Assessment of Karyorrhexis Incidence in Exfoliated Buccal Mucosa Epithelial Cells among Fuel Station Employees in Sleman, Special Region of Yogyakarta, Indonesia

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Abstract

Introduction: Benzene exposure from petrol vapor possesses a health risk to humans, particularly fuel station employees through the inward breath of the unstable portions of petrol during vehicles refueling. This may trigger an increase in nuclear abnormalities and lead to DNA impairment pieces of evidence. The purpose of this study was to evaluate the incidence of karyorrhexis occurred in buccal mucosa epithelial cells among fuel station employees in Sleman, Special Region of Yogyakarta, Indonesia, to explore the possible cytogenetic risk on occupational exposure to petrol derivatives.

Materials and methods: A total of 15 fuel station employees and 15 control subjects within the age group of 20–55 years were initiated. Buccal smears were obtained from the oral cavity with cytobrush and smeared into slides. Afterward, these specimens were stained with Papanicolaou's method and then analyzed for nuclear abnormalities. Karyorrhexis incidence was recorded as per 1,000 cells counted using a light microscope and then statistically analyzed with an independent T-test ($p < 0.05$).

Results: The result revealed that there were statistically significant higher frequencies ($p < 0.05$) of karyorrhexis incidence between the exposed employees compared to the controls. The result revealed that among the exposed employees compared to the controls there were statistically significant higher frequencies ($p < 0.05$) of karyorrhexis incidence.

Conclusion: This research concluded that exposure to petrol derivatives such as benzene increases the karyorrhexis incidence of exfoliated buccal mucosa epithelial cells on fuel station employees in Sleman, Special Region of Yogyakarta, Indonesia.

Clinical significance: This study indicates increased karyorrhexis incidence of exfoliated buccal mucosa epithelial cells caused by repeated exposure to benzene.

Keywords: Benzene, Buccal cells, Fuel station employees, Karyorrhexis incidence.

International Journal of Experimental Dental Science (2020): 10.5005/jp-journals-10029-1216

Introduction

Benzene is a product of crude oil refinement, which is derived from petroleum refining.[1–3] International Agency for Research on Cancer (IARC) 1989 expressed that risk to fuel vapors is conceivably carcinogenic to humans, mainly due to the premise of the well-established carcinogenic nature of benzene.[4,5] A great concern was historically given to this well-known genotoxic as an occupational health hazard.[6]

The Environmental Protection Agency of the United State of America (1998)[7] limits the current permissible benzene exposure level in the air to one part per million (ppm) for 8 hours.[8] However, petrol station employees are continuously subjected to petroleum refinement products essentially through inhalation of the volatile fraction of petrol during vehicle refueling, furthermore retain the fuel fumes and the products transmitted by the engines.[4,5,9,10]

Grandjean and Andersen[11] stated that excess risk for lung cancers was accounted for in petrol station employees who were exposed to petroleum products for a drawn-out stretch of time.[5] A study by Carere et al.[12] has demonstrated that benzene can induce different types of genetic impairment such as chromosome aberrations, sister chromatid exchanges (SCE), micronuclei formation, and DNA damage. A similar study conducted by Metgud et al.[8] about nuclear irregularities in exfoliated buccal

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epithelial cells of petrol station employees in Udaipur, Rajasthan. They found that the cytogenotoxic effects of petrol exposure cause a significant increase in the incidence of nuclear abnormalities such as micronucleus (MN), binucleation, karyorrhexis, and karyolysis in the buccal cells of petrol station employees.

Nuclear abnormalities (NAs) are biomarkers to observe individuals or populations exposed to the mutagenic, genotoxic, or teratogenic event. According to Tolbert et al.,[13] besides physiological cellular differentiation, NA is also observed in cell death with DNA damage.[14] Nucleus cells may be degraded into concise chromatin, fragmented nuclei (karyorrhexis), pyknotic

nuclei, or totally lose their nuclear material (karyolysis). Some cells are rarely may be blocked in a binucleated stage or may show nuclear buds, a biomarker of gene amplification.[15]

The oral cavity is the port of entry of various hazardous agents into the body and its affection can indicate systemic condition.[16] It reflects an individual's health-being since oral mucosa often shows disease changes.[14] Oral cavity has a moist lining that communicates with the exterior known as oral mucosa. The oral mucosa primarily is to protect the deeper tissues of the oral cavity.[17] It serves as the first barrier against potential carcinogens. Consequently, it is vulnerable to damage by these agents and shows pathological changes before reflecting a systemic condition.[14]

Rosin[18] stated that approximately 90% of human cancers are originated from the epithelial origin. It might be a suitable approach to using these types of cells for tracking down elevated cancer risk in humans.[19] The buccal mucosa epithelial cells serve as a target site that presents to evaluate earlier cytotoxic and genotoxic events initiated by carcinogenic agents entering the body through inhalation and ingestion. Buccal mucosa epithelial cells provide the first barrier which is adequate for metabolizing proximate carcinogens to reactive products.[20] Therefore, oral buccal mucosa has been proposed as a reflection that shows an individual's health, owing to its vulnerability to harm by hazardous agents before reflecting a systemic condition.[14]

On top of that, petrol gets absorbed more easily in tropical than in temperate countries.[21] Indonesia is a tropical country with five main islands, namely Sumatra, Java, Kalimantan, Sulawesi, and Papua.[22] Accordingly, petrol pump employees pose a higher risk for exposure to toxic chemicals of petroleum products.[23] Moreover, the employees engaged in filling petrol mostly do not take any protections and hence remain unhygienic.[21] As a consequence, occupational exposure to benzene derivatives poses genotoxic risks. In light of this issue, employees are more prone to have nuclear abnormalities in their buccal cells.[9]

Taking into consideration that occupational hazards to such by-products may carry genotoxic risk, this study is done to examine the cytogenotoxic damage of exfoliated buccal cells obtained from petrol station employees and control subjects in Sleman, Special Region of Yogyakarta, Indonesia.

MATERIALS AND METHODS

Subjects of this study consist of 15 fuel station employees who are occupationally exposed to petrol vapor and vehicle exhaust for at least one year regularly at the petrol stations in Sleman, Special Regency of Yogyakarta. The control group consists of 15 healthy volunteers from UGM students and staff who are without indication of any exposure to petrol derivatives or other potential genotoxic substances.

Before sampling, an issued letter of ethical clearance was obtained in accordance with the Ethics and Advocacy Unit of the Faculty of Dentistry, University of Gadjah Mada (UGM) for approving this current study, as well as a permission letter from the Faculty to conduct study case in PT. Pertamina, Laboratory of Pathology Anatomy at Faculty of Medicine, Public Health and Nursing UGM and Laboratory of Clinical Pathology at Faculty of Veterinary Medicine UGM.

The study was briefed to all the participants in detail, explanation of the procedures, their roles in the study, and the content of the informed consent form. The participants were asked to sign informed consent forms under ethical clearance as an agreement to take part in this study. Then, participants have to complete a standardized questionnaire to obtain necessary data on lifestyles and personal factors (age, gender, working period, medical health, alcohol consumption, smoking habit, etc.).

Before buccal epithelial cell collection, rinsing their mouth thoroughly with distilled water were advised to subjects to avoid contamination of debris in the oral cavity and to remove exfoliated dead cells. Buccal epithelial cells were collected from each individual by swabbing the buccal mucosa gently with a sterile cytobrush moistened with 0.09% NaCl. Epithelial swabbing was done by rotating cytobrush at least 360° on the buccal mucosa.

Exfoliated buccal mucosa epithelial cells were smeared on the object-glass containing two drops of physiological solutions (NaCl 0.09%) by rotating the cytobrush oppose the direction of rotation on the buccal mucosa. Cytologic smears were dried and fixed in a 95% ethanol solution. Slides were stained by means of the Papanicolaou reaction. Specimens had undergone hydrolysis by immersing the slides briefly in 95% ethanol solution at room temperature for 15 minutes and then rinsed with a tap water bath for 10–15 minutes. The specimens were firstly nuclear stained by soaking in Mayer's hematoxylin for 3–5 minutes and then rinse with running taped water for 2 minutes until clear water was visualized. Then, specimens were dehydrated by immersing the slides in 95% ethanol solution with two changes for 10 dips each. After that, the specimen was contrasted by cytoplasmic stained with OG-6 counterstain for 2 minutes, followed by rinsing it with 95% ethanol solution in 10 dips three times. Then, the slides were specifically stained with a Second EA-50 stain for cytoplasmic and nucleolar staining (RNA) in 3–4 minutes. After that, specimens were dehydrated by immersing the slides in 95% ethanol solution with two changes for 10 dips each, followed by absolute ethanol solution with three changes for 10 dips each. Later, the specimens were cleared by immersing into xylene in 10 dips three times at different jars. Last, the specimen was allowed to dry, mounting with a coverslip, and evaluated to determine nuclear anomalies.

Interpretation of cells was performed under a light microscope (magnification of 1,000 times) with the aid of immersion oil. A total of, at least, 1,000 exfoliated cells were screened per slide for karyorrhexis incidence. Before the karyorrhectic (KR) nuclei were interpreted, the cells which fulfill Tolbert's criteria below were chosen for scoring. It consisted of the following parameters:

- Intact cytoplasm and relatively flat cell position on the slide.
- Little or no overlapped with adjacent cells.
- Little or no debris.
- Normal nucleus and intact, nuclear perimeter smooth and distinct.

Criteria needed for identification of KR nucleus was a fragmented nucleus that had undergone integrity loss which has more extensive nuclear chromatin aggregation relative to cells with condensed chromatin as shown in Figure 1. Karyorrhectic nuclei that were identified and fulfilled the criteria above were counted by using a handy counter. The incidence of KR nuclei was recorded as per 1,000 cells counted.

The normality and homogeneity of the research data were tested by using the Shapiro–Wilk test and Levene's test. The collected data were then analyzed by using an independent T-test with a value $p < 0.05$.

RESULTS

The assessment of karyorrhexis incidence in exfoliated buccal epithelial cells of petrol station employees was performed about the buccal smear from that of the control subjects. Karyorrhectic cells were characterized by a densely speckled nuclear pattern indicative of nuclear fragmentation. Both of the groups showed distinct alterations in the total incidence of KR cells in the cell count. The normal nucleus and the other typical nuclear abnormalities of exfoliated buccal epithelial 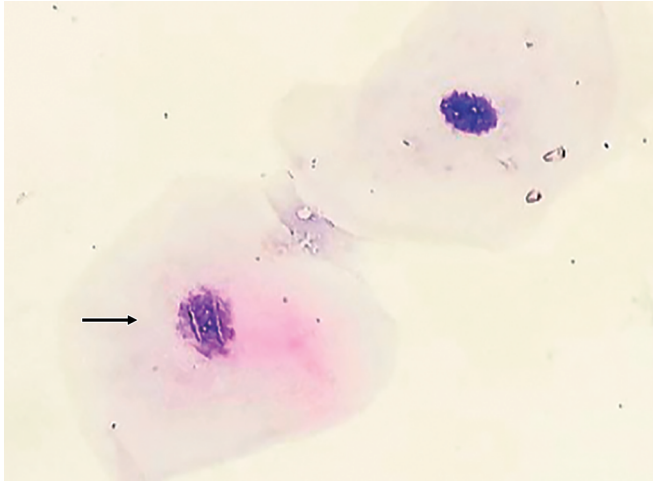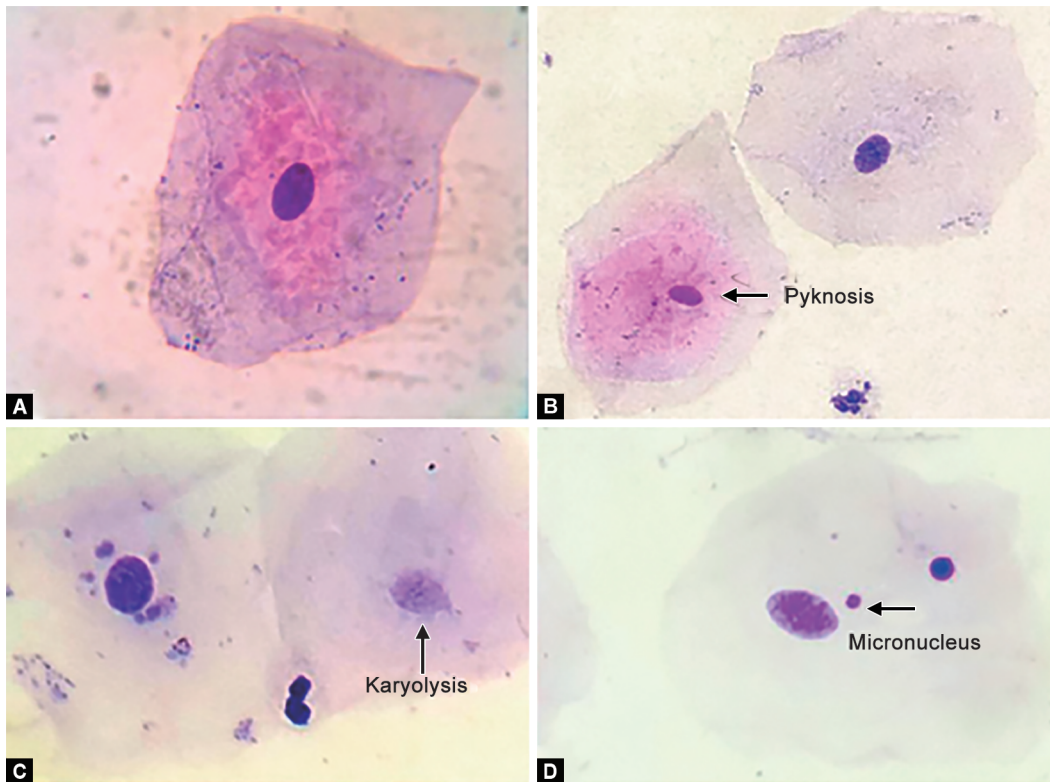cells were seen in both the control group and petrol station employees as shown in Figure 2. However, there is a higher incidence of nuclear abnormalities in petrol station employees. There was an increase in karyorrhetic incidence in petrol station employees compared to control groups as shown in Figure 3.

In the present study, the exfoliative cytology technique was used to evaluate cellular changes in the buccal mucosa of petrol station employees in comparison with control subjects. Thirty male subjects with ages ranging from 20 to 55 years were enrolled in this study. Demographic characteristics of the groups studied were summarized in Table 1.

Both of the groups were almost similar in age distribution. In addition, the petrol station employees chosen were matched with the inclusion and exclusion criteria.

With regard to the mean values and standard deviation of karyorrhexis incidence in the exfoliated buccal epithelial cells of petrol station employees comparison demonstrated that there were no apparent differences in key characteristics between both of the groups, thus providing reasonable assurance that the results of the incidence of karyorrhexis analyzes are representative of the entire study population, and does not appear to have introduced a source of bias by disproportionately the representing study groups and control subjects, an intercomparison bar chart (Fig. 4) was made.

Based on the facts shown in Figure 4, it was noticed that intercomparison of mean values and standard deviations for karyorrhexis incidence were relatively higher in petrol station employees in contrast to control subjects. Normality and homogeneity of the data were calculated using Shapiro–Wilk and Levene's test, respectively, as shown in Table 2. The data of control subjects and petrol station employees were normally distributed and have the same variance or in homogeny, as determined by Levene's test for equality of variances. Furthermore, the data were

Fig. 1: Photomicrograph indicates that the exfoliated buccal epithelial cells undergo karyorrhexis (arrow) that are observed and counted under a light microscope with a magnification of 1,000× with the aid of immersion oil (Papanicolaou, 1,000×)

Figs 2A to D: Exfoliated buccal epithelial cells showing various nuclear anomalies at 1,000× with PAP: (A) Normal cell; (B) Pyknosis; (C) Karyolysis; (D) Micronucleus
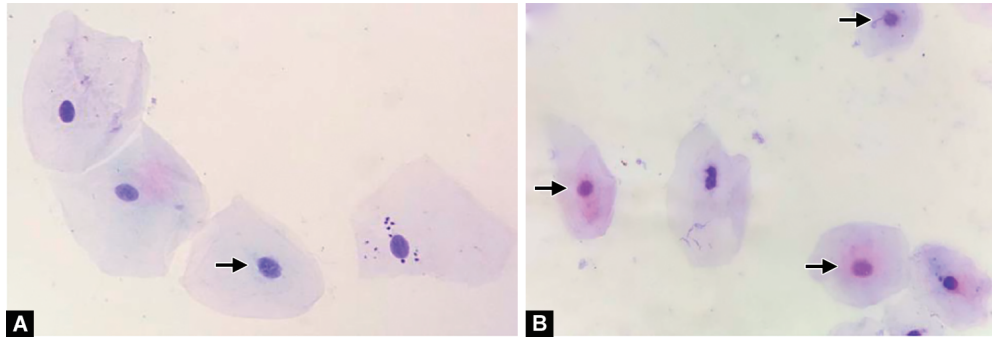
Figs 3A and B: Photomicrographs indicate that the exfoliated buccal epithelial cells undergo karyorrhexis (arrow) found in (A) Control subjects is less than that of (B) Petrol station employees (Papanicolaou, 400×)
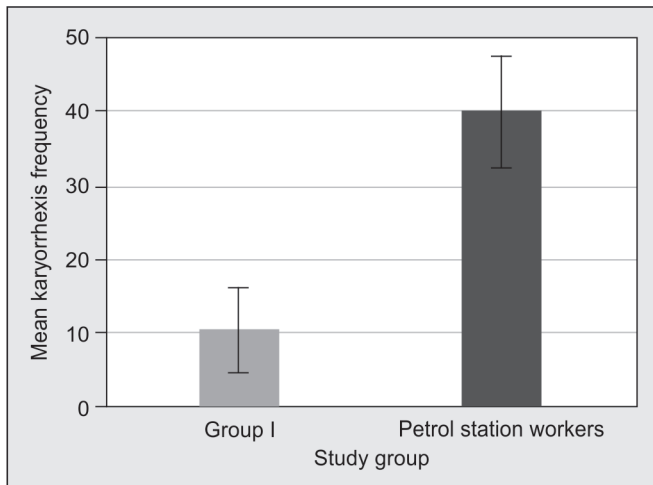

Fig. 4: Means and standard deviations of karyorrhexis incidence in petrol station employees and control subjects

Table 1: Demographic characteristics of controls and petrol station employees

Study group	N	Age (years)	Duration of employment (years)	Weekly exposure (hours)
Control subjects	15	29.93 ± 9.74		
Petrol station employees	15	31.60 ± 10.87	7.40 ± 6.51	46.00 ± 2.93
Total	30			

tested using an independent sample *T*-test with a value $p < 0.05$ after the background assumptions are satisfied. For the mean difference, an independent *T*-test was run on the data with a 95% confidence interval (CI). Table 3 explains the statistical analysis through comparison of mean karyorrhexis incidence in both study groups.

The result indicated the difference in mean karyorrhexis incidence of exfoliated buccal epithelial cells across the control subjects and petrol station employees was statistically highly significant. The results illustrated that petrol station employees had a significant increase of karyorrhexis incidence in exfoliated buccal mucosa epithelial cells who are occupationally exposed to petrol derivatives such as benzene as compared to the control group.

Discussion

In the current study, we examined an increase in the mean karyorrhexis incidence in petrol station employees, regardless of the population age, total working time, and exposure dose compared to the control group (as shown in Table 1 and Fig. 4). These findings are consonant with the results of the studies by Çelik et al.,[4] Martins et al.,[24] Rajkokila et al.,[9] Gadhia et al.,[3] and Metgud et al.[8] who used different stains and they determined a significant increase in the incidence of karyorrhexis and other nuclear abnormalities in the buccal cells of petrol station employees than the control

individuals. Martins et al.[24] deemed that such results support the notion that petroleum was also a cytotoxic agent. In relevant to the present study demonstrated that petrol station employees are at risk of significant cytogenetic damage. The literature postulated that petrol station employees are at a higher risk of exposure to the carcinogenic characteristics benzene and had a higher rate of cellular destruction compared to the control group.

Benzene is one of the hydrocarbons and is widely distributed as an environmental contaminant. IARC (International Agency for Research on Cancer)[25] has classified benzene as carcinogenic to humans (group I). Benzene, derived from the petrochemical and petroleum refining industries, is also an additive derivative that easily evaporates with an aromatic smell. A prolonged exposure to benzene in humans generally noticed in factories, refineries petrol stations, poses a risk of cancer development.[5] In Yogyakarta, petrol station employees are employed rather than self-service which is significantly increasing the opportunity for exposure. In the context of occupational exposure, a petrol station worker in the study group is a representative of an occupational group who is permanently exposed to this hazardous air pollutant as the previous study reported by Elesawy et al.[26]

Petrol station employees who pump fuel to vehicles usually and inevitably absorb the products of fuel fumes and the products of combustion.[8] Benzene is readily absorbed by petrol station employees in the study group during refueling which is mainly via nasal or oral inhalation. Experimental data indicate that humans can absorb up to 80% of inhaled benzene (after 5 minutes of exposure).[7,27,28] This is asserted during the interview and questionnaire section with the petrol station employees in the petrol station. A questionnaire analysis showed a population with low median age, albeit with long-period exposure at the petrol station. Besides, in the current investigation, levels of

Table 2: Normality and homogeneity test of controls and petrol station employees

	Population samples	Shapiro–Wilk (normality test)			Levene's test for equality of variances	
		Statistics	df	Sig.	F	Sig.
Karyorrhectic incidence	Control subjects	0.925	15	0.231	1.163	0.29
	Petrol station employees	0.91	15	0.137		

Table 3: Comparison of petrol station employees and control subjects with respect to karyorrhexis incidence in exfoliated buccal epithelial cells

	Control subjects		Petrol station employees		p value
Parameter	Mean	SD	Mean	SD	
Karyorrhectic incidence	10.47	± 5.82	39.93	± 7.54	0.000**

**The significance is $p < 0.05$

benzene and other petrol derivatives in ambient air were found to be higher in petrol stations as compared to the control group's working environment. This indicates that petrol station employees are directly in contact with benzene vapor with a higher incidence compared to the others. Odewabi et al.[29] alleged that during vehicles refueling in the petrol station, the vapor concentrations of air around the petrol pump are between 20 and 200 ppm.

Buccal cells are the first barrier for the inhalation or ingestion route and are capable of metabolizing proximate carcinogens to reactive products.[15] The buccal cavity includes the space and gaps in the cheek adjacent to the mouth and extends from the lips to the oropharynx. The oral mucosa lines the buccal cavity and consists of a multi-layered epithelial lining that includes the basement membrane, the lamina propria, and a layer of connective tissues supplied by blood vessels and nerves.[30] Because the oral mucosa is highly vascularized, chemical substances that are absorbed through the oral mucosa directly enter the systemic circulation, bypassing the gastrointestinal tract and first-pass metabolism in the liver.[31] Inhalation can incur the oral cavity exposed to the benzene and show adverse effects on the condition of buccal mucosa epithelial cells. Therefore, Holland et al.[15] previously explained that it could be argued that oral epithelial cells represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion.

Exfoliative cytology was used in the study by cytological analysis of exfoliated oral buccal mucosal cells using Papanicolaou (PAP) smears. Kumar et al.[32] stated that the most commonly used DNA specific stains are Feulgen and around 30% of the studies were done using non-specific DNA stains such as Giemsa, May–Grunwald–Giemsa (MGG), and PAP. PAP, which is the most commonly used cytological stain, was found to show better staining results as compared to the MGG, a Romanowsky stain that is used widely in field studies. As for the PAP stain, the smear was wet-fixed in alcohol which gave a clear background when compared to MGG, where the smear was air-dried and resulted in a background that was full of cell debris and salivary proteins.[33] An investigation done by Kumar et al.[32] specifies that PAP stained nuclei are not dependable and should be used with caution, only for meta-nucleated analysis. Whereas Feulgen stain is technique sensitive and time-consuming but it yielded low values with a positive result. So, PAP stain can be used to identify abnormal cytological changes but not to score MN.

The sample collection was performed by using a cytobrush to access the oral buccal mucosa. It provides a noninvasive, cost-limited, and time-saving tool for evaluating cytological changes. Moreover, this method is relatively easy to interpret.[26] It is well tolerated by participants and shows the first candidate tissue that serves to evaluate cytotoxic and genotoxic effects.[14]

The oral mucosal permeability in different regions of the mouth is an important aspect to consider while analyzing the local effects of carcinogenic agents. The non-keratinized tissues (buccal mucosa) are much more permeable than keratinized tissues, such as the palate and gingiva.[26] In addition, oral mucosa is covered by a stratified epithelium composed of multiple layers of cells that show different stages of differentiation (maturation) between the deepest cell layer and the most superficial layer. Buccal mucosa has a higher turnover rate, which is 25 days.[17,33,34] The complete turnover rate of these epithelial cells in the buccal region is quite rapid, ranging from 5 to 6 days, but the mature and non-viable cells remain on the surface.[35] High turnover rate induces a continuous cell renewal in which new cells produced by mitosis in the basal layer migrate towards the surface layer to replace those that are exfoliated to maintain epithelial homeostasis.[8]

However, several systemic conditions and treatments will limit the proliferation rate of oral buccal epithelial cells.[14] Factors such as inflammation, the time of the day, and stress can also affect mitotic activity. Mitosis defects result in various nuclear abnormalities, such as micronuclei, binucleation, broken egg appearance, pyknotic and KR nuclei, and increased numbers of abnormal mitotic figures.[36] Hence, there is an increase of karyorrhexis incidence among the petrol station employees compared to the control group in the current study. Besides, factors that upset the balance of the rate of cell replacement by cell division (mitosis) and the rate of programd cell death (apoptosis) may lead to malignant transformation.[37]

In molecular aspects, it is known that some of the reactive metabolites of benzene, such as phenol, catechol, and hydroquinone, can bind to and damage macromolecules, including DNA. These reactive metabolites may also generate reactive oxygen species (ROS) that can exacerbate the DNA damage. Additionally, alterations in DNA methylation, notably mitochondrial DNA have been recently verified to result from low-dose benzene exposure as a direct effect of ROS-induced DNA damage.[26] It also has been demonstrated that repeated exposure to cytotoxicants can result in chronic cell injury, compensatory cell proliferation, and ultimately tumor

development.[8] Hence, these hazardous agents cause oxidative stress and result in irreversible cell injury that they are no longer able to adapt.

Apoptosis is a fundamental biological process that is genetically controlled and required for both normal development and tissue homeostasis.[38] The morphological hallmarks of apoptosis result, including DNA fragmentation, membrane blebbing, cell shrinkage, mitochondrial remodeling, ROS production, and cleavage of a variety of proteins.[39,40] During the apoptosis stage, cells will undergo morphological alterations, such as karyolysis, karyorrhexis, and pyknosis. These nuclear abnormalities act as biomarkers to monitor individuals or populations exposed to mutagenic, genotoxic, or teratogenic events. The present study suggested that benzene exposure induced apoptotic response in buccal mucosal epithelial cells. Heretofore, the investigation on increase karyorrhexis incidence plays an important role in apoptosis events and acts as a versatile biomarker for evaluating the genotoxic, mutagenic, and teratogenic event or as reliable predictive parameters for cancer.

Apoptosis is also a form of programd cell death that is regulated by the Bcl-2 family and caspase family of proteins.[39] There are various apoptotic pathways exist that can be distinguished by the adapters and initiator caspases involved. Caspases involved in apoptosis have been subclassified by their mechanism of action and are either initiator caspases (caspase-8 and -9) or executioner caspases (caspase-3, -6, and -7).[40] Activating caspase-9 can directly cleave and activate caspase-3 and caspase-7.[39] The activation of caspases also is a marker for cellular damage. Heretofore, further research in the molecular aspect on activation of caspases plays an important role in apoptosis events should be conducted to affirm that apoptosis events happened in this study.

In accordance with Metgud et al.,[8] the analysis of buccal epithelium cells in this research also provides information about nuclear abnormalities, which is a useful index of chemical exposure and toxic response. The previous research[4,41] indicates that karyorrhexis and karyolysis are indicators of apoptosis. DNA damage sustained from normal DNA replication or cell processes, stress, mitotic catastrophe, agents such as radiation, toxins, hormones, growth factors, cytokines, and drugs, and ROS can induce apoptosis if left unrepaired. Early apoptosis is characterized by cell shrinkage, dense cytoplasm, tightly packed organelles, and pyknosis due to chromatin condensation. This is followed by budding which involves extensive plasma membrane blebbing, karyorrhexis, and separation of cell fragments into apoptotic bodies.[42]

Karyorrhectic cells have a dense network of nuclei chromatin elements that lead to fragmentation and disintegration of the nucleus.[5] Kumar et al.[32] stated that these cells showed the disintegration of the nucleus and may be highlighting the late stage in the cell death process of apoptosis. In karyolytic (KL) cells, the nucleus is devoid of DNA and appears to have no nuclei.[5] An increased number of KL cells has significance as these appear in the pre-keratinization process, which depicts an adaptive event to cellular trauma because of the chronic effects of the masticatory process in the oral mucosa. This anomaly is also evident in necrotic cells and is related to cytotoxicity. Kumar et al.[32] suggested that KR and binucleated cell formation should be considered as precious morphological stages of karyolysis and MN in squamous epithelium. Meta-nucleated anomalies were examined jointly for nuclear degeneration followed by carcinogenesis. During this process, series of sequential events lead to the formation of initially binucleated cells followed by MN and ultimately KR and KL cells, supporting homeostasis of oral epithelium.[32,43]

To monitor cytotoxic effects, the incidence of karyorrhexis was evaluated into this experimental design. As seen in the results section (Fig. 3), the findings revealed that karyorrhexis incidence was significantly higher in exfoliated buccal mucosal cells of petrol station employees in contrast to the control group. This may be due to the presence of benzene in automobile exhaust and fumes of the petroleum products which can contribute to the induction of cytogenetic damage and cellular death including micronuclei; binucleation and cellular death (pyknosis, karyolysis, and karyorrhexis) in buccal mucosal epithelial cells from petrol station employees.

Benzene exposure of petrol station employees can vary widely due to several factors, such as the quantity of fuel pumped, type and number of vehicles filled, protective measures, and the total content of benzene in the petroleum.[4] According to data obtained from the petrol stations included in this study, each worker pumps a different quantity of petroleum, containing 5% (v/v) benzene, during their 7- or 8-hour daily work shift. On the other hand, it should also be emphasized that petrol station employees are not only exposed to hydrocarbons present in petrol vapors but also the emissions produced by engines during fuel combustion. These factors are to be considered as an implicative parameter for high degree nuclear anomalies in buccal smears of petrol station employees.

Genomic damage is probably the most important fundamental cause of developmental and degenerative disease. It is also well established that genomic damage is produced by environmental exposure to genotoxins. In the present study, the duration of occupational exposure to benzene on employees varied and can be categorized based on their total duration of employment in the petrol station. The collected data indicated that 4 petrol station employees served for >10 years while the other 11 of them served for <10 years. We observed a higher degree of karyorrhexis incidence among employees who work >10 years (45–57) than employees who work <10 years (31–43). The study results are in agreement with the study conducted by Singaraju et al.[44] Employees who serve longer and probably had an increased occupational exposure. This may also be a causal nexus for a higher degree of karyorrhexis in their buccal smears.

Age factor may play a role in genotoxic effect in humans. In the current research, the study group for petrol station employees has a mean age of 31.6 ± 10.87. Six employees with the age group of 35–55 have an average karyorrhexis incidence of 46.5 while 9 employees with the age group 20–34 have an average karyorrhexis incidence of 35.56. The aging of the oral mucosa so far has been characterized mostly in relation to changes in the oral epithelium such as decreased mitotic activity and consequently a slowdown in tissue regeneration and healing rates. Most of these observations have been understood as progressive atrophy of the oral mucosa from the previous study by Eid et al.[45] Lamster and Northridge[46] were provided evidence for alterations in the oral mucosal with age are equivocal. Due to aging, as a consequence reduction in micronutrients that are critical for mucosal turnover and integrity will manifest themselves with an oral mucosal lesion at a relatively early stage. Mucosal atrophy with reductions in the attachment apparatus between the mucosa and the dermis is evidenced. This could be considered as a supportive factor for the low incidence of nuclear abnormalities in employees who are at a young age.

Apart from age, the incidence of karyorrhexis is also influenced by gender. Singaraju et al.[44] documented that the number of female petrol station employees is less than male subjects as it is customary for men to be engaged more in this occupation. To consider those female hormones, such as estrogen and progesterone, influence the growth and development of epithelial cells, and male hormones will affect bone metabolism and connective tissue matrix, cytomorphometric alterations, or oral mucosa are certainly affected by hormones.[47,48] Considering these, the current study only included male individuals as the study groups.

In addition, lack of personal protective equipment (PPE) application during petrol refuel handling however can be one of the contributing factors for the reduced quality of health among the petrol station employees. Most of them were not concerned about any health problems due to their occupational exposures and thereby the use of personal protection is abysmally low. The use of appropriate PPE should give a significant preventive impact on benzene exposure among petrol station employees. However, the usage of PPE, as observed in this study can be regarded as unsatisfactory. During field observation of the working environment, most of the employees do not use a facial mask or hand gloves as minimum personal protective measures. Hence, they are more liable to inhale or contact volatile organic compounds emitted during the vehicle refueling, which highly likely may cause cytotoxic and genotoxic effects on oral epithelial cells.

On the other hand, it is known that the genomic and cytotoxic damage can be affected by demographic factors, such as genetic/ethnic/cultural background, medical procedures (radiotherapy or chemotherapy within 1 year), local or systemic disease, dental procedures (wearing a denture or orthodontic appliances within 1 month), micronutrient deficiency and lifestyle factors (e.g., diet, alcohol, smoking, drugs, and stress). In the questionnaire analysis for the study group, we did not detect inappropriate health behaviors on lifestyle questions. The self-reported medical history showed that the main health problems were related to acute symptoms; determined slight changes appeared to be mainly associated with the direct irritant action of fuel vapor in eyes, and also to have an oral ulcer in a frequent manner previously. Borthakur et al.[35] suggested that chemical substances limit the proliferative ability of the epithelium, producing thinning and ulceration manifested predominantly in the oral mucosa. However, due to a lack of complete medical history and nutritional status data, a direct association of the manifested symptoms with benzene exposure could not be established. Hematological and neoplastic diseases were not alleged from petrol station employees, which was most likely due to the quiet character of the natural history of these diseases and the fact that the diseases lead quickly to absence from work. Also, these late manifesting diseases might not be present in the average young population studied. Therefore, all these demographic factors were set as controlled to avoid bias in the current study.

Conclusion

Petrol station employees had a significant increase in karyorrhexis incidence in exfoliated buccal mucosa epithelial cells who are occupationally exposed to petrol derivatives such as benzene.

Clinical Significance

This study indicates increased karyorrhexis incidence of exfoliated buccal mucosa epithelial cells caused by repeated exposure to benzene.

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