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FLUORIDE CONCENTRATIONS IN SALIVA OF CHEMICAL PLANT WORKERS WITH RESPECT TO THE GENERAL CONDITION AND MICROBIAL STATUS OF THE ORAL CAVITY

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ABSTRACT: The aim of this study was to examine fluoride concentration in saliva and its relation to general condition and microbial status of the oral cavity in chemical plant workers occupationally exposed to fluorine compounds. The study included 90 men (control group: n=30 men, study group: n= 60 men) who took part in the examination of the clinical condition of teeth and oral hygiene status. In the saliva, microbial status was examined using a caries risk test (CRT® bacteria) and fluoride concentrations were measured using potentiometric method. The results show that *Streptococcus mutans* and *Lactobacillus acidophilus* counts were higher in the study group (with occupational exposure to fluoride) than in the control group. This means that a high fluoride concentration in saliva alone, without maintaining good oral hygiene, does not protect from dental caries and its consequences.

Keywords: Fluoride; Microbial status; Occupational exposure; Oral cavity; Saliva.

INTRODUCTION

According to a report by the Polish Ministry of Health, in comparison with other countries, the population of Poland tops the rankings in terms of the highest frequency of dental caries. Regrettably, such a high incidence of dental caries recorded in Poland, i.e., 86.9%, is exceptional—compared with 60.3% recorded in South Africa and 30% in the USA. In Poland, the percentage of edentulous adults between 35–44 and 65–74 years of age is rising rapidly. Additionally, in these periods of life, the average number of preserved natural teeth in the aforementioned group is decreasing sharply and the incidence of periodontal diseases has reached a very high level. The main reason for the occurrence of these dental problems in Poland may be a generally low health awareness in the Polish population.[1]

Delivering fluoride (F) to the enamel and dentin is one of the factors preventing caries. However, according to numerous studies, F can have a negative impact on the structure of the enamel and dentin.[2-7] Fluorosis in enamel manifests as subsurface hypomineralization, which expands towards dentine. This is most likely associated with delayed hydrolysis and removal of proteins from the enamel, especially amelogenins, during enamel maturation.[8] It was observed that high F doses in rat feed resulted in severe disturbance of dentin mineralization, manifested by the occurrence of extensive interglobular spaces filled with the proteins of organic matrix of dentin and blurred interface between unmineralized predentin and mineralized dentin.[9,10] Apart from the extensive interglobular spaces, there were wide decalcification stripes which identified the regions of poor mineralization.[9] Therefore, the question arises whether it is appropriate to promote the widespread use

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of F for the purpose of caries prevention and whether F is still an important aspect of safety and caries prevention.[11,12]

The aim of this study was to examine F level in saliva and investigate its correlation with the general condition and microbial status of the oral cavity in chemical plant workers with primary education level and occupational exposure to F compounds in comparison with patients with higher education level who were not occupationally exposed to F.

MATERIAL AND METHODS

Patients: The study included 90 men who self-reported as healthy. The study group (n=60, age: 20–62 years) comprised workers with primary education level and with direct exposure to F in gaseous form (1.9–2.7 $\mu g/m^3$), employed at the Phosphoric Acid Department of the Chemical Plant in Police, Poland. The time of exposure at their workstations is eight-hour shifts five days a week. The average employee tenure in the study group was 19.27 years, but half of those surveyed were employed in the aforementioned facility for more than 20 years. The control group (n=30, age: 25–60 years) comprised workers who were not exposed to F compounds in gaseous form at their place of employment. The controls were randomly selected from the patients with higher education level reporting to the Dental Practice of the Specialist Dental Clinic of the Pomeranian Medical University in Szczecin, Poland. The patients gave their consent to participate in the study.

F levels in drinking water in the area of residence for the members of both groups did not exceed 0.3 mg/L.[13]

Approval for the present study was given by the Bioethics Committee of the Pomeranian Medical University in Szczecin (number KB-0012/108/11). All individuals taking part in this research project underwent examination voluntarily and anonymously.

PHYSICAL EXAMINATION:

Examination of the clinical condition of teeth: The clinical condition of teeth was assessed according to the criteria of the World Health Organization, based on the number of teeth that are Decayed (D), Missing (M), and Filled (F). These numbers were used to calculate the DMF index for each patient using the following formula.

$$\text{DMF Index} = D + M + F$$

Where:

D = Number of decayed teeth
M = Number of missing teeth
F = Number of filled teeth

Examination of oral hygiene status: Oral hygiene status was evaluated according to the API (Approximal Plaque Index) as modified by Lange, which is an excellent method for assessing the effectiveness of the patient's oral hygiene practices. For the purposes of calculating the index, ten interdental spaces were checked for the presence of plaque, five on the vestibular side and five on the lingual side of the oral cavity. The index was calculated using the following formula:

$$\text{Approximal plaque index (API)} = \frac{\text{Sum of dental spaces with plaque}}{\text{Sum of all examined interdental spaces}} \times 100\%$$

The results were interpreted as follows:

API	=	70% – 100%	Inadequate oral hygiene
API	=	40% – 69%	Average oral hygiene
API	=	25%– 39%	Reasonably good oral hygiene
API	=	< 25%	Optimal oral hygiene

Microbial evaluation of saliva: The material used in microbial testing was stimulated saliva samples collected from patients at the beginning of the dental examination. To stimulate the saliva for collection, the patients were asked to chew a sterile paraffin tablet for 2 min and then spit all generated saliva into a sterile container. Salivary microbial testing was carried out using a caries risk test (CRT® bacteria) made by Ivoclar-Vivadent (Lichtenstein). After depositing a tablet of $NaHCO_3$ in the test tube and removing the protector films from test surfaces, saliva samples were applied using a sterile pipette onto the plate with selective culture media:

(i) CRT® bacteria by Ivoclar-Vivadent for growing *Streptococcus mutans* (SM);

(ii) CRT® bacteria by Ivoclar-Vivadent for growing *Lactobacillus acidophilus* (LB).

The plate with the culture media was placed in the tube which was then securely closed. Culture inoculation was performed according to the manufacturer's instructions. The tubes were deposited in an incubator (37ºC/48 hr). The tests were interpreted by comparing the number of bacterial colonies with the reference cards provided by the manufacturer:

CFU < 10^5 —low count of SM and LB (low risk of caries development)

CFU > 10^5 —high count of SM and LB (high risk of caries development)

Where:

CFU stands for colony-forming unit.

DETERMINATION OF FLUORIDE CONTENT IN SALIVA:

The procedure for material collection: F levels were measured in samples of approximately 3 mL of unstimulated mixed saliva, collected following an overnight fast or no sooner than 2 hr after a meal. Prior to sample collection, the patients did not brush their teeth using an F-containing toothpaste.

Saliva samples were centrifuged (10 min/4ºC/2,500 × g), and the resulting supernatant was used to determine F levels by potentiometric analysis using an ion-selective electrode (Orion 9609).

Measurement: TISAB II buffer (0.5 mL) was added to the 0.5 mL sample of post-centrifugation saliva. The ion-selective electrode was placed into the vessel and its potential was read after 5 min. Then, 0.1 mL of the relevant reference material was added and the sample was tested again. The potential of the electrode was read after 5 min. F concentration in the examined saliva samples was calculated from the difference in potential before and after adding the reference material. The electrode had been calibrated using standard solutions.

Statistical analysis: Statistical analysis was performed using Statistica 12.5 (Stat Soft, Poland) and Microsoft Excel 2010. The arithmetic means (AM) and standard deviations of the AM (SD) were calculated for each group. All continuous variables were checked for normal distribution using the Kolmogorov-Smirnov test. Statistically significant differences between the two groups were checked using Student's t-test for independent variables (normal distribution) or the Mann-Whitney U test (non-normally distributed data). Non-continuous variables were described in terms of quantity and prevalence. To examine the statistical relationships between non-continuous variables, the Pearson χ^2 test was employed. The level of significance was $p \leq 0.05$.

RESULTS

Results of salivary fluoride level analysis: The findings in terms of salivary F concentrations in patients from the study and control groups are presented in Table 1.

Table 1. Fluoride concentration in the saliva of patients from study and control groups

Group	n	Fluoride concentration in saliva [mg/L]					p
		Mean	SD	Min	Max	Median	
Study	60	0.992	0.811	0.205	3.456	0.717	0.0000
Control	30	0.045	0.060	0.007	0.324	0.028	

The average salivary F concentration in patients from the study group (0.992 mg/L) was twenty-two times higher than that in the patients from the control group (0.045 mg/L) and the difference was statistically significant. The lowest F level in the study group was twenty-nine times higher than the lowest level in the control group (Table 1).

Results of physical examination - dental status: During the clinical examination of the oral cavity, the teeth of patients from the study and control group were examined for such parameters as the number of teeth that were decayed (D), missing (M) and filled (F). On this basis, the average DMF index was determined for the study group and the control group.

Both in the study group and in the control group, the number of people with an active caries process was high. It was found in 85% of people in the study group, and in only a little fewer (80%) in those in the control group. The average number of decayed teeth in the study group (4.35) was higher than in the control group (2.77). The difference was borderline significant. In the study group, the average number of missing teeth was statistically significantly higher than in the control group. Half of the participants in the study group had 7 and more teeth missing, whereas in the control group the median number was 1 tooth. In the study group, the average number of filled teeth was less than one third of that in the control group. This difference was highly statistically significant (Table 2).

Table 2. Average number of decayed (D), missing (M) and filled (F) teeth in study and control groups

Group	n	D (decayed teeth)			p
		Mean	SD	Median	
Study	60	4.35	3.56	4.00	0.0517
Control	30	2.77	2.30	2.50	
Group	n	M (missing teeth)			p
		Mean	SD	Median	
Study	60	8.47	7.86	7.00	0.0005
Control	30	3.23	4.07	1.00	
Group	n	F (filled teeth)			p
		Mean	SD	Median	
Study	60	2.55	3.19	1.00	0.0000
Control	30	7.83	2.57	8.50	

The average DMF index calculated for both groups was similar, amounting to: 15.63 in the study group and 13.83 in the control group. The difference between the groups was not statistically significant (p=0.247).

For the study group, the dependence between tenure (number of years worked), and the numbers of D, M, and F teeth, and the DMF index was analysed in subgroups (Table 3). For the purpose of statistical analysis, the study group was categorized according to tenure into three subgroups: from 1 to 5 years, from 15 to 26 years, and 27+ years. The differences between the tenure-based subgroups in terms of the number of teeth with active decay (D) and number of missing teeth (M) were statistically significant.

Table 3. Relationship between years of work and numbers of decayed (D), missing (M), and filled (F) teeth, and total DMF Index (D+M+F) in the study group

Group	Duration of work [years]	n	D	M	F	D+M+F
Study	1–5	15	5.73 ± 3.75	1.93 ± 2.69	3.87 ± 3.89	11.53 ± 6.82
	15–26	27	4.78 ± 3.67	8.00 ± 5.99	2.26 ± 3.12	15.04 ± 4.99
			p=0.0003	p=0.0003	p=0.1453	p=0.0872
Study	1–5	15	5.73 ± 3.75	1.93 ± 2.69	3.87 ± 3.89	11.53 ± 6.82
	≥27	18	2.56 ± 2.55	14.61 ± 8.70	1.89 ± 2.42	19.06 ± 6.38
			p=0.0000	p=0.0000	p=0.1325	p=0.0058
Study	15–26	18	4.78 ± 3.67	8.00 ± 5.99	2.26 ± 3.12	15.04 ± 4.99
	≥27	27	2.56 ± 2.55	14.61 ± 8.70	1.89 ± 2.42	19.06 ± 6.38
			p=0.0114	p=0.0114	p=0.8183	p=0.0410

Results of physical examination – according to API (Approximal Plaque Index): The results in terms of the Approximal Plaque Index API for participants of both groups are presented in Table 4.

Table 4. Average Approximal Plaque Index (API) values in study and control groups

Group	n	API [%]			p
		Mean	SD	Median	
Study	57	61.83	33.24	60.00	0.0000
Control	30	21.43	22.53	13.00	

The average API in the study group (61.83%) fell in the 40–69% range, pointing to average oral hygiene, while in the control group it amounted to less than 25%, which is indicative of optimal oral hygiene. The difference was highly statistically significant (Table 4).

Results of salivary microbial tests: One of the methods of evaluating caries risk is to determine the cariogenic bacterial load (titre) of *Streptococcus mutans* and *Lactobacillus acidophilus* in saliva. Table 5 presents a comparison of the study and control group (percentages of participants), according to the bacterial load of SM and LB.

Table 5. Comparison, using the CRT® bacteria caries risk test by Ivoclar-Vivadent (CRT) of the number of patients in studied groups depending on the bacterial load of *Streptococcus mutans* (SM) (A) and *Lactobacillus acidophilus* (LB) (B)

(A) CRT SM test	Group				All
	Study		Control		
	n	%	n	%	n
< 10^5	29	48.33	21	70.00	50
> 10^5	31	51.67	9	30.00	40
Sum	60	100.00	30	100.00	90
χ^2 Pearson	3.80		df=1		p = 0.051

(B) CRT LB test	Group				All
	Study		Control		
	n	%	n	%	n
< 10^5	21	35.00	19	63.33	40
> 10^5	39	65.00	11	36.67	50
Total	60	100.00	30	100.00	90
χ^2 Pearson	6.50		df=1		p = 0.011

More than half of the people in the study group (51.7%) were found to have a high load of SM in the CRT SM test (CFU>10^5). In the control group, that percentage was lower and amounted to 30%. The analysis demonstrated a difference in the number of SM bacteria between the study and control group which was borderline significant (Table 5A). A similar correlation was observed in the CRT LB test. The difference between the study and control group in terms of the bacterial load of LB (Table 5B] was statistically significant.

In the evaluation of the bacterial load of SM and LB, no statistically significant correlations were found between the bacterial load and salivary F levels both in the study and control groups.

DISCUSSION

Saliva constitutes a natural environment for teeth, periodontium, and mucous membranes of the oral cavity and creates a microenvironment, determining the proper course of many biochemical and physical reactions.[14] The levels of salivary minerals, including F, are a consequence of the general health status (including age, smoking, alcohol intake, and environmental influence), as well as local factors (periodontal and dental status).[15,16] At present, little evidence exists concerning the association between salivary electrolytes and oral health. In our study, statistically significant higher concentrations of F in saliva were demonstrated in individuals with occupational exposure to high doses of this element (0.992 mg/L), as compared to the control group (0.045 mg/L) (Table 1). It might be supposed that the concentration of F in total saliva is related to F consumption, depending on its content in the environment, especially in drinking water. No statistically significant differences were found in terms of preferences in toothpaste choice. A substantial majority of the participants declared that they used F-containing toothpaste. The statistically significant higher concentration of F in the saliva of the study group could be explained by its increasing supply associated with occupational exposure. F ions can get into dental tissues from saliva, which constantly washes dental structures and creates a specific environment in the oral cavity.[14]

The tissues of the tooth consist of both organic and inorganic matter. The resistance of these tissues is determined by the quantitative content of the inorganic and organic matrix. It is well known that teeth with the right levels of minerals and a proper degree of mineralization are more resistant to the activity of external cariogenic factors.[17] In the crystalline hydroxyapatite matrix, some of the sites that are occupied by calcium atoms, and hydroxyl or phosphate groups may be replaced by other elements, among which a special role is played by F ions.[18] Due to its bacteriostatic properties, F blocks the activity of enzymes and inhibits the growth of dental plaque, thus lowering its cariogenicity. The presence of F in appropriate concentration reduces enamel mineral loss by diminishing the solubility of dental hydroxyapatite which becomes more resistant to demineralization.[19] On the other hand, it has been shown that F oversupply and intake into hard tissues could interfere with the crystal nucleation process.[20] Crystallization would occur continuously toward c-axis at the peripheral area, whereas the central area would remain amorphous, resulting in crystal defects with a fuzzy structure appearing in the teeth and bone.[20] It may ultimately weaken the tooth structure and accelerate the onset and progression of the decay process. This was observed in our study. The dental status of workers exposed to F evaluated according to the average DMF index was comparable to that of the control group. However, in the analysis of the individual components of the index, it was demonstrated that the employees of “Police” Chemical Plant had a higher average number of decayed and missing teeth than the members of the reference group (Table 2).

The presence of plaque not only has an adverse effect on teeth, but also contributes to the development of periodontal disease. Poor oral hygiene leads to accumulation of bacterial plaque on tooth surfaces and in interdental spaces, thus constituting an important risk factor for caries and periodontal disease.[15] In our study, the participants of the study group were found to have statistically significantly higher amounts of plaque (expressed as a percentage) than those in the control group (Table 4). One would have expected that higher F content in the saliva of people with occupational exposure to that mineral should somehow protect their teeth from plaque formation. Yet, our findings suggest that good hygiene and frequency of tooth brushing may still be of primary importance.

According to the current state of knowledge, the development of a carious lesion is dependent on a combination of factors including: the presence of plaque, supply of certain carbohydrates, susceptibility of tooth surfaces, and sufficiently long time of cumulative operation of the three aforementioned factors. SM and LB are regarded as cariogenic bacteria because of their ability to rapidly produce acids from fermenting carbohydrates, to survive in an acidic environment, and to adhere to tooth surfaces due to synthesising viscous extracellular polysaccharides from sugars. Many authors demonstrated a significant correlation between the number of cariogenic bacteria and the intensity of caries.[21-27] The research conducted by our team suggests that more than half of the participants with occupational exposure to F (51.7%) have a high count of SM bacteria in saliva, whereas in the control group a high count was found in only 30% of participants (p=0.051). A similar regularity was observed for LB: a high count of LB was found in 65% of the study group, while in the control group a high count of LB bacteria was noted in approximately 37% of participants (p=0.011). It is suggested that the high count of LB may have been related to the larger number of teeth with active caries in the study group, as this genus is believed to be responsible for the development of caries, whereas SM is considered to initiate them.

Kuch and Szkaradkiewicz conducted a study wherein they compared the intensity of caries in adults with the number of cariogenic bacteria.[28] The authors demonstrated a statistically significant higher average DMF index in the group with a high count of LB. With regard to SM, statistically significant higher DMF values were found in the group of participants with a low count of SM. In our study, the same correlations for LB and SM were found. However our results were not statistically significant.

We made some interesting observations with regard to the F levels in saliva vs. the number of bacteria. One would expect that the higher salivary levels of F found in the “Police” Chemical Plant workers, as compared to the control group, would bring down the number of cariogenic bacteria. F acts as a catalyst in the process leading to the formation of stronger crystalline structures (fluorapatite) with a decreased dissolution rate, and also inhibits bacterial adhesion to dental tissues, thus reducing plaque accumulation. Moreover, F inhibits glucose transport across bacterial cellular membranes (by inhibiting enolase activity), as well as blocking bacterial metabolism. Interestingly, the results observed in our study point to the opposite: higher bacterial counts of SM and LB were observed in the study group (with occupational exposure to F) than in the control group. This means that high F concentration in saliva alone, without maintaining good oral hygiene, does not protect from dental caries and its consequences.

CONCLUSIONS

The priority in terms of caries prevention should be to increase health awareness of the population, regardless of education level. Oral health awareness should not be reserved only for people with higher education. Particular emphasis should be placed on the correct technique and frequency of effective mechanical removal of dental plaque combined with the use of toothpaste since the two approaches are complementary in reducing the incidence of dental caries. Simply increasing the F concentration in enamel and dentin is not effective or safe in caries prevention.

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