Original Article

Cytogenetic Study in Lung Carcinoma of North Coastal Andhra Pradesh, India

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ABSTRACT

Tobacco smoking is the principal risk factor for the cause of lung cancer in South Asian countries. In India, males are more prone to risk than the females. Besides smoking, occupational exposure to industrialization and second-hand smoking are of minor causes. The change in cell metabolism and its function due to exposure of carcinogens that lead to structural abnormalities which are known as chromosomal aberrations. The present study is focused on the cytotoxicity and genotoxicity of the cells in lung cancer patients. Micronucleus assay (MN) was performed to assess the genotoxicity and cytotoxicity of the cells damaged in lung cancer cases and compared it with the control group. A sample size of 20 cases and 20 controls were taken. Males showed statistically high risk towards DNA damage than females. The mean age in the study is 50.5±13.642. The frequency of chromosomal aberrations showed significant in cases than the control groups.

Introduction

Cancer is defined as the abnormal cells without control over the cell division and its multiplication to divide. These cells have the capability to invade to other parts of the organ destroying the metabolism and immune system of the body. In lung cancer the disease appears in the tissues of the lungs, usually in the cells lining the small air passages know as bronchioles [1]. It is categorized into two groups based on the diagnosis: small cell lung cancer (SCLS) which constitutes 20-25% of lung cancers and non-smaller cell lung cancer (NSCLS) accounting a total of 85%. Squamous cell carcinoma (30%), adenocarcinoma (35%) and large cell lung cancer (15%) are subtypes of NSCLS.

In the early twentieth century, the disease lung cancer sounds rare. According to the current scenario the estimated incidence of lung cancer in India unabated to 70,275 of all ages and in both sexes and the males [2]. From the GLOBOCAN report 2012, the rough incidence rate per 100,000 was 5.6 for both sexes, the age-standardized rate per 100,000 worldwide was 6.9 and the increased risk was estimated to be 0.85. Lung cancer was ranked fourth among all other cancers globally. In the cancer populace worldwide, males affected with lung cancer are ranked second place and females are positioned 6th. For every year the new cases registered worldwide is of 1.6 million whereas in India a near value of 63,000 cases were reported [3].

Smoking tobacco or cigarette smoke is one of the major risk factor being the sole reason for 80-90% of lung cancers. Cigarette smoke is a composite aerosol, weighed by more than 4,000 chemical compounds: 95% of it constitutes 400-500 varied gaseous components and 3,500 particulate compounds [4]. The smoke consists of mainstream components (including potential carcinogens as of like polycyclic aromatic hydrocarbons, aromatic amines, N-nitrosamines, benzene, vinyl chloride, arsenic, chromium and many more) and side-stream smoke components. IARC (The International Agency for Research Centre) identified ~50 carcinogens in tobacco smoke [5, 6]. From different studies in tobacco smoke, it was shown that radioactive materials, such as radon, bismuth and polonium were present. Nearly, 80% of women in South Asia affected by lung cancer are due to never smokers [7]. In the western countries, smokers to non-smoker’s ratio is higher than in India [2]. Many studies on candidate gene analysis, GWAS (Genome wide-association studies) and SNPs (Single Nucleotide Polymorphisms) have been reported revealing an evidence of family history of lung cancer. Besides these, age, gender, and other epidemiological factors play a key role as a causative for lung cancer.

Chromosomal aberrations (CAs) can be a structural abnormality or the numerical abnormality of micronuclei. CAs findings in peripheral blood lymphocytes (PBL) have been functional for over 30years in occupational and environmental exposure as abiomarker for early detection of genotoxic carcinogens [8, 9]. The structural abnormalities of CAs include chromosomal breaks, gaps and...
pellet 2-3ml of pre-warmed hypotonic solution (0.75M KCL) was added and incubated for 10 min at 37°C. The tubes are centrifuged at 1200 rpm for 10min. To the pellet pre-chilled fixative (3:1 ratio of methanol and acetic acid) was added a side to the walls of the tubes to avoid clumping of the cells. The tubes were mixed thoroughly and allowed to stand for 2-3min and centrifuged later. The process with fixative is repeated 3-4times until white pellet is obtained. The final cell suspension was made up of 1ml fresh fixative. The above obtained cell suspension is stored at 4°C for further use.

Slide preparation:

Two drops of cell suspension were dropped on the clean microslide with the help of a dropper. The slides were heated on a hot plate for 1-2min. The slides were dipped into 2% Giemsafor about 5 min.

Results and discussion:

Karyotypic changes normally are extensive with many numerical and structural changes which are often near-triploid [13]. Prominent numerical changes include loss of chromosomes. For every individual sample, 50 fine spread metaphases were screened. The study showed significantly high frequency of numerical abnormalities and structural aberrations of chromosomes in lung cancer patients than the controls. The resulted chromosomal aberrations and the frequency of MN assay are illustrated in Table 1 and Table 2 respectively.

Statistical analysis was done using SPSS software. In the present study, 52.4% of male and 42.5% of female lung cancer patient were involved. 84.2% were smokers. For the patients tested for micronuclei assay, 1000 metaphases were identified for 20 samples out of which 1.55±0.51 showed gaps in the chromosomes with a frequency of 42.9%, 1.7±0.47 breaks with a frequency of 28.6% and dicentric chromosomes being 1.8±0.41 by a frequency of 19%. In the test controls, same numbers of metaphases were observed but the chromosomal aberration varied with that of the patients showed 15% gapped chromosomes with 1.85±0.33, chromosomal breaks being 1.8±0.41 with the frequency of 20% and 5% (1.95±0.22) dicentric chromosomes. The different chromosomal aberrations and micronuclei cells were shown in Figure 1, 2, 3 and 4.

The proportion of mononucleated, dinucleated and trinucleated per 500 cells each were shown to have 19%, 31.8%, and 23.8% respectively with mean±SD 1.8±0.41, 1.06±0.50 and 1.75±0.44 significantly higher than the controls were the dinucleated cells to be 20%. The absence of mono and trinucleated cells in the controls showed statistically significant for cancer group. Similar studies showed highly significant proportion in the cases than the controls [14]. The cells with neoplasmic bridges were 57.1%. 33.3% and 30% respectively with mean±SD 1.8±0.41, 1.06±0.50 and 1.75±0.44 significantly higher than the controls showed highly significant proportions of nuclear buds in the diseased patients and the controls correspondingly.

From the above results, it is shown that lung cancer people with a habit of smoking and occupational exposure to environmental smoke or industrial gases are at risk for chromosomal aberrations than the normal healthy groups.
Table 1: Assessment of chromosomal aberrations in patients and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Number of Metaphase</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Gap</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Breaks</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Dicentric</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total no. of chromosomal aberrations</td>
<td>18</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2: Frequency of micronuclei in patients and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Number of cells</td>
<td>20000</td>
<td>20000</td>
</tr>
<tr>
<td>Mononucleated cell with micronuclei</td>
<td>4</td>
<td>--</td>
</tr>
<tr>
<td>Binucleated</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Trinucleated</td>
<td>4</td>
<td>--</td>
</tr>
<tr>
<td>Cells with Neoplastic Bridges</td>
<td>6</td>
<td>--</td>
</tr>
<tr>
<td>Nuclear bud Cells</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Total abnormalities</td>
<td>33</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure 1: Picture depicts the micronucleus division: into A) Mononucleated cell B) Binucleated cells C) Trinucleated cells at the metaphase stage.

Figure 3(b): As shown in the figure pointed arrow is the metaphase chromosome with gaps and breaks.

Figure 3(c): An arrow in the picture indicates the arrested metaphase chromosome from the cases with dicentric chromosomal structures.

Conclusion:

The mankind is experiencing a high risk of exposure to the environmental pollutant like pesticides, chemical contact and occupational exposure to tobacco smoking which is of far above the ground and leading cause for lung cancer. DNA adducts, the metabolites of smoke carcinogens bound covalently with DNA, are regarded as an indicator of cancer risk in smokers. Cytogenetic analysis has helped to unravel the clinical implication of chromosomal changes and chromosomal damage in lung and other cancers. The work carried out showed the risk of smoking and occupational exposure to smoke as a major factor for lung cancer. Further study implications with large sample size and advanced methods in detecting chromosomal aberrations at early stages of the disease must come forth in order to provide and prevent the risk of cancer in near future.
References:


