NUCLEAR ANOMALIES IN EXFOLIATED BUCCAL EPITHELIAL CELLS OF PRALLETHRIN BASED MOSQUITO REPELLENT USERS OF SOUTH INDIA

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ABSTRACT

Cytogenetic damage associated with prallethrin based mosquito repellent use was analyzed by micronuclei (MN) and other nuclear abnormalities (NA) in South Indian human volunteers. The mean MN frequency of exposed subjects showed a significant difference when compared with controls (p<0.05). The presence of other NA such as binucleated cells (BNC), karyorrhexis (KRC) and karyolysis (KLC) were found to be notably higher in exposed individuals (p<0.05). These results show that chronic exposure to prallethrin increases a slight DNA damage. Though the present investigation involving a limited number of human subjects indicates the onset of both protective changes as well as derangement in metabolism, a detailed and rigorous study is greatly warranted to arrive at a definite conclusion about any toxic effects of prallethrin based mosquito repellents.

INTRODUCTION

Man is exposed to a great deal of environmental perils that may affect the functioning of specific biomolecules and thereby damage health at various levels. Potentially hazardous environmental toxicants like pesticides display a broad spectrum of biological effects, being toxic not only to target organisms but also to humans. Usage of pesticides in the ecosystem leads to development of various types of morphological, physiological, biochemical and behavioral changes in individuals [1, 2]. Many have the potential to cause genetic alterations in the target tissues of exposed humans. Such alterations, if they occur in tumor suppressor genes, may lead to the development of cancer in the target organs [3]. Common pesticides used in homes and lawns are now being shown in medical research to accelerate aging of the immune and nervous system resulting in serious health problems years after exposure.

Indoor pyrethroid exposure is of considerable magnitude in India [4-6] due to their high insecticidal activity. Prallethrin, an active ingredient in the liquid vaporizer, is a synthetic pyrethroid and is one among the top few commonly used insecticides having maximal human exposure for prolonged periods as it is used as a chief component of mosquito repellents [7, 8]. In addition to inhalation, slow but significant absorption and accumulation in epidermis [9], these pyrethroids when used in closed and poorly ventilated areas increases the risk of severe toxicity [10]. There is dearth of information concerning the effect on humans due to prolonged and long-term use of mosquito repellents. The exfoliated-cell MN assay is a relatively new cytogenetic technique for monitoring the effects of exposure to carcinogens and mutagens. Micronuclei, which are the primary cytological structures scored in the CBMN assay, are thought to contain chromat (from one or more chromosomes) that was not incorporated (“lagging” or “lost”) into the daughter binucleates following nuclear division [11]. An increased frequency of MN indicates an increased frequency of structural and/or numerical chromosome aberrations [12]. Continuous exposure of humans to pyrethroid-based mosquito repellents for longer durations may lead to adverse health effects. No information is available on long-term use of these mosquito repellents pertaining to the genetic alteration in human subjects.

The primary objective of this study was to evaluate the genotoxicity in human volunteers exposed to pyrethroids, prallethrin based mosquito repellent in terms of nuclear changes by MN assay as important information in conducting risk assessments for humans.

MATERIAL AND METHODS

The study population comprised of 220 individuals of south Indian ethnicity. The subjects was asked the following questions: (a) are you in the practice of mosquito repellent usage and (b) how long (years) have you been using and (c) on average, what was the number of hours of exposure per day. If the answer was ≥5 years of exposure and ≥8 hrs per day exposure to the questions, the subject was considered exposed to repellents. 110 exposed subjects were obtained from the questionnaire and an equal number of controls (unexposed to repellents) were recruited for the study. Among control and exposed subjects, smokers constituted 20.9% whereas alcoholics were 20% and 22.72% respectively (Table1). Individuals exposed to any occupational genotoxic agents were excluded from this study. Participants were informed about the objectives of the study. Individuals satisfying the study criteria were enrolled after obtaining their informed consent and were asked to complete a questionnaire to obtain necessary information on their lifestyle and personal factors (age, working environment, alcohol consumption and smoking habits, health, etc) [13]. The investigation was carried out according to the principles of the Declaration of Helsinki.

Prior to buccal cell collection the exposed and control subjects were advised to rinse their mouth thoroughly with water to remove unwanted debris. Exfoliated buccal cells were obtained by gently rubbing the inside of both cheeks with an extra soft toothbrush for 1 min each. The participant then rinsed the mouth with 20 ml of 0.9% saline and expectorated into a 50 ml conical-based tube (Tube Swube CS200, BD Biosciences, US). The toothbrush was then rinsed in the tube and 30 ml saline was added before the cells were pelleted. The cells were washed with phosphate buffer saline (pH 7.4). Cell suspension of 10 µl was smeared on a microscopic slide and stained with Acridine Orange (Sigma-Aldrich). Slides in triplicate were prepared for each subject. 1000 cells were scored per subject to determine the frequency of the various cell types outlined in the buccal cytome assay, consisted of micronuclei cell (MNC), binucleated cells (BNC), karyorrhectic (KRC), and karyolytic cells (KLC). A total of 1000 differentiated cells were scored in order to determine the frequency of MN. Cells were scored by using light microscope under 40X magnification (Olympus CH20i).

### TABLE 1: Demographic Characteristics of Study

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N (%)</th>
<th>Age Mean ± SD</th>
<th>Smokers</th>
<th>Alcoholics</th>
<th>Duration of exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Per day (Hrs)</td>
<td>Years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>50 (45.45)</td>
<td>33.36 ± 7.03</td>
<td>23 (46)</td>
<td>22 (44)</td>
<td>-</td>
</tr>
<tr>
<td>Women</td>
<td>60 (54.54)</td>
<td>33.65 ± 6.99</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Exposed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>55 (50)</td>
<td>32.42 ± 7.39</td>
<td>23 (41.81)</td>
<td>25 (45.45)</td>
<td>8.68 ± 0.68</td>
</tr>
<tr>
<td>Women</td>
<td>55 (50)</td>
<td>33.82 ± 7.90</td>
<td>-</td>
<td>-</td>
<td>8.71 ± 0.69</td>
</tr>
</tbody>
</table>

The results of the study are expressed as mean ± standard deviation. The synergistic effect between smoking and exposure were tested with a two-way analysis of variance. Multiple comparisons were made by using a least significant difference test. The error rate was accepted as 0.05 by student’s t test. All statistical analysis was performed using the program SPSS 10.0 (SPSS, Chicago, IL) and the SAS system for windows.

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RESULTS

The assessment of cytotoxic effects of prallethrin compounds on humans has been conducted by determining the induction of MN in the buccal cells. Differences in mean MNC among exposed subjects with respect to age were not statistically significant (1.85 ± 0.61 and 5.65 ± 1.76, p > 0.05) analogous to controls. The mean value of MNC in smokers was considerably elevated when compared to non smoker subjects in both groups (3.04 ± 0.87and 5.72 ± 1.69 Vs 1.87 ± 0.81and 1.87 ± 0.81, p < 0.05). Correspondingly, MNC frequency was significantly higher in subjects consuming alcohol in comparison with non alcoholics (5.77 ± 1.77 and 4.13 ± 1.35). In addition to this, both control and exposed shows an increased level of all nuclear abnormalities (MNC, BNC, KRC and KLC) in males than the female subjects (p < 0.05) with respect to age, smoking, alcohol consumption and duration of exposure. None of the females smoked or chewed tobacco and all female subjects are non-smokers, because culturally Indian women do not smoke or consume alcohol. This could be a supportive factor for the low frequency of nuclear abnormalities in women than men (Table 2). There was a significant difference between exposed workers and control groups as regards MNC, BNC, KRC and KLC (p < 0.05). Among the three nuclear anomalies; KLC was found to be more predominant.

<table>
<thead>
<tr>
<th>TABLE 2: Cytological Observations in Control and Exposed Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Controls (n=110)</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Female(n=60)</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>No (n=87)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
</tr>
<tr>
<td>No (n=88)</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Female(n=55)</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>No (n=85)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
</tr>
<tr>
<td>No (n=88)</td>
</tr>
<tr>
<td>Years of exposure</td>
</tr>
<tr>
<td>&gt;5 (n=59)</td>
</tr>
</tbody>
</table>

* p < 0.05; MNC- Micronucleated cell, BNC- Binucleated cell, KRC- Karyorhectic cell, KLC- Karyolytic cell

DISCUSSION

MNC are regarded as markers of abnormal mitoses involving chromosomal breakage and missegregated chromatin [14]. The MN in exfoliated buccal epithelial cells is useful biomarkers of exposure to genotoxic chemicals and reflects genotoxic events that occurred in the dividing basal cell layer one to three weeks earlier. Different laboratories have reported variable normal background MNC frequency in human buccal epithelial cells: 0.04% [15], 0.16% [16], 0.1-0.3% [17] and 0.33% [18]. The MN assay is regarded as an important biomarker to predict the relative risk of cancer in upper aero-digestive tract [19].

Cigarette smoking is one of the factors that may influence the rate of cytogenetic damage, such as MNC in humans. It has been reported that cigarette smoking significantly increases the frequencies of MNC and other nuclear abnormalities in both controls and exposed subjects [20-22]. Increase in MNC frequency was reported in fire fighters [23], gas station workers [24], welders [25], beedi smokers [26] and foundry workers [27]. Increase in exposure to toxic chemicals induces a significant increase in the buccal MNC [28, 29]. During the malignant process, the path to carcinogenesis appears to be correlated with increasing MNC frequencies. This idea is supported by quite a few preceding investigations [30-33].

Studies on mice exposed to pyrethroid based mosquito coil smoke reported histopathological lesions, including the loss of cilia and an increase in vascularity of the alveolar wall [34]. An exposure of rats to mosquito coil smoke for 60 days resulted in focal deciliation of the tracheal epithelium, metaplasia of epithelial cells and morphological alterations of the alveolar macrophages [35]. Prallethrin induced biochemical changes in erythrocyte membrane and red cell osmotic hemolysis in human volunteers and in wistar rats was reported earlier [36, 37].

A higher frequency of MNC, BNC, KRC and KLC was observed in the buccal cells of study subjects, probably due to the genotoxic effect of pyrethroid derivatives to which they are exposed. Our findings assist in understanding the impact of synthetic pyrethroids on health and environmental safety. Considering the wide spectrum of synthetic pyrethroids, a more comprehensive understanding of the negative effects is indispensable for planning future application and regulation of these pesticides.
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Authors’ Statement

Competing Interests
The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

REFERENCES


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