatid-type aberrations (CTAs), with different mechanisms of formation [12]. CA studies also act as a biomarker of health outcome which measures the genetic damage due to exposure of various mutagens and probably the only one which has been internationally standardized and validated ([11,13]). DNA double strand breaks (DSBs) are the principal lesions in the process of CA formation ([14,15]).

Micronuclei (MN) are small, extranuclear bodies that arise in dividing cells from acentric chromosome/chromatid fragments or whole chromosomes/chromatids. It is primarily formed due to chromosome segregation machinery defects such as deficiencies in the cell cycle controlling genes, failure of the mitotic spindle, kinetochore or other parts of the mitotic apparatus or by damage to chromosomal substructures and mechanical disruption [11].

Comet assay or single cell gel electrophoresis (SCGE) is a rapid, simple, and sensitive technique for measuring and analyzing DNA breakage in individual cells ([16,17]) and extensively used in human biomonitoring [18]. In Comet assay, the intensity of DNA damage is assessed by computing the tail moment by three tail parameters (tail length, tail intensity and tail moment) [19]. The present study is framed to ascertain the cytogenetical damages in buccal epithelial cells and peripheral blood lymphocytes by evaluating the frequencies of CAs, CTs and MN due to the inhalation of tobacco (cigarette) smoke in the smokers.

2. Subjects and methods

2.1. Subject selection and sample collection

The subjects ($n = 80$) are selected based on pedigree analysis, smoking duration and an initial short survey in self report format which integrated age, duration of cigarette smoking, hereditary problem, medical status and facts about their profession. Subjects are the local residents of Tiruchirappalli district in Tamil Nadu, India. The foremost inclusion criteria in the present study embrace the analysis of pack years (No. of packs of cig. smoked/day × duration of cigarette smoking in years) [20], life style factors (alcohol intake and smoking) and age. The exclusion criteria included the elimination of subjects from viral infection, occupational history, exposure to radiation and chemicals, surgery, chemotherapy, autoimmune diseases, immunology, and genetic disorders. All the controls (non-smokers) are physically and mentally normal subjects who had no history of any genetical disorders.

The study is conducted according to the Institutional Human Ethical clearance and Helsinki [21] procedure. Informed consents are obtained from both smoker and non-smoker subjects. The smokers (exposed subjects) and the non-smokers (controls) are divided into two groups based on their age (Group I: 20–33 years, Group II: 34–50 years). Based on their comparison of age and pack years, smokers are classified into three groups as light smokers ($\leqslant$15 years), medium smokers (16–25 years) and heavy smokers ($>$25 years). Venous blood (5 ml) samples are collected [22] and transferred into an EDTA containers from each subject (separately).

2.2. Micronuclei assay in buccal epithelial cells

The Subjects are instructed to wash their mouth with sterile water. Buccal cells are collected by gentle scrapping of wooden spatula on their cheek. The spatula is stored in saline and centrifuged at 8000 rpm for 5 min. The cell pellet are collected and fixed in methanol:acetic acid (3:1) solution. The fixed cells onto a slide are air dried and stained with Felugen: Fast Green stain and observed under Leica Microscope for MN. For each sample 1000 cells are scored according to the criteria described [23].

2.3. Cytokinesis-block micronuclei (CBMN) assay

Lymphocyte cultures are set up according to the following standard method [24]. At 44 h, Cytochalasin B (6 μg/ml) is added to the lymphocyte cultures. At the end of incubation time (72 h), cells are harvested by centrifugation and hypotonic solution (0.075 M KCl) is added then left undisturbed for a minute. The cells are transferred into the slides and fixed in Carnoy's fixative (methanol:acetic acid, 3:1) and stained. About 1000 cells are scored from each subject.

2.4. Chromosome aberration assay

0.5 ml whole blood is added to 4.5 ml RPMI 1640 medium and then incubated at 37 °C. At the end of 71 h, 0.0l g/ml colcemid is added to block cells in mitosis. Lymphocytes are harvested at 72 h by centrifuging cell culture medium at 800–1000 rpm for 7 min and adding hypotonic solution (KCl 0.075 M) at 37 °C for 20 min to swell the cells and fixed with methanol and acetic acid (3:1 v/v) fixative. Cell suspension is put onto slides wetted with ice-cold acetic acid (60%) and dried on a hot plate (56 °C for 2 min). 100 complete metaphase cells of the first cell cycle are evaluated under a microscope (100×) to identify numerical and structural CAs according to the International System for Human Cytogenetic Nomenclature ([25,26]).

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