

Evaluation of salivary enzymes and oral lesions among gas station workers compared to nearby shopkeepers in Tehran

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Objective Studies on the association of air pollution and oral and dental health status are limited. This study was aimed to evaluate salivary enzymes and oral lesions among gas station workers compared to nearby shopkeepers, in relation to air pollution.

Methods In this study, we compared the level of total antioxidant capacity, superoxide dismutase and glutathione peroxidase in the saliva and oral lesions between gas station workers and shopkeepers. Fifty-two participants including 26 gas station workers and 26 shopkeepers were evaluated. These participants had at least 1 year of working experience and worked for 5 days a week. The inclusion criteria for the two groups were as follows: Absence of systemic diseases, no history of using immunosuppressive medications or oral spray, no history of radiotherapy or chemotherapy, absence of xerostomia and age range of 20–60 years. Shopkeepers were recruited from the nearby shops (less than 1 km distance from the gas stations). Saliva samples were collected from all 52 participants and evaluated.

Results The mean age was 33.08 ± 8.82 years for the gas station workers and 34.19 ± 12.28 years for the shopkeepers. The mean work experience was 8.38 ± 6.25 years and 8.38 ± 9.17 years for the gas station workers and shopkeepers, respectively. Level of salivary enzymes were not different between the two groups. No difference was observed about oral lesions among the two groups.

Conclusion This study showed that no difference existed between gas station workers and nearby shopkeepers regarding salivary enzymes and the appearance of oral lesions. Further studies are required to assess the effect of environmental pollutants on the saliva.

Keywords Total antioxidant capacity, superoxide dismutase, glutathione peroxidase, oral lesions, air pollution.

Introduction

Air pollution can have hazardous effects on human health. It negatively affects the nervous, respiratory, cardiovascular, renal, gastrointestinal system, pregnancy and cell function. According to recent statistics, about 3 million people lose their life annually due to air pollution, 90% of which, live in developed countries.¹

Environmental and occupational factors play an important role in health status of individuals. Occupational and environmental exposures may be related to disease symptoms. Environmental exposures in a specific patient may necessitate some interventions to prevent future side effects and minimize the risk of occupational diseases.² Environmental lead contamination is a global health dilemma. Lead is one of the most important environmental pollutants.^{3–5} It is mainly produced by the exhaustion of fossil fuels. Extensive use of lead in industries has resulted in air pollution and water and food contamination. Thus, the serum and salivary level of lead and its accumulation in different tissues in the human body have significantly increased in the recent years.⁶ As mentioned earlier, long-term deposition of lead in hard tissues is much higher than that in soft tissues.⁷ Heavy metals such as lead and cadmium have no physiological activity and are toxic even in low concentrations.⁸ Some studies have shown that dental hard

tissue can uptake and store lead and other heavy metals from the environment.

Dental hard tissue can become carious or may undergo abrasion, erosion or attrition. In the recent years, many studies have evaluated the effect of heavy metals on dental caries. Moreover, some authors believe that acidic vapors can cause non-carious dental lesions. Extensive dental problems can compromise the quality of mastication, mental power and work quality and result in gastrointestinal and other systemic problems. They can also cause serious economic and social problems.⁹

Antioxidants play an important role in inhibition of synthesis of reactive oxygen species such as O_2 , H_2O_2 and alkyl peroxy and repairing the damage caused by their activity.¹⁰ The oral cavity is the main route of entry of foods, drinks and inhalation substances. Saliva is the first protective barrier against environmental factors. Saliva possesses many protective mechanisms such as secretory immunoglobulin A, enzymatic-protein defense system, histatins, lysozyme and lactoferrin. The salivary antioxidant system is another defense mechanism of the saliva that includes uric acid, superoxide dismutase (SOD), total antioxidant capacity (TAC) and glutathione peroxidase (GPX).¹⁰ Antioxidants comprise a great

portion of our nutritional regimen. These antioxidants along with intracellular antioxidants and the enzymatic system protect the human body against inflammation, infections and tumoral processes.¹¹

Tabrizzadeh et al.¹² showed that lead can cause acute and chronic toxicity with a wide range of oral and systemic manifestations. They evaluated mine workers and workers of a textile company in Yazd city and reported that the observed signs and symptoms were not related to systemic intoxication with lead. Instead, they were most probably due to direct exposure of oral mucosa to lead during respiration. Those occupationally exposed to lead vapor show gingival margin pigmentation, a blue line along the gingival margin due to deposition of lead sulfide as the result of the activity of bacteria in the gingival sulcus, grayish areas on the buccal and tongue mucosa, decreased salivary flow, periodontal disease, metallic taste in the mouth, and tongue tremor when compressed.¹²

Winkler et al.¹³ evaluated and compared mine workers working at two different depths in a coal mine and found no significant difference in the saliva of the two groups. However, they proved that excessive contamination of soil in the working environment decreased the saliva flow and consequently decreased the salivary level of antioxidants.¹³

Air pollution adversely affects the human health. On the other hand, no previous study has evaluated the effect of air pollution on. Thus, we aimed to assess oral lesions and level of TAC, SOD and GPX in the saliva and compared them between gas station workers and nearby shopkeepers.

Materials and Methods

Study population

This study was conducted on 52 individuals out of which, 26 were gas station workers and 26 were shopkeepers. All participants signed written informed consent forms prior to participation in the study. Gas station workers were chosen from three gas stations in Tehran city and had a minimum of 1 year of work experience in gas station and work for 5 days a week. The inclusion criteria for the two groups were as follows:

Absence of systemic diseases, no history of using immunosuppressive medications or oral spray, no history of radiotherapy or chemotherapy, absence of xerostomia and age range of 20–60 years. Shopkeepers were recruited from the nearby shops (less than 1 km distance from the gas stations).

Sample size calculation

The following equation was used to calculate the sample size for the comparison of TAC, SOD and GPX salivary levels between the two groups:

$$n = \frac{(Z_{1-\alpha/2} - Z_{1-\beta})^2 (SD_1^2 + SD_2^2)}{d^2}$$

The standard deviation for the level of TAC, SOD and GPX was calculated to be 0.13 according to Ceretti et al.¹⁴ Minimum sample size was calculated to be 26 in each group to compare a difference with a magnitude of 0.1.

Data collection

1. Data were collected through clinical examination and mucosal status. The results of laboratory tests were also recorded.

2. Spectrophotometry and chromatography were used for the assessment of TAC, SOD and GPX.
3. Disposable dental mirrors and dental explorers were used for clinical examination. Also, 15 ml falcon tubes were used for saliva collection.

Saliva collection

Patients were instructed to spit into falcon tubes when comfortably seated straight to collect unstimulated saliva. They were requested to spit into the tubes until 5 mm of the tube was filled with saliva. The tubes were then capped, placed on dry ice and stored at –20°C. Using saliva kits, the salivary level of the three enzymes was measured.

Oral lesions

Oral mucosa was evaluated with a dental mirror and a spotlight to determine presence/absence of lichen planus, white and red lesions, ulcers, or aphthous lesions. The history of aphthous lesions was also evaluated.

Ethical considerations

This study was conducted on patients who signed written informed consent forms. The study was approved in the ethics committee of Tehran University of Medical Sciences, International Campus (IR.TUMS.VCR.REC.1395.1252).

Results

A total of 26 gas station workers and 26 shopkeepers were evaluated in this study. The mean age was 33.08 ± 8.82 years for the gas station workers and 34.19 ± 12.28 years for the shopkeepers. The mean work experience was 8.38 ± 6.25 and 8.38 ± 9.17 years for the gas station workers and shopkeepers, respectively. The difference in this regard was not significant between the two groups (Table 1).

Assessment and comparison of TAC, SOD and GPX salivary levels between the two groups are shown in Table 2. Comparisons were made using *t*-test. The difference between the two groups was not significant in any of these values (*P* > 0.05, Table 2).

Oral lesions were also compared between the two groups. The results revealed that 7.7% of shopkeepers had oral lesions while no gas station worker had oral lesions. However, this difference was not statistically significant between the two groups (*P* > 0.05, Table 3).

Discussion

By the growing population, number of motor vehicles has significantly increased. Also, by the increase in industries and factories, demand for fossil fuels has increased, resulting in significant and irreparable environmental destruction. This also has resulted in increased air pollution. Air pollution is due

Table 1. Demographic information of participants (n = 52)

		Mean	Standard deviation
Age	Shopkeepers	34.19	12.28
	Gas station workers	33.08	8.82
Work experience	Shopkeepers	8.38	9.17
	Gas station workers	8.38	6.25

Table 2. Level of TAC, SOD and GPX enzymes in the gas station workers and shopkeepers (n = 52)

Enzyme		Mean	Standard deviation	P-value
TAC	Shopkeepers	9.37	6.23	0.69
	Gas station workers	9.08	5.04	
SOD	Shopkeepers	96.63	46.91	0.91
	Gas station workers	93.86	44.14	
GPX	Shopkeepers	21.74	61.57	0.59
	Gas station workers	62.04	23.80	

Table 3. Oral lesions in gas station workers and shopkeepers (n = 52)

	Oral lesions	
	Present	Absent
Shopkeepers	2 (7.7%)	24 (92.3%)
Gas station workers	0	26 (100%)

to the presence of solid and liquid pollutant particles as well as carbon dioxide, sulfur dioxide, nitrogen dioxide and ozone in the air. High level of pollutants in the air can cause significant health problems for children and the elderly and result in irreversible long-term health complications.¹

Polluted air includes 10 µ or smaller particles, sulfur dioxide, nitrogen dioxide, photochemical oxidants such as ozone, carbon monoxide and lead.¹⁵ Ozone and solid particles produced by the exhaustion of motor vehicles have created serious public health concerns.¹⁶

Gas station workers are the true representatives of occupational groups constantly exposed to gases and vapors of fossil fuels. This exposure has created some concerns due to probable long-term effects on human health.¹⁷ When entered into the blood stream, these materials exert their cytotoxic and genotoxic effects.¹⁷⁻¹⁹

Exposure to genotoxic materials can occur through environmental exposure, non-specific contamination, occupational exposure or accidental industrial exposure.²⁰ Evidence shows that gases produced by the exhaustion of gasoline and diesel are mutagenic and carcinogenic for laboratory animals and probably humans.²¹

We evaluated the level of TAC, SOD and GPX in the saliva and compared them between the two groups of gas station

workers and shopkeepers. We found no significant difference between the two groups in this respect. However, Hallare et al.²² in a similar study reported that gas station workers compared to controls that had higher number of micronucleated cells.²² Also, Santos-Mello and Cavalcante¹⁷ cytologically evaluated gas station workers in Brazil and reported a significant increase in chromosomal deletions in the metaphase in gas station workers (0.829%) compared to controls (0.126%).¹⁷ Similarly, a study by Benites et al.²³ showed that gas station workers experienced a significant increase in micronucleated cells. However, Fredga et al. did not notice any increase in number of chromosomal disorders in gas station workers in Sweden.²³ Similar to gas station workers, police officers are also exposed to air pollutants. Such occupational exposures may cause mutagenic damage to epithelial cells of the buccal mucosa.²⁴

A previous study showed that the TAC of the saliva decreases by an increase in number of carious teeth and advanced age.²⁵ Our study indicated no difference in level of antioxidants between the two groups. Thus, it may be concluded that the level of exposure of gas station workers to pollutants was not high enough to affect TAC, SOD and GPX, or the level of air pollution was the same up to 1 km distance from the gas station. Oral lesions were also evaluated in our study. The results showed that the two groups were not different in this regard.

To our knowledge, this is the first study of its kind in Iran, which is considered as a strength of our study. The present study had some limitations. It was difficult to encourage people to participate in the study. Also, it was hard to find adequate number of eligible participants to reach the required sample size. We offered free dental clinical examination in order to persuade them to participate in the study.

Acknowledgement

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Conclusion

This study revealed no significant difference in salivary level of antioxidants and oral lesions between gas station workers and shopkeepers. Further studies are required to assess the effect of environmental pollution on the saliva.

Conflict of Interest

None ■

References

- Kampa M, Castanas E. Human health effects of air pollution. *Environ Pollut*. 2008;151:362-367.
- Kales SN, Christiani DC. Acute chemical emergencies. *N Engl J Med*. 2004; 350:800-808.
- Frank RM, Sargentini-Maier ML, Turlot JC, Leroy MJ. Comparison of lead levels in human permanent teeth from Strasbourg, Mexico City, and rural zones of Alsace. *J Dent Res*. 1990;69:90-93.
- Cleymaet R, Bottenberg P, Slop D, Clara R, Coomans D. Study of lead and cadmium content of surface enamel of schoolchildren from an industrial area in Belgium. *Community Dent Oral Epidemiol*. 1991;19:107-111.
- Cleymaet R, Bottenberg P, Retief DH, Slop D, Michotte Y, Coomans D. In vivo use of a dual acid etch biopsy for the evaluation of lead profiles in human surface enamel. *Caries Res*. 1991;25:256-263.
- Karahalil B, Aykanat B, Ertas N. Dental lead levels in children from two different urban and suburban areas of Turkey. *Int J Hyg Environ Health*. 2007;210:107-112.
- Hu H, Rabinowitz M, Smith D. Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms. *Environ Health Perspect*. 1998;106:1-8.
- Alomary A, Al-Momani IF, Massadeh AM. Lead and cadmium in human teeth from Jordan by atomic absorption spectrometry: Some factors influencing their concentrations. *Sci Total Environ*. 2006;369:69-75.
- Cenić-Milosević D, Mileusnić I, Kolak V, Pejanović D, Ristić T, Jakovljević A, et al. Environmental lead pollution and its possible influence on tooth loss and hard dental tissue lesions. *Vojnosanit Pregl*. 2013;70:751-756.

10. Raju PK, Vasanti D, Kumar JR, Niranjani K, Kumar MS. Oral hygiene levels in children of tribal population of Eastern Ghats: an epidemiological study. *J Int Oral Health*. 2015;7:108–110.
11. Goldie MP. Antioxidants in oral health care: making the connection. *Int J Dent Hyg*. 2005;3:93–95.
12. Tabrizzadeh M, Boozarjomehri F, Akhavan Karbasi M, Maziar F. Evaluation of the relationship between blood lead level and prevalence of oral complication in Koushk lead mine workers, Yazd province. *J Dent Tehran Univ Med Sci*. 2006;19:91–98.
13. Winkler O, Hadnagy W, Idel H. Cytokines detectable in saliva of children as appropriate markers of local immunity of the oral cavity—an approach for the use in air pollution studies. *Int J Hyg Environ Health*. 2001;204:181–184.
14. Ceretti E, Feretti D, Viola GC, Zerbini I, Limina RM, Zani C, et al. DNA damage in buccal mucosa cells of pre-school children exposed to high levels of urban air pollutants. *PLoS One*. 2014;9:e96524.
15. Cruz Ddl, Siador JC, Peralta T, Aguilar P, Barlis E, Socorro Jd, et al. National Air Quality Status Report (2003-2004). Philippines: Environmental Management Bureau, Department of Environment and Natural Resources; 2005.
16. Torres E, Subida R, Gapas J, Sarol J, Villarín J, Vinluan R, et al. Public health monitoring of the Metro Manila air quality improvement sector development program. Manila, Philippines: World Health Organization (WHO), Asian Development Bank (ADB), Philippines Department of Health (DoH). 2004;156.
17. Santos-Mello R, Cavalcante B. Cytogenetic studies on gas station attendants. *Mutat Res*. 1992;280:285–290.
18. Hadnagy W, Seemayer NH. Cytotoxic and genotoxic effects of extract of particulate emission from a gasoline-powered engine. *Environ Mol Mutagen*. 1988;12:385–396.
19. Crebelli R, Tomei F, Zijno A, Ghittori S, Imbriani M, Gamberale D, et al. Exposure to benzene in urban workers: environmental and biological monitoring of traffic police in Rome. *Occup Environ Med*. 2001;58:165–171.
20. Anderson D. Factors contributing to biomarker responses in exposed workers. *Mutat Res*. 1999;428:197–202.
21. Crebelli R, Conti L, Crochi B, Carere A, Bertoli C, Del Giacomo N. The effect of fuel composition on the mutagenicity of diesel engine exhaust. *Mutat Res*. 1995;346:167–172.
22. Hallare AV, Gervasio MK, Gervasio PL, Acacio-Claro PJ. Monitoring genotoxicity among gasoline station attendants and traffic enforcers in the City of Manila using the micronucleus assay with exfoliated epithelial cells. *Environ Monit Assess*. 2009;156:331–341.
23. Benites CI, Amado LL, Vianna RA, Martino-Roth Mda G. Micronucleus test on gas station attendants. *Genet Mol Res*. 2006;5:45–54.
24. Burgaz S, Demircigil GC, Karahalil B, Karakaya AE. Chromosomal damage in peripheral blood lymphocytes of traffic policemen and taxi drivers exposed to urban air pollution. *Chemosphere*. 2002;47:57–64.
25. Hershkovich O, Shafat I, Nagler RM. Age-related changes in salivary antioxidant profile: possible implications for oral cancer. *J Gerontol A Biol Sci Med Sci*. 2007;62:361–366.

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