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Determination of cytogenetic abnormalities in buccal mucosa of dental laboratory technicians

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ABSTRACT

This study aims to investigate the toxic effects of metals, which dental technicians are exposed to, on the oral mucosa cells in dental prosthesis laboratories (DPL). To monitor cytotoxic effects, mutations of pyknosis, Karyolysis Karyorrhexis, binucleus, micronucleus, and broken-egg were evaluated. Experimental group comprised of a total of 30 volunteer DPL employees from various DPLs, and 30 teachers and office workers were volunteered to be a part of the control group. Age range of DPL employees and whether they consume alcohol or smoke cigarettes were also considered as sub-variables. Peripheral smear technique was applied by taking samples from the oral mucosa of the experimental group and the control group. Anomalies determined between technicians and control group were statistically significant ($p < .05$). However, our findings revealed that the sub-variables (ie, age range, alcohol, and smoking) did not significantly affect the anomalies.

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KEYWORDS
Buccal mucosa cells; dental laboratory technicians; micronucleus; nuclear anomalies; occupational risk

1. Introduction

Despite the technological developments, today's professions where manual production is common continue their existence, and one of these professions is the employees of dental prosthesis laboratories (DPL). DPL employees are exposed to many metals such as cobalt, chromium, nickel, and many chemicals such as methyl methacrylate (MMA) plaster, wax due to the manual fine smoothing of metal and porcelain materials in the production process of prostheses.[1–5] Metal bases used in the construction of metal prostheses generally consist of 35–65% Co, 20–30% Cr, 0–30% Ni, and Be, Si, boron, and carbon.[6] Also, Ni and Co are associated with lung cancers. Occupational respiratory system diseases, especially pneumoconiosis, occupational asthma and dermatitis, autoimmune diseases, neurological damages, and lung and bladder cancers are common in this occupational group due to contact with chemicals, and the inhalation of very fine particulate materials and metals contained in those materials.[4,7–14] The increase in the number of micronuclei (MN) is accepted as an indirect indicator of the numerical and structural chromosome irregularities created by environmental agents in cells.[15,16] The frequency of MN in dental prosthesis laboratory workers is one of the important issues that has been investigated in recent years and observed a significant increase in DPL workers compared to the control groups .[15,17] Not only the frequency of MN but also the presence of altered cells based on chromosomal instability or gene amplification such as Pyknotic nuclei, Chromatin condensation, Broken-egg, Karyolytic cells, Karyorrhexis, Binucleated cells, Nuclear Bud are also important.[18,19] Buccal cells form the primary barrier of the digestive system and the secondary barrier of the respiratory system organs. With oral inhalation, some toxic substances can enter the oral mucosa and be metabolized causing genotoxic damage.[20] Therefore, buccal cells constitute the cells of choice for determining the genotoxicity that may be caused by carcinogenic agents at occupational exposure. As can be seen in this review, there are many anomalies related to the exposure of small particles and metals in DPLs, and to further the knowledge about this issue, and to raise awareness regarding the occupational risks of DPL employees, this study aims to investigate the genotoxic effects of dental prosthesis laboratory employees on buccal mucosa cells as a result of their inhalation exposure to fine particles and their toxic, carcinogenic metals during dental prosthesis construction stages, and to raise.

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Table 1. Demographic information of the participants.

Variable	Experimental ($n = 30$)	Controls ($n = 30$)	p Value
Age (years)			.119
17–30	3	4	
31–40	15	13	
41–56	12	13	
Mean (±S.D.)	41.73 ± 10.57	38.33 ± 7.6	.073
Smoking	21	18	.046
Non-smoking	9	12	
Alcohol consumers	9	15	.094
Non-alcohol consumers	21	15	
Gender			.378
Female	7	16	
Male	23	14	
Duration of exposure (years) mean (±S.D.)	21.34 ± 12.47	–	

2. Materials and methods

2.1. Sample

A total of 30 technician from DPLs serving in Kocaeli, who volunteered to participate in the study constituted the experiment group; 30 teachers working in schools and office workers serving under the Ministry of National Education were chosen as the control group. Questionnaire forms aiming to collect demographic data were given to the participants after scanning the relevant literature. Participants' names were not taken, but for further investigation feedback was given to the participants who were found to have a high level of an anomaly via their phone numbers collected in the questionnaire forms. None of the control group members was exposed to metal alloys or other chemicals during their work or hobbies. Oral mucosa samples were collected approximately 2–4 h after having meal; smear procedures were carried out at the workplaces of the participants following participants had a glass of water. After the collected samples were dried on a microscope slide, they were transported in a closed box between ice batteries to the laboratory where the analysis would be carried out.

2.2. Examining the samples

The collected samples were spread on cold slides and fixed with methanol–acetic acid (3:1, v/v). They were washed out and dried with air. Slides were stained with May-Grünwald. Olympus BX51 branded research microscope with computer image transfer and fluorescence attachment was used in cytological examinations. Five hundred cells per individual were analyzed in each of two different areas of the mouth of the 30 experimental and 30 control group participants. By examining the cells of the oral mucosa; nuclear anomaly types (micronucleus, Pyknotic nuclei, Chromatin condensation, Broken-egg, Karyolytic cells, Karyorrhexis, Binucleated cells, Nuclear Bud, etc.) and frequencies were investigated. All of the analyses were conducted two times repeatedly.

Table 2. Analysis of variance for buccal cell abnormalities frequencies by taking into account age range, smoking, alcohol.

Buccal cell abnormalities	Mean square	F-ratio	p Value
Micronucleus			
Age range	339.321	4.480	.036
Smoking	40.880	0.540	.464
Alcohol	0.408	0.005	.942
Pyknotic nuclei			
Age range	1.966	0.043	.836
Smoking	33.704	0.739	.392
Alcohol	22.018	0.483	.489
Chromatin condensation			
Age range	270.903	7.648	.007**
Smoking	14.349	0.405	.526
Alcohol	2.359	0.067	.797
Broken-egg			
Age range	4.388	0.312	.578
Smoking	78.704	5.589	.020
Alcohol	0.063	0.004	.947
Karyolytic cells			
Age range	330.553	4.231	.042
Smoking	90.797	1.162	.283
Alcohol	278.434	3.564	.062
Karyorrhexis			
Age range	0.499	0.007	.933
Smoking	87.103	1.229	.270
Alcohol	158.217	2.232	.138
Binucleated cells			
Age range	0.014	0.003	.956
Smoking	8.412	1.892	.172
Alcohol	0.213	0.048	.827
Nuclear bud			
Age range	44.333	8.476	.004*
Smoking	13.692	2.618	.108
Alcohol	0.000	0.000	.999

*$p < .005$.
**$p < .1$.

2.3. Statistical methods

Statistical analyses were conducted using SPSS 19. The amount of nuclear abnormalities were calculated by descriptive statistics including arithmetic mean ± standard deviations. To determine the significance of differences between nuclear abnormalities in DPL technicians exposed to metal powders and the control group. The parametric t test was used. Besides Pearson correlation analysis was applied to group comparisons according to age range, gender, smoking, and alcohol consumption. The control and experimental groups were comparable when the age range, smoking, and alcohol use are considered. The similarity was not observed in terms of gender. Statistical significances were set at a p value of $<.05$. All p values were two-tailed. The mean and standard deviation were determined for each biomarker. The biomonitoring and cytogenetic were performed via multifactorial analysis of variance (MANCOVA) for the concurrent

Table 3. Analysis of buccal cell abnormalities for the experimental and control groups.

Buccal cell abnormalities	Mean	Std. deviation	Mean square	*F*-ratio	*p* Value
Micronucleus			14287.8	188.623	.000*
Exposed	28.67	12.151			
Control	6.08	2.714			
Total	17.38	14.333			
Pyknotic nuclei			1846.5	40.481	.000*
Exposed	16.30	8.532			
Control	8.50	4.127			
Total	12.40	7.738			
Chromatin condensation			511.6	14.444	.000*
Exposed	10.50	6.880			
Control	14.58	5.133			
Total	12.54	6.383			
Broken-egg			736.0	52.267	.000*
Exposed	5.30	5.312			
Control	0.42	0.766			
Total	2.86	4.505			
Karyolytic cells			3322.2	42.525	.000*
Exposed	14.92	12.507			
Control	5.08	2.895			
Total	10.00	10.300			
Karyorrhexis			201.3	2.841	.095
Exposed	14.48	11.057			
Control	12.50	4.444			
Total	13.49	8.450			
Binucleated cells			41.5	9.356	.003*
Exposed	2.67	2.747			
Control	1.50	1.127			
Total	2.08	2.172			
Nuclear bud			145.1	27.742	.000*
Exposed	4.00	3.215			
Control	1.58	0.869			
Total	2.79	2.641			

*$p < .005$.

assessment of the impact of smoking, alcohol, and age range. In all analyses, group difference was used as a covariate.

3. Results

The data obtained *via* the questionnaire forms are presented in Table 1. Mean age, smoking, and alcohol consumption of DPL employees were not found to be significantly different from the control group ($p > .05$). Due to the difficult working conditions, male employees were more in number in DPLs. Therefore, the gender factor is different from other factors.

Table 2 illustrates whether age range, smoking, and alcohol use affect buccal mucosal cell anomalies for DPL technicians and the control group. Based on these results, it was seen that age range, smoking, and alcohol consumption did not have any impact on the formation of Micronucleus, Pyknotic nuclei, Broken-egg, Karyolytic cells, Karyorrhexis, Binucleated cells, whereas age range significantly affected the formation of Chromatin Condensation ($p = .007$) and Nuclear Bud ($p = .004$).

In Table 3, intraoral cell core abnormalities of the experimental and control groups were compared. Based on these results, statistically significant difference were found between the two groups in all anomalies observed except for Karyorrhexis at $p < .05$ level. Some of the nuclei and morphological anomalies that occurred in oral cells are shown in Figure 1.

4. Discussion

Dental lab workers are open to a range of toxic substances, such as metal alloys, determined as potential health risks. Metal alloys namely Vitallium, Duralium, Wironit, and Remanium are utilized in the manufacturing of crowns, bridges, and dental prostheses. Commonly used elements of these alloys are cobalt, chromium, molybdenum, nickel, silica, and other metals in various amounts[3,6,21,22] During the fine smoothing processes performed in DPLs, employees are exposed to very fine particulate matter that can easily pass through the respiratory tract.[2,4,5] Previous studies have shown that there are abundant chromium and cobalt in the lung tissues of dental technicians with pneumoconiosis and that abundant chromium exposure increases the risk of cancer in the respiratory tract.[1,23–26] It has been stated by the researchers that chromium and nickel coatings increase the risk of genotoxicity and carcinogenicity of stainless-steel welders and workers in the hard metal and cobalt industry.[24,27,28,29] The frequency of MN occurring in buccal cells due to the intake of genotoxic carcinogens during respiration and digestion is accepted as an early indicator of carcinoma development.[20,30,31,32] In our study, the micronucleus data related to the loss of allele and contributing to the carcinogenesis observed in the experimental group are significantly different compared to the control group. In our study, the double-nucleated cells were found to be statistically significant compared to the control group. Such cells contain two nuclei and are mostly found in cancer cells. In this study, in the buccal mucosa cells of dental laboratory technicians, micronucleus cells were encountered four times more, Pyknotic nuclei cell approximately two times more, Broken-egg cells approximately 12 times more, Karyolytic cells approximately three times more, Nuclear Bud cells approximately two times more compared to the control group. Similarly, Tadin et al.[17] found that the frequency of MN in buccal cells of DPL workers was 2.5 times more than dentists and dental assistants. Shahsavari et al.[33] found that the frequency of MN in buccal cells of DPL workers was 2.6 times higher than

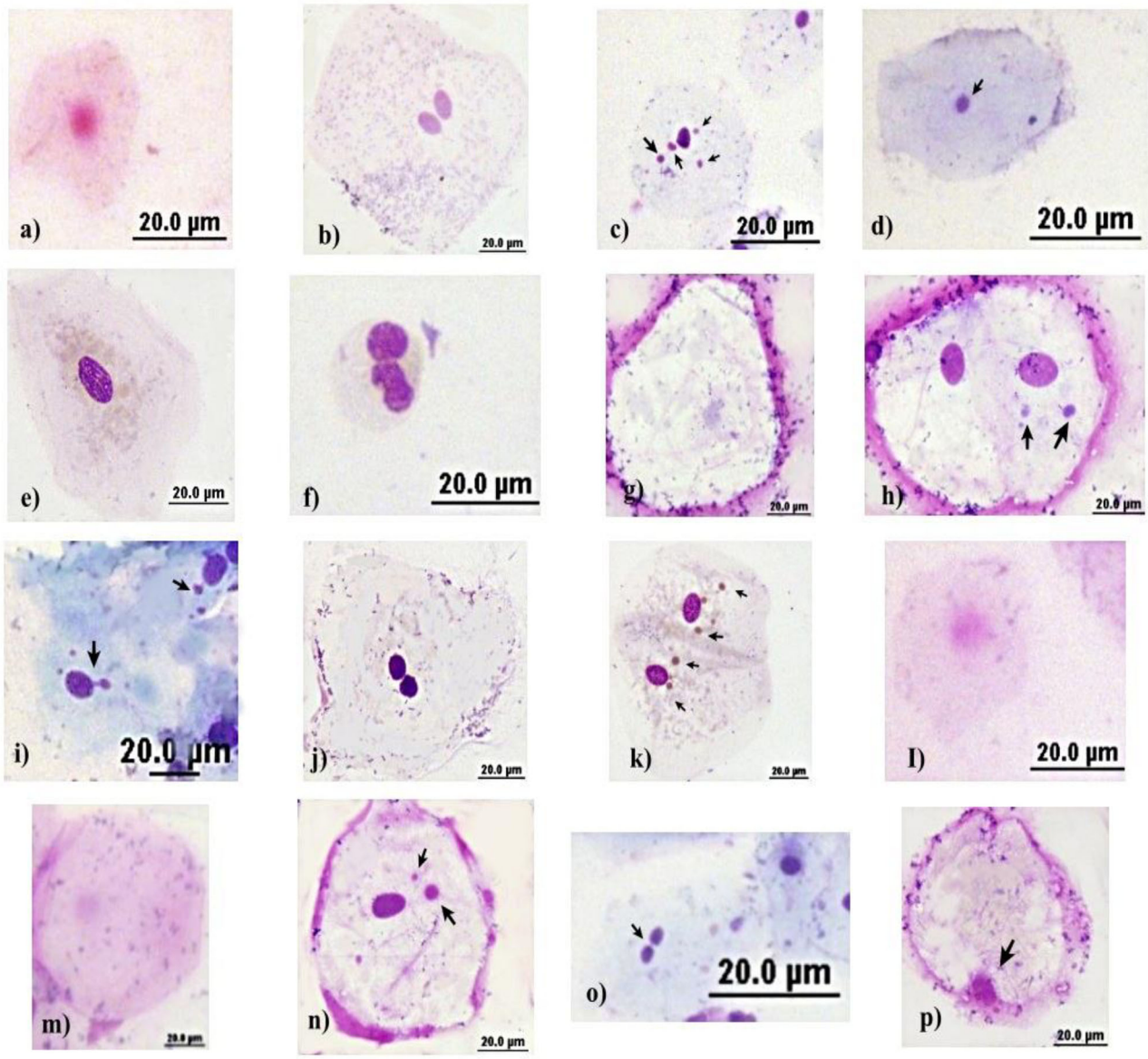


Figure 1. Buccal cell abnormalities observed in epithelial cells: (a.ı); karyorrhexis (b.h); Binucleated cells (c.k.h.n.); Micronucleus (d.p); Pyknotic nuclei (e); Chromatin condensation (f.j); Nuclear bud (g.m); Karyolytic cells (i.o); Broken-egg.

in the control group. Ishikawa et al.[34] found that the frequency of MN in peripheral lymphocytes was approximately 2.5 times higher in DPL workers compared to office workers. Benova et al.[35] supported our results by showing a significant increase in the frequency of MN in buccal cells in chromium coating workers in their study. Also, Burgaz et al.[15] performed micronucleus level analysis in blood samples of DPL workers in lymphocytes and samples taken from the nasal mucosa. As a result of their study, they found that the metal concentrations in the urine of DPL workers, lymphocyte, and micronucleus levels in the nasal mucosa were statistically significantly higher than the control group. Considering these differences between DPL employees and control groups, the anamolies may be caused by materials used such as metal, MMA, porcelain powder and resin found in the DPL indoor air. The reason for the higher frequency of MN in our study compared to other studies may be the inadequate implementation of occupational health and safety standards in the specific DPLs, and also long and intense working hours.[5,36] *In vitro* and *in vivo* research has demonstrated that DNA damage can be caused by base metal alloys used in dental laboratories.[15,17,33,34,37] This study suggests that the number of metal alloys, even if these metals are within the range of biological tolerance levels, absorbed may be sufficient to induce mutations in oral mucosa cells of dental technicians. Additionally, since DPL technicians are simultaneously open to

different metals, there may be synergetic and antagonistic interactions between them.[15,34,38] Therefore, it is hard to identify which compounds were in charge of the genotoxic damage seen in this study. Also, the authors acknowledge that exposure to metals in dental laboratories concerns both the elements of base metal alloys and the metals located in refractory materials and the abrasives utilized in the course of all casting and prosthesis finishing processes.[2,39] Cadmium and some inorganic compounds in the powder chemical used for acrylic polymerization in the production of prosthesis base in DPL enter the body through respiration, digestion, and skin and cause severe intoxication.[40,45,4646] In addition to iron and chromium used in the same laboratories, nickel used in dental alloys and orthodontic appliances is extremely carcinogenic due to its high reactivity.[42–44] Among the alloys used in the production of dental prostheses, beryllium is taken into the body, especially with the respiratory system, and its systemic effects can be seen, as well as local effects can be seen by contact or contact with the eyes.[45] Likewise, nickel, cobalt, and molybdenum among the alloys used in the production of dental prostheses may cause discoloration in the mouth and stomatitis, gastrointestinal bleeding, abdominal pain and liver involvement due to oral ingestion in the digestive system.[47]

Similarly, in our study, in the buccal cells of DPL workers and the control group, normal differentiated cells were found to be significant in cells with pycnotic nuclei, which were approximately one-third to two-thirds of a nucleus size. Tadin et al.[17] found that the frequency of pycnotic cells was greater among DPL technicians compared to dentists and residents. Cells with chromatin condensation, a sign of cell apoptosis, and nuclear bud cells with small amounts of genetic material attached to the main cell nucleus, are regarded as indicators of genotoxic exposure that causes chromosome abnormalities.[48] In the buccal samples of the DPL technicians and the control group, a statistically significant difference was found in this cell frequency. Tadin et al.[17] found that the frequency of nuclear bud cells is higher in DPL workers compared to dentists and dental assistants. When these results are evaluated together, it is possible to conclude that mutation factors in genes related to DNA repair, separation of chromosomes, and aberrations may have caused these anomalies to increase. Finally, there was no statistically significant difference in the karyolytic cells reflecting the later stages of necrosis and apoptosis compared to the control group. However, Tadin et al.[17] found a higher frequency of this cell group in DPL workers compared to dentists and dental assistants. Genotoxicity studies show that DNA damage in buccal cells increases with age.[49,50] In our study, the relationship between the size of the damage and the age of DPL workers was found only between nuclear bud and condensed chromatin. No such relationship was observed with other abnormalities.

In our study, the relationship between lifestyle factors (ie smoking and alcohol consumption) and the frequency and size of DNA damage was not found to be statistically significant. Tobacco smoke includes numerous genotoxic elements that can enhance the frequency of MN and other nuclear abnormalities in smokers in comparison to nonsmokers. Celik et al.[51] found a high amount of two-core cells in among tobacco users, while Bolognesi et al.[52] did not report a significant difference in micronucleus frequency among smokers. Similar findings indicating that lifestyle does not affect core anomaly frequencies were also observed in other studies.[48] Alcohol is also defined as a genotoxic substance;[53] but, in this study alcohol use was not significantly associated with the increase in the amount of nuclear abnormalities in buccal epithelial samples. This study may be useful in providing information on the level of cytogenetic damage in tissues that are the target of human carcinomas and where carcinomas can develop, and in detecting genotoxic effects associated with the production of dental prostheses in oral mucosa cells.

5. Conclusion

Our results apparently demonstrated that the work-related exposure of DPL technicians significantly increased the amount of cells with nuclear abnormalities because of the genotoxic impact of the metals and metal alloys they were exposed to. Previous biomarker studies that combine investigation of buccal cells and nuclear abnormalities demonstrated potential changes in genomic stability events. This study provides insightful information in identifying potentially degenerative illnesses and about the early pre-symptomatic diagnosis. Consequently, it is recommended that occupational conditions in dental laboratories should be improved, precautionary measures against exposure in the workplace should be reviewed, and the implementation of a health research program should also be considered.

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Ethical approval

Compliance with Ethical Standards the Ethics Committee of Kocaeli University approved this study with the ID number KU GOKAEK 2018/284.

Consent to participate

All participants volunteered to participate in the study and signed consent forms.

Consent to publish

Both authors agreed to publish their manuscript in this journal and gave consent to publish.

Author contributions

Burcu Yuksel Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing – original draft; Writing – review & editing.

Serap Arsal Yildirim Conceptualization; Data curation; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization.

Competing interest

Authors declare no conflict of interest.

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