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Genotoxic Effect of Methyl Methacrylate and Nickel-cobalt-chromium in Dental Lab Technicians: A Micronuclei and Cytomorphometric Study of Buccal Mucosal Cells

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Abstract

Dental laboratory technicians are exposed routinely to non precious metal alloys such as chromium, cobalt, nickel, and methyl methacrylate (MMA). These chemicals are considered potentially toxic substances and are known to cause chromosomal damage and nuclear alterations. Buccal mucosa cells are very sensitive to toxicity as they readily form micronuclei (MN), in response to toxic exposure. Thus, the present study was conducted to evaluate MN frequency and analyze the cellular alterations, if any, using quantitative cytomorphometric analysis in the buccal mucosal cells of dental lab technicians. The study comprised 28 dental lab technicians who were exposed to acrylic (MMA) and metal (nickel, cobalt, and chromium) for a minimum of 5 years with 8h daily exposure. Twenty-eight individuals with no known exposure were selected as control group. For MN frequency, cytosmears were prepared from both study subjects and control group and were stained with Feulgen reaction. Cytosmears were made and stained by Papanicolaou's stain, and cytomorphometric analysis was done using Caliper Pro4.2 Image Analysis Software. The MN frequency and cytoplasmic area (CA)/nuclear area (NA) was compared using one-way ANOVA among dental lab technicians who were categorized into three groups, i.e., exposed only to acrylic (MMA), exposed only to metal (nickel, cobalt, and chromium) and exposed to both metal and acrylic. The mean occurrence of MN frequency was significantly higher ($P < 0.05$) in the study individuals when compared to the control group. When individual exposure was considered, subjects exposed to both acrylic and metals showed an increased mean frequency of MN, which was not statistically significant ($P > 0.05$). Quantitative analysis showed a definite increase in the NA and CA/NA ($P < 0.05$) and decrease in CA ($P < 0.05$) in study subjects when compared to the control group. Comparison of these cellular and nuclear parameters in the three divided groups did not show any significant changes. The findings of the present study signify that exposure to nickel, chromium, cobalt, and MMA induce genotoxic effect in exfoliated buccal epithelial cells, in addition, the significant quantitative cellular alterations are also seen which indicate a potential health risk for dental lab technicians. Therefore, to ensure maximum occupational safety, biomonitoring is of great value for assessing the risk for dental lab technicians.

Keywords: Cytomorphometry, Dental Lab Technicians, Feulgen Reaction, Genotoxicity, Micronuclei

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1. Introduction

Dental laboratory technicians are routinely exposed to non precious metal alloys such as chromium, cobalt, molybdenum, beryllium, nickel, small amounts of gallium, ruthenium, or aluminum which are used in the manufacturing of crowns, bridges, dentures, silica which is used as an abrasive, and methyl methacrylate (MMA) in preparing dentures. All these compounds are considered potentially toxic substances[1] and have been classified as human carcinogens by the International Agency for Research in Cancer (1990). Exposure to these compounds

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may occur via inhalation, local action or by ingestion of salts, solutions, mists, and fumes of these metals.[2]

MMA monomer is the principle ingredient of many dental restorative and prosthetic resins. Regular contact and chronic inhalation of MMA has caused toxic side effects, ranging from allergic contact dermatitis, stomatitis, paresthesia of the finger tips to neuropathy, liver toxicity, hemorrhage, and necrosis of lung tissue.[3]

Chronic exposure to heavy metals may lead to the generation of micronuclei (MN), which are the genetic material fragments not incorporated in the nuclei of daughter cells during mitosis.[2] The two basic phenomena leading to the formation of MN in mitotic cells are chromosome breakage and dysfunction of the mitotic apparatus. MN are formed from a centric chromosome or chromatid fragments and whole chromosome or chromatids that lag behind in anaphase and are left outside the daughter nuclei in telophase.[4]

Epithelial cells of the buccal mucosa form the first barrier to these toxic compounds either inhaled or ingested and are capable of metabolizing their proximate carcinogens to reactive products.[5] Buccal mucosa cells are used as a source, for monitoring human exposure to toxic substances encountered in occupational and environmental settings as the cells are very sensitive to toxicity leading to the formation of MN in response to toxic exposure.[3]

"Micronucleus test" is a technique which determines the chromosomal damage and thus the mutagenic influences on the mucosa.[6] Micronucleus assay could be used to assess chromosome damage as they are examined in routine cytopathological preparations. Their frequency of occurrence is a measure of chromosome breakage in early cell divisions, and the number of MN is known to increase with carcinogenic stimuli, long before the development of clinical symptoms. Thus, micronucleus in a cell represents an "internal dosimeter" to estimate exposure to genotoxic and carcinogenic agents.[7]

Exfoliated buccal cells from the oral cavity of carcinogen-exposed individuals have demonstrated increased frequencies of MN.[8] Since there are no studies

done to evaluate MN frequency using Feulgen reaction in the buccal mucosal cells of dental lab technicians, this present study was undertaken to determine the extent of genotoxic damage in relation to MMA and nickel-cobalt-chromium in dental laboratory technicians via MN induction test.

Quantitative cytomorphometric analysis of the exfoliated oral mucosal cells has been done in association with exposure to tobacco in any forms, alcohol consumption, premalignant, and malignant lesions, in systemic conditions such as diabetes, renal dysfunctions, and alterations in cytological pattern seen may be attributed to the changes occurring in the epithelial layer.[8-12]

As the dental lab technicians are exposed to chemical compounds routinely and these compounds are known to cause chromosomal damage in the buccal mucosal cells, there can be cellular alterations taking place because of this chromosomal damage. Thus, the present study also intended to analyze the cellular alterations, if any, occurring in the buccal mucosal cells via quantitative cytomorphometric analysis.

2. Methodology

2.1 Source of Data

The present study was conducted in the Department of Oral Pathology and Microbiology of Sri Hasanamba Dental College and Hospital, Hassan. The study population was selected among the dental laboratory technicians working in various institutions and dental laboratories in and around Karnataka. Students and patients attending the outpatient Department of Oral Medicine and Radiology were included as control group.

An approval from the Institutional Ethical Committee was obtained before beginning the study and all the participants were given complete information about the purpose of the study and informed written consent was taken before undertaking the study.

The sample size for this study comprised 28 dental laboratory technicians and 28 control individuals with no known exposure. The study sample was selected using the following inclusion and exclusion criteria.

2.2 Inclusion Criteria

- Dental laboratory technicians exposed to MMA and nickel-cobalt-chromium for a minimum period of 5 years
- Individuals working for not <8h/day.

2.3 Exclusion Criteria

- Dental Laboratory technicians with a history of tobacco usage in any forms
- Dental Laboratory technicians with a history of alcohol intake

- Dental Laboratory technicians with any oral lesions affecting any part of the oral cavity
- Subjects with any systemic disorders which could affect the oral mucosa
- Individuals with history of radiation therapy.

The samples were collected from all 28 dental lab technicians and 28 controls. Buccal mucosal smears were obtained from each using sterile cytobrush and were then fixed with spray fixative (95% methanol). Totally six smears were obtained from each . The obtained smears were stained using two different stains such as four smears were stained by Feulgen reaction using Schiff 's reagent for MN assessment and two smears were stained by Papanicolaou's stain for cytomorphometric analysis.

Cytosmears stained with Feulgen reaction were used to analyze 1000 cells per individual for MN frequency. Identification of MN in the slide was done using the criteria given by Tolbert *et al.*[13]

Criteria to include the cell for count were: Cells with intact cytoplasm and lying relatively flat; little or no overlap with adjacent cells; little or no debris; nucleus normal and intact; nuclear perimeter smooth and distinct.

Criteria for identifying MN among the cells included: round or oval structures with similar stain intensity as main nucleus; usually have 1/3–1/16 diameter of main nucleus; cell should contain both main nucleus and micronucleus; located in cellular cytoplasm; scored in basal and differentiated cells only.[14]

2.4 Quantitative Cytomorphometric Analysis

Two smears of each stained by Papanicolaou's stain were used for cytomorphometric analysis. Fifty cells were analyzed per individual.

Selection of cells for cytomorphometry was done based on the following criteria: Only clearly defined cells where in the nuclear and cell boundaries could be clearly traced were measured; cells were selected that were not clumped together or overlapping with each other.[15]

The images of the cytologic fields were captured at ×40 magnification. Nuclear and cytoplasmic areas (CAs) were measured using the image analysis software (Image Caliper Pro4.2).

CA, nuclear area (NA) and CA/NA ratio, was determined in each cell and was measured in microns. All the data were entered in the master chart and were subjected to statistical analysis.

3. Results

The study group selected was between 5 and 26 years of age and the mean age was 19.93 ± 7.10 years. Of 28 lab technicians, 25 were males and 3 were females. The mean duration of exposure was 10.11 years. Out of 28 lab technicians, 11 were exposed only to acrylic (MMA), 8 were exposed only to metal (nickel-cobalt-chromium), and 9 were exposed both to acrylic and metal [Table 1].

MN frequency was compared between dental lab technicians and controls using *t*-test. The mean ± standard deviation occurrence of MN in lab technicians was found to be 13.5 ± 2.5 [Figure 1] when compared to controls which was 2.57 ± 1.3 and was statistically significant ($P < 0.001$ [Table 2].

The MN frequency was also compared among dental lab technicians who were categorized into three groups, i.e., exposed only to acrylic (MMA), exposed only to metal (nickel, cobalt, and chromium) and exposed to both

Table 1. Distribution of lab technicians according to age, sex and exposure to acrylic (MMA) and metals (nickel, cobalt, and chromium)

Factors	*n* (%)
Age	
Mean±SD	19.93±7.10 years
Sex	
Male	25 (89.29)
Female	3 (10.71)
Exposure to acrylic	
Yes	20 (71.43)
No	8 (28.57)
Exposure to nickel, cobalt, and chromium	
Yes	17 (60.71)
No	11 (39.29)
Total	28 (100.00)
Duration	
Mean±SD	10.11±6.61 years

MMA = Methyl methacrylate, SD = Standard deviation

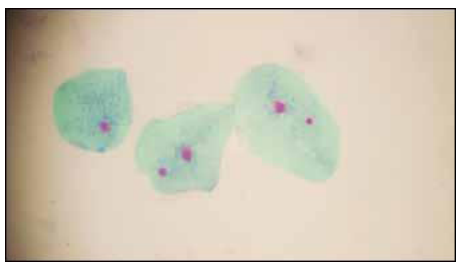


Figure 1. Buccal mucosal cells showing micro nuclei in study group.

metal and acrylic. Mean MN frequency was 12.63, 13.5, and 14.56, respectively. One-way ANOVA was used to compare the inter group MN frequency and was found not to be statistically significant (*p*-0.632).

Quantitative cytomorphometric analysis of the buccal mucosal cells in both study subjects as well as controls was done in the cytosmears stained by Papanicolaou's stain. All the measurements were compared between dental lab technicians and controls using *t*-test.

In buccal mucosal smears of the lab technicians, the NA was significantly greater than the NA of the controls ($P < 0.013$) [Table 3] and the CA was comparatively lower than the control subjects ($P < 0.001$) [Figure 2]. When CA/NA ratio was calculated, the mean ratio in lab technicians was found to be 32.31, when compared to controls which was 39.92, was statistically significant ($P < 0.001$) indicating a definite increase in the NA in the exposed study individuals

The cytomorphometric analysis was compared among dental lab technicians who were again categorized into three groups, i.e., exposed only to acrylic (MMA), exposed only to metal (nickel, cobalt, and chromium) and exposed to both metal and acrylic. Mean CA/NA ratio was found to be 32.40, 32.58 and 31.98 respectively .

Table 2. Comparison of mean MN analysis by unpaired *t*-test between control and lab technicians with

Groups	*n*	Mean±SD	*t*-test	P
Control	28	2.57±1.3	12.83	<0.001*
Lab technicians	28	13.5±2.5		

SD = Standard deviation, MN = Micronuclei. *Statistically significant

Table 3. Comparison of mean NA of the exfoliated buccal mucosa cells among controls and lab technicians using unpaired *t*-test

Groups	*n*	Mean±SD	*t*-test	P
Control	28	90.95±4.2	2.57	0.013*
Lab technicians	28	96.06±9.6		

SD = Standard deviation, NA = Nuclear area. *Statistically significant

Inter group comparison among the study subjects was done using one-way ANOVA to find if there are any significant differences in cellular alterations and was not statistically significant (p-0.982) .

Table 4. Comparison of mean CA of the exfoliated buccal mucosa cells among controls and lab technicians using unpaired *t*-test

Groups	*n*	Mean±SD	*t*-test	P
Control	28	3543.476±483.6	4.36	0.001*
Lab technicians	28	2905.474±602.8		

SD=Standard deviation, CA=Cytoplasmic area. *Statistically significant

The MN frequency and cellular alterations among dental lab technicians was correlated with their duration of exposure. Spearmen's co- relation test was used to co-relate MN frequency with duration of exposure and was found not to be statistically significant (*p*-0.066). Spearmen's co-relation test was also used to co-relate cellular alterations with duration of exposure and was found not to be statistically significant (*p*-0.847)

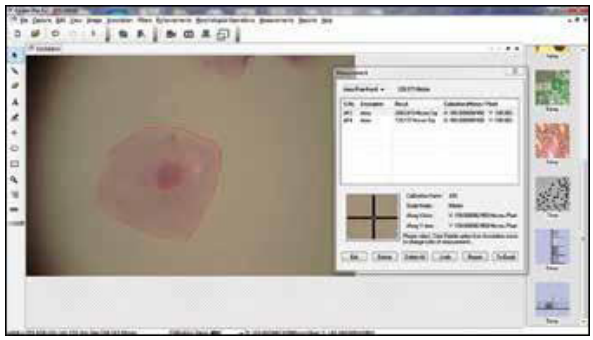


Figure 2. Cytomorphometric measurements of buccal mucosal cells in study group.

4. Discussion

Populations chronically exposed to occupational inhalants are at high risk of developing cytogenetic disorders. Increased MN frequency in the buccal mucosa cells of fire fighters after prolonged exposure to combustion fumes unveiled their heightened susceptibility to cancer. Similarly, increased MN frequency detected in petrol station attendants and calcite factory workers put them under significant risk of developing overall types of cancer. Individuals who should periodically undergo biological monitoring and proper care include hair dressers, car battery repair workers, and carpenters because of their daily exposure to harmful chemicals.[3]

In the present study MN frequency in subjects exposed to cobalt, chromium, nickel, and MMA was found to be significantly higher ($P < 0.05$) when compared to MN occurrence in control group [Table 2]. This increased frequency was in accordance to the studies done by Burgaz *et al.*[16] where increased MN frequency was noted in peripheral lymphocytes and exfoliated nasal cells of dental lab technicians exposed to Ni, Co, and Cr. The present study was also in accordance to the studies done by Sellappa *et al.*, Benova *et al.*, and Qayyum *et al.*[2,17,18] who noted an increased MN frequency in workers exposed to Ni, Co, and Cr in different occupational settings.

The present study also evaluated MN frequency in

lab technicians who were categorized into three groups, i.e., individuals exposed to acrylic (MMA), exposed to metals (NI, Co, and Cr) and exposed to both. Individuals who were exposed to both metals and acrylic showed an increased mean frequency of MN when compared to individual exposure but was not statistically significant. When MN frequency was correlated with the duration of exposure among dental technicians, there was no statistical significant correlation. Burgaz *et al.*[16] also correlated MN frequency with the duration of exposure among dental technicians and found a positive correlation in the lymphocytes and negative correlation in nasal cells of technicians.

Cytological stains also play an important role in MN assay. The present study used Feulgen reaction for identifying MN, as it is known that Feulgen stain is most commonly used for quantifying DNA content in the cells.[19] Similar study was conducted in MMA-exposed dental lab technicians to evaluate the MN frequency using Papanicolaou's stain and found to be insignificant in contrast to the present study.

Quantitative cytomorphometric analysis of exfoliated buccal mucosal cells stained with Papanicolau's stain of dental lab technicians exposed to these chemical compounds routinely was done to analyze the cellular alterations if any.

When NA of the exfoliated buccal mucosal cells was compared with the NA of cells in the control group, there was statistically significant increase in the NA in the cells of the study individuals ($P < 0.05$) [Table 3]. CA was reduced in the cells when compared to cells of the control group ($P < 0.05$) [Table 4]. When CA/NA ratio was assessed, there was statistically significant increase in the ratio when compared to the cells in the control group ($P < 0.05$).

The present study also evaluated cytomorphometric analysis in lab technicians who were categorized into three groups, i.e., individuals exposed to acrylic (MMA), exposed to metals (NI, Co, and Cr) and exposed to both. Individuals who were exposed to both metals and acrylic showed an increased NA when compared to individual exposure but were not statistically significant. When cytomorphometric analysis was correlated with the duration of exposure among dental technicians, there was no statistically significant correlation. Similar results are seen in the studies done in association with exposure to tobacco, premalignant and malignant disorders, and systemic conditions such as diabetes, renal dysfunction. In the above-mentioned conditions, studies have shown both an increased MN frequency along with the cellular alterations.[8-12]

Thus, the observed cytomorphometric changes in the lab technicians could be attributed to the exposure of carcinogenic agent ssuch as Ni, Co, Cr, and MMA carcinogens reduce the ability of the cytoplasm to mature, so that there is a greater immaturity to the cytoplasm of the cell with greatly increased activity of the nucleus leading to increase in the nuclear parameters and decrease in cellular parameters.[20]

The present study showed that both qualitative and quantitative changes in the exfoliated buccal mucosal smears of lab technicians exposed to Ni, Co, Cr, and MMA. However, it cannot be conclusively determined which compound(s) are responsible for the genotoxic damage observed in the present study.

5. Conclusion

An increase in the MN frequency observed in the present study demonstrates that MN assay performed in the exfoliated buccal mucosal cells is an ideal methodology to measure the genotoxic effects of non precious metals and MMA. Significant quantitative cytomorphometric changes observed in the present study indicate a potential health risk for dental lab technicians.

A study involving a larger sample size is required to establish the genotoxic effects of non precious metals and MMA in the dental lab technicians. However, the results of the present study are not enough to establish any causal connection, although there is experimental evidence that supports the genotoxicity of chromium, nickel, and resin monomers. confounding factors contributing to the results is inevitable In addition, possibility of other unrecognized in the present study.

Therefore, there is a need to educate dental lab technicians exposed to non precious metals and MMA about the potential hazard of occupational exposure and the importance of using protective measures.

5.1 Financial Support and Sponsorship
Nil.

5.2 Conflicts of Interest
There are no conflicts of interest.

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