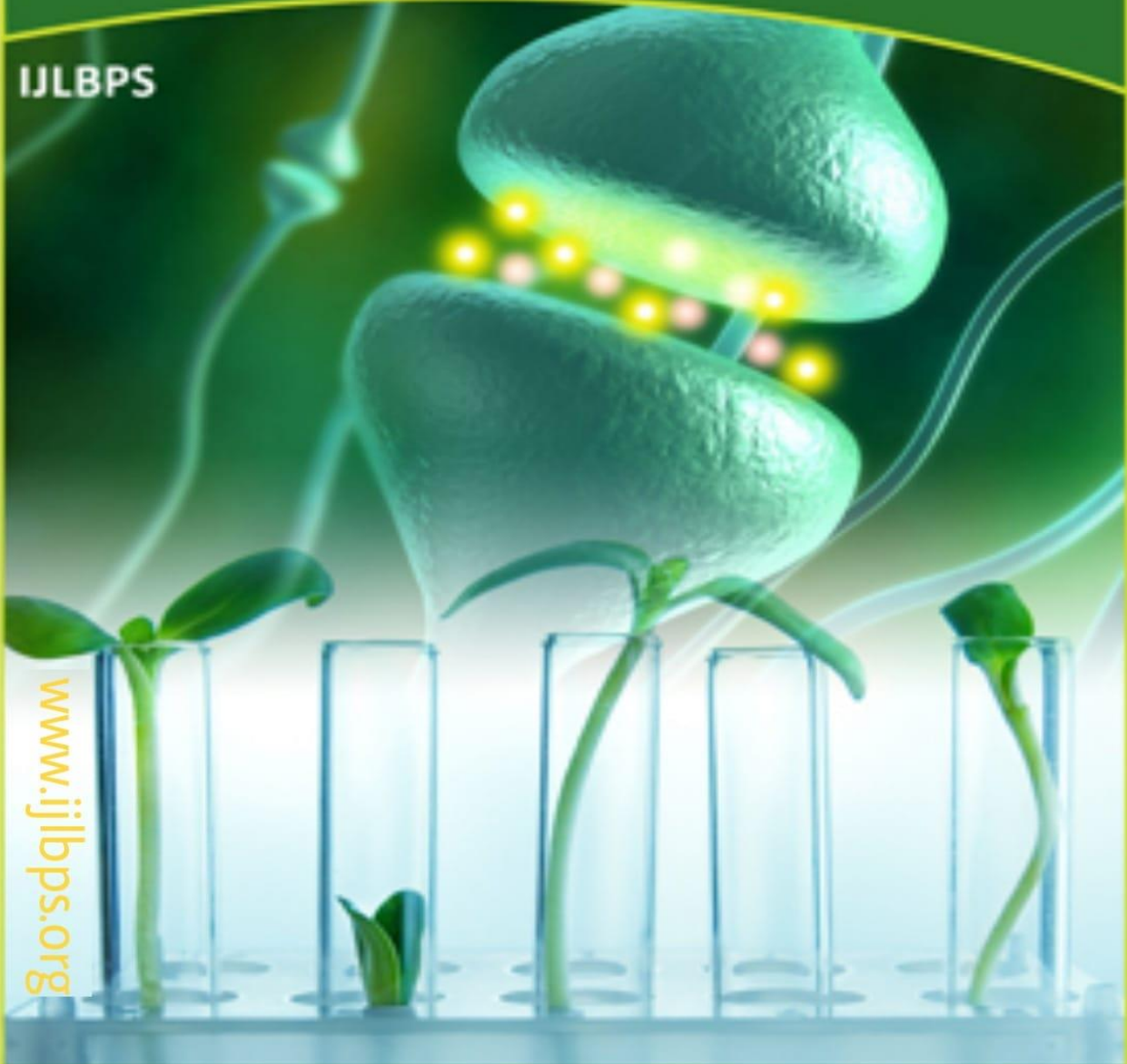




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Elevated frequencies of micronuclei and other nuclear abnormalities  
in buccal epithelial cells of spray painters in South India

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**Abstract**

To evaluate genetic damage in exposed populations, the micronucleus (MN) test is performed on exfoliated buccal cells as part of human biomonitoring. MN test was performed on exfoliated buccal epithelial cells from 94 spray painters and 80 controls to identify genotoxic effects. Two thousand buccal cells were collected by exfoliation from each participant. Participants were classified into subsets according to whether or not they were regular smokers or drinkers. Workers who were exposed had a considerably higher rate of micronucleated cells than controls. When spray painters were compared to controls of the same age, gender, education level, and number of years in the industry, the former were shown to have much higher rates of MN. Important data on cytogenetic damage from spray painting exposure should be used in the risk assessment process, and attention should also be made to the use of protective gear.

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Key-Words: Micronucleus, Occupational exposure, Spray painters, Exfoliated cells, Genotoxicity

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**INTRODUCTION :**

Chemicals that cause mutations, cancer, and birth problems are often inhaled by the residents in industrial regions. Variability, metabolic rate, DNA repair mechanisms, and other variables all contribute to the wide range of reactions that humans have to external exposures.<sup>2</sup> Primary prevention of risks is a major emphasis of occupational health, which is concerned with all aspects of workplace health and safety. Several variables influence worker health, and one of

them is exposure to carcinogens in the job. Organic solvents (aliphatic, aromatic, and chlorinated), metals (lead, chromium, cadmium), and many other substances with possible carcinogenic qualities are often included in complex mixes that painters are exposed to on a daily basis. Three different forms of cancer, including those of the urinary system, skin, larynx, pancreas, and leukemias, may be caused by exposure to substances in the workplace.

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4 Spray painters for automobiles are subjected to a wide variety of chemicals, including ketone-based solvents, aliphatic and aromatic compounds and esters, organic and inorganic pigments, and various resins. Natural Workplace exposure to organic solvents has been linked to an increase in micronuclei in lymphocytes and buccal epithelial cells among those employed in the paint business. Both Silva and Santos-Mello<sup>6</sup>, who found higher rates of aneuploid cells and chromosomal deletions in auto painters, and Fuchs et al.<sup>7</sup>, who found a temporary uptick in DNA strand breaks in auto painters, made similar observations. Biomonitoring studies, in which relevant biological indicators with a short-term manifestation are used, are one approach to studying the impacts on an exposed population; for example, cytogenetic examination may detect changes to DNA or chromosomes as a consequence of exposure. The genotoxic effects of carcinogenic chemicals and carcinogenic combinations at the low concentrations to which human populations are exposed have been measured using the micronucleus (MN) test for exfoliated cells.<sup>8</sup> There is a pressing need to learn more about the dangers that chemicals in the environment pose to human health<sup>9</sup>. Auto repair businesses in South India are often found on busy city streets, putting their employees in danger of pedestrians and passing traffic. In order to get a deeper understanding of the genetic risk on an exposed population, we used the MN and other biomarker for nuclear abnormalities (NA). Our goal was to compare cytogenetic damage in exfoliated buccal cells from spray painters and controls, and to determine whether or not MN frequency is related to non-occupational variables like smoking and alcohol use.

#### The Stuff and How We Did It

##### Subjects

Ninety-four male spray painters and eighty non-exposed men made up the research population. Twenty-one car repair businesses in Coimbatore City's outlying areas were sampled; their exposed

group comprised 28 smokers, 22 non-smokers, 26 alcoholics, and 18 sober individuals. Age- and sex-matched control groups (24 smokers and 18 non-smokers, 21 alcoholics and 17 non-drinkers) were not exposed to harmful chemicals in the workplace. The participants completed an informed consent form at the time of sample collection. Each participant filled out a questionnaire that asked them about their age, profession, smoking history, drug (including alcohol) usage, history of sickness, and receipt of any recent immunizations or x-rays. Healthy volunteers completed a comprehensive questionnaire structured after the one released by the International Commission for the Protection against Environmental Mutagens and Carcinogens.<sup>10</sup> A second survey was taken by those in the exposed group to assess the effectiveness of safety equipment. Workers in this category put in an average of more than 8 hours each day at the office over the course of at least 5 years. There were no discernible variations between the groups in this research when it came to issues like diet, exercise, and mental health. The ethical review board of the participating institution gave its stamp of approval to the research protocols performed here.

#### A Cellular Sample

Each participant gargled with running water to prepare their mouth for sampling. Tolbert and his colleagues developed criteria for collecting buccal cells (BCs) from willing participants at the conclusion of the workday.<sup>11</sup> The patient's mouth was properly washed with water to eliminate any food particles or other debris before the BC was collected. The inside of both cheeks were rubbed with a wooden spatula to collect buccal cell samples. Three milliliters of sterile saline were used to collect the cells.

#### Dissecting microscopic nuclei

After spreading 10 l of buccal mucosal cell suspension on a microscope slide, letting it dry in

the air, and then fixing it in a cold methanol: acetic acid (3:1) solution in 0.1M phosphate buffer (pH 7.5) for 20 minutes, we could see the cells clearly. After that, Feulgen reaction staining was used to colorize the slides.

modification of Belien and coworkers' original method.<sup>12</sup> Staining with new Schiff reagent (Sigma Chem, USA) for 90 minutes, followed by washing in tap water for 15 minutes, and then hydrolysis in 5N HCl for 10 minutes at room temperature. After 2-5 minutes in Coplin jars with 1% Fast Green reagent, the cells were washed with distilled water to remove any excess stain. The slides were examined using a 1000x light microscope (Leitz, Germany). Micronuclei were counted in a minimum of 2,000 cells per person. The slides were shuffled, and a single reader rated them. Normal cells had their micronuclei counted. Binucleates, karyolysis, and the frequency with which eggs were shattered were also noted. Tolbert and coworkers' system was used to categorize MN and other nuclear abnormalities. The following requirements must be met by MN:

Nuclear material, b) detached from the parent nucleus, c) less than a third the diameter of connected nuclei, d) smooth, oval, or circular in form, e) in the same plane of focus, and f) of the same color, texture, and refraction as the primary nucleus. Binucleate cells were those that have two nuclei. In addition to MN, we also kept track of other types of NA, such as Binucleates (BN), broken eggs' (nuclei that emerged constricted), and karyolysis (dissolution of nucleus).

#### Number crunching

During the process of setting up and evaluating the samples, codes were assigned. In order to compare them statistically, they had to be deciphered first. Student's t-test was used to determine whether or not there was a statistically significant difference in means between the control and exposed groups, and Pearson's rank correlation analyses were used to determine the strength of the relationship between the dependent and independent variables. The Mann-

Whitney test was used to compare the MNC, BNC, BEC, and KLC distributions of individuals split into two classes. SPSS was used for all the computations.

SPSS (Chicago, Illinois) version 11.01 statistics program.

#### Discussion and Results

Table 1 displays the demographic information of the research participants. Different groups were created for each person based on their age, duration of employment, whether they smoked cigarettes or chewed tobacco, and if they drank alcohol. Table 2 displays the results of the analysis of the frequency of micronuclei and nuclear abnormalities. When comparing the MN frequencies in exfoliated buccal cells of smokers in the exposed group (12.52 0.21) and smokers in the control group (3.18 1.36) there was a statistically significant difference ( $p < 0.05$ ). The frequency of MN was similarly significantly affected by smoking in the unexposed control group (3.18 vs. 1.43) exposures of different people are being monitored.<sup>24</sup> smokers in each group). Nonsmokers who were exposed to secondhand smoke had an MN frequency of 8.64 1.32, whereas those who were not were only exposed to 1.43 1.08 ( $p < 0.05$ ). Among alcoholics who were exposed to micronuclei, the average frequency was 11.59, whereas among those who were not, it was only 3.96 0.47. In addition to MN, spray painters also had a higher prevalence of other NA compared to controls ( $p < 0.05$ ). Smokers and drinkers are more likely to develop karyolysis than anybody else because to the three NA. Micronucleated cells were found to be significantly higher in smokers than in nonsmokers ( $p < 0.001$ ), as reported by Burgaz et al.<sup>13</sup> Similarly, we found that routine use and occupational exposure had a synergistic impact. In buccal epithelial cells, we found an increase in the prevalence of micronuclei production and nuclear alterations. Smokers and drinkers in both the exposed and control groups had a higher micronucleus frequency compared to those who did not engage in these behaviors. Some people

believe that alcoholic drinks contain mutagenic chemicals.<sup>8</sup> However, in vivo, alcohol induces a wide range of genetic effects, including sister chromatid exchange and the production of micronuclei, for which evidence is currently limited to specific test systems or organisms, as reported by Bishop et al.<sup>14</sup>. Alcohol use, as reported by Dittberner et al.<sup>15</sup>, is associated with an uptick in micronuclei. Several articles have addressed the potential for cytogenetic harm in organic solvent-exposed professions.<sup>5,6,16-18</sup> Concerns regarding the dangers of occupational exposure to solvents have been prompted by their expanding usage and variety. The frequency of MN induction is shown to increase in the alcohol-exposed person in the current study. Analysis of buccal epithelial cells offers information about nuclear abnormalities such as KL (nuclear disintegration), the BE effect (broken-egg structures), and BN (two nuclei inside a cell), thus we utilized them to determine the extent of cytogenetic damage in our research. Early signs of apoptosis include KR and KL.<sup>19,20</sup> Workers exposed to vehicle coatings and painters in general have been shown to have higher levels of chromosomal aberrations (CAs), sister chromatid exchange (SCE), micronuclei (MN) (in lymphocytes and in oral mucosa cells), and DNA damage revealed by the Comet test in leukocytes.<sup>21,22</sup> Our data also show that the buccal cells of the control group, in comparison to the unexposed non-smoking population, have higher rates of cigarette smoking and alcohol use, and community of alcoholics. In populations exposed to genotoxicants by ingestion or inhalation, the prevalence of MN in human exfoliated cells is regarded as a valuable indicator of genotoxic effects.<sup>23</sup>

Health monitoring programs that focus on chemical carcinogens have sparked a lot of interest in the use of genotoxicity biomarkers as techniques for identifying human genotoxic exposure and consequences. Human biomonitoring of genotoxic exposures will benefit from the investigation of relationships between biomarkers, and this will aid in the

selection of suitable biomarkers for more effective

### Conclusion.

The specificity of biomarker assays varies greatly, but most occupational and environmental exposures are really combinations of genotoxic chemicals. Workplace exposure studies should prioritize genotoxicity testing. Guaranteeing environmental endowment and occupational health often benefits from evaluation based on genotoxic characteristics. The current study reveals that exfoliated buccal cells examined by the MN test showed strong evidence of genotoxicity in spray painters under their specific exposure settings. This demonstrates the additive impact of regular use and occupational exposure. Spray painters who were exposed to organic solvents had an increased incidence of not just MN but also BN, BE, and KL. When cells are damaged, anomalies like this tend to increase. This study's findings demonstrate that cytogenetic damage was significantly elevated among the spray painters. There is a possibility that these personnel have been exposed to genotoxic substances without realizing it or knowing the full extent of their exposure. Workers who may be exposed to paint in any capacity need to be informed of the potential genotoxic consequences and given access to a safe and healthy work environment.

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