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Original Research

The effect of asthma medications on ion concentration in carious and non-carious primary molar teeth

Asthma medications and teeth caries

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Abstract

Aim: Asthma is one of the most common childhood diseases. Evidence shows a positive correlation between the use of asthma medications and dental caries.
Material and Methods: The participants included in the study were categorized into four groups: healthy children with carious teeth (CH), children on asthma medication with carious teeth (CAM), healthy children with non-carious teeth (NCH), and children on asthma medication with non-carious teeth (NCAM). The element analysis of the extracted teeth was performed using inductively coupled plasma mass spectrometry (ICP-MS).
Results: The Mn levels of the CAM group were found to be statistically significantly higher than that of the NCAM group ($p < 0.001$). The CAM was found to have significantly higher levels of Cu than CH ($p = 0.002$). It was determined that the S levels of CH and CAM groups were statistically significantly higher than the NCH and NCAM groups, respectively. ($p = 0.003$, $p < 0.01$, $p = 0.004$, and $p < 0.01$, respectively). The levels of Sr, Al, and Zr were also detected to be statistically significantly higher in the NCAM than in NCH ($p = 0.002$, $p = 0.007$, and $p = 0.009$, respectively).
Discussion: Regardless of whether there is tooth decay or not, higher levels of Sr, Zr, Cu, and Al elements were found in the teeth of children using asthma medications compared to the teeth of healthy children. The results obtained regarding the relationship between dental caries and Pb, Mn, Cu, Sr, Li, and S levels were similar to those reported by other studies in the literature. The results of our study regarding the correlation of Mo and Al with dental caries are different from those reported in some previous studies.

Keywords

Asthma, Dental Caries, Trace Element, Inductively Coupled Plasma Mass Spectrometry

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Introduction

Asthma is a heterogeneous respiratory disease. It has symptoms such as wheezing, shortness of breath, chest tightness, and cough and is characterized by chronic airway inflammation. Furthermore, asthma can be accompanied by variable expiratory airflow limitation, which might become permanent [1]. The World Health Organization (WHO) has reported that asthma is one of the most common chronic childhood diseases [2]. Long-term controller medications and quick-relief medications are often used to manage asthma symptoms [3].

Dental caries is a biofilm disease that develops over time through complex interactions among various factors, including acidogenic bacteria, sugar-containing food, teeth, and saliva, thereby causing mineral loss in the teeth [4]. For decades, asthma has been referred to as a significant risk factor for caries formation, with recent studies corroborating this relationship [2]. This positive correlation between asthma and dental caries may be explained by the fact that asthma medications reduce salivary flow and reduce the oral pH below 7 [5]. In addition to the physiological effects of the medications, the method of their administration can also increase caries risk. For example, the prolonged oral stay of the drugs in case of improper inhaler use increases the risk of caries formation [6].

Previous studies have reported some trace elements to be closely associated with dental caries [7-14]. In some studies, molybdenum (Mo) [7], vanadium (V) [7], fluorine (F) [7, 8], strontium (Sr) [8-11], and lithium (Li) [7, 9] were reported to have a cariostatic effect, whereas selenium (Se) [7], lead (Pb) [7, 12, 13], manganese (Mn) [9, 14], copper (Cu) [7, 12] and zinc (Zn) [7] were reported to have a cariogenic effect.

Accordingly, the current study examines extracted carious and non-carious primary molars of healthy children and children on asthma medication to analyze changes in their ion concentrations and elaborate on how asthma medication affects the predisposition of children with asthma toward dental caries.

The null hypotheses of the current study are (a) there is no difference between the element levels in the carious teeth of healthy children and children on asthma medication, (b) there is no difference between the element levels in the non-carious teeth of healthy children and children on asthma medication.

Material and Methods

Patient Selection and Sample Collection

Pediatric patients who applied to our institution met the inclusion criteria and wanted to participate in the study were included in the study. For all the pediatric patients included in the study, their parents or legal guardians provided consent via written informed consent forms.

The sample size was determined based on the study of Tvinnereim et al. [15] and the calculations were made with alpha error = 0.05, beta error = 0.20, and an effect size of 1.3. According to these calculations, nine teeth for each group and a total of 36 teeth would be adequate for the sample size. However, considering the possible data losses, a total of 40 primary molars were included in the study.

In this study, primary molars extracted from different patients were evaluated. Carious teeth were extracted due to the presence of extreme caries, while non-carious teeth were extracted due to delayed physiological root resorption. The teeth included in the study were not restored. Dental pulp was not removed before the tooth extracts were prepared. The included patients were divided into four groups (n = 10 teeth for each group) of carious and non-carious teeth of healthy children and children on asthma medications. Group 1 included the carious teeth of healthy children (CH), group 2 included the carious teeth of children on asthma medication (CAM), group 3 included the non-carious teeth of healthy children (NCH), and group 4 included the non-carious teeth of children on asthma medication (NCAM).

Preparation And Analysis Of Samples

The teeth were extracted using suitable extraction techniques for primary molars without damaging the teeth. The extracted teeth were then rinsed with physiological saline and stored in formaldehyde fluid in a glass container until the completion of sample collection. The carious teeth had crowns and roots; however, some of the non-carious teeth that were extracted due to physiological resorption did not have roots. After the completion of sample collection, the teeth were ground along with the crown and roots using a ring grinder they were formed a powder, with the particles being less than 100 microns in size. Following the grinding procedure, the powdered tooth samples were dissolved with aqua regia, after which the element levels in the samples were analyzed with ICP-MS (Perkin Elmer Nexion 2000).

Statistical Methods

Subsequently, the SPSS (Statistical Package for the Social Sciences, SPSS Inc. Chicago, IL, USA) 17.0 statistical package program was used to analyze the data obtained in the study. Descriptive statistics were given as mean ± standard deviation or median and min–max values. Kruskal–Wallis and Mann–Whitney U tests, as well as Bonferroni correction, were utilized for multiple intergroup comparisons, with Mann–Whitney U test being used for paired comparisons. Spearman's correlation test was performed to assess the element levels in the carious and non-carious teeth of children who were on asthma medication. Since the values obtained in the analyses of some element levels were under the threshold value, the number of samples was inadequate for some groups for the analysis of these elements. Therefore, the groups where the number of samples collected was insufficient for the statistical analysis of these elements were excluded from the comparison, and their critical p values were calculated again by the Bonferroni correction. $p < 0.05$ was considered statistically significant.

Ethical Approval

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Results

There was no statistically significant difference among the groups for Pb levels ($p > 0.05$). The Mn levels of the CAM group were found to be statistically significantly higher than those of the NCAM group ($p < 0.001$). The Cu levels of the CH group were statistically significantly lower than that of the CAM group, and the CH group was statistically significantly higher than that of

Table 1. Comparison of the analysis results of Pb, Mn, Cu, S, Sr, Mo, Li, Al, and Zr elements among the groups

	CH	CAM	NCH	NCAM
	mean±SD;median (min-max)			
Pb (ppm)	2.16±1.06;2	3.13±1.61;3.05	1.41±0.86;1.5	2.51±1.81;1.9
	(1-4.8)	(1-6)	(0.5-2.8)	(0.6-5.9)
Mn* (ppm)		9.69±3.67;9	4.6±1.53;4.45	4.85±1.59;4.65
		(5-16)a	(2.8-7.8)	(2.6-7.8)b
Cu (ppm)	7.61±1.85;7.9	13.5±4.71;11.8	3.75±0.8;3.7	3.93±0.78;3.8
	(4.9-10.2)c	(7.8-19.4)d	(2.5-5.3)e	(3.1-6)f
S (%)	0.059±0.003;0.06	0.075±0.015;0.07	0.05±0.01;0.05	0.054±0.017;0.05
	(0.05-0.06)g	(0.06-0.1)h	(0.04-0.09)i	(0.04-0.09)j
Sr (ppm)	67.94±14.92;71.9	732.08±2035.21;81.05	76.63±19.73;78.2	1111.95±1770.11;118.05
	(34.2-86.4)	(50.1-6523.7)	(40.2-109.2)k	(83.5-5342.2)l
Mo** (ppm)	1.01±0.41;1	1.24±0.54;1.15		0.81±0.20;0.7
	(0.5-1.9)	(0.6-2.2)		(0.6-1.3)
Li*** (ppm)	1.17±0.15;1.1	1.39±0.5;1.15		1.48±0.51;1.2
	(1.1-1.6)	(1-2.4)		(1-2.2)
Al (%)	0.023±0.013;0.02	0.091±0.11;0.02	0.02±0.021;0.01	0.094±0.11;0.02
	(0.01-0.06)	(0.02-0.33)	(0.01-0.08)m	(0.02-0.36)n
Zr (ppm)	4.16±7.92;1.55	15.93±20.66;6.7	4.7±7.9;1.15	13.98±15.68;8.45
	(0.7-26.6)	(0.7-55.4)	(0.6-25.1)o	(2.5-50)p

* According to Bonferroni correction; * for Mn (critical p<0.025)
* * for Mo (critical p<0.025)
*** for Li (critical p<0.025)
p value ; a-b (p<0.001), c-d p=0.002;p<0.01, c-e (p<0.001), d-f (p<0.001), g-i p=0.003;p<0.01, h-j p=0.004;p<0.01 k-l p=0.002;p<0.01, m-n p=0.007;p<0.01, o-p p=0.009;p<0.01

Table 2. Correlation coefficients for elements in carious teeth of children using asthma medication

	Al	Cu	S	Sr	Mn	Mo	Pb	Zr	Li
Al				0.778					
Cu						0.689			
S						0.758			
Sr									
Mn						0.738			0.654
Mo									
Pb									-0.654
Zr									
Li									

*Spearman correlation coefficients

Table 3. Correlation coefficients for elements in non-carious teeth of children using asthma medication

	Al	Cu	S	Sr	Mn	Mo	Pb	Zr	Li
Al				0.849					
Cu									0.834
S									
Sr							-0.802		
Mn						0.726		-0.661	
Mo									
Pb									
Zr									
Li									

*Spearman correlation coefficients

the NCH group ($p = 0.002$, $p < 0.01$, and $p < 0.001$, respectively). The Cu levels of the CAM group were found to be statistically significantly higher than those of the NCAM group ($p < 0.001$). It was determined that the S level of CH was statistically significantly higher than the S level of NCH, and the S level of CAM was statistically significantly higher than the S level of NCAM. ($p = 0.003$, $p < 0.01$, $p = 0.004$, and $p < 0.01$, respectively). Sr levels in the NCAM group were found to be statistically significantly higher than those in the NCH group ($p = 0.002$; $p<0.01$, respectively). The Al levels of the NCH group were found to be statistically significantly lower than that of the NCAM group ($p = 0.007; p < 0.01$, respectively). The Zr levels of the NCH group were found to be statistically significantly lower than that of the NCAM group ($p = 0.009; p < 0.01$, respectively). There was no statistically significant difference among the groups in terms of Mo and Li levels ($p > 0.05$) (Table 1).

According to the correlation coefficients of the element levels detected in the carious teeth of children using asthma medication, the element pairs Cu–Mo and Mn–Li was in the moderate positive correlation of statistical significance ($r = 0.689$, $p = 0.028$; $p < 0.05$, $r = 0.654$, $p = 0.04$; $p < 0.05$, respectively), Al–Sr, S–Mo, and Mn–Mo were in the strong positive correlation of statistical significance ($r = 0.778$, $p = 0.008$; $p < 0.01$, $r = 0.758$, $p = 0.011$; $p < 0.05$, $r = 0.738$, $p = 0.015$; $p < 0.05$, respectively), Pb–Li were in the moderate negative correlation of statistical significance ($r = -0.654$, $p = 0.04$; $p < 0.05$) (Table 2).

According to the correlation coefficients of the element levels detected in the non-carious teeth of children on asthma medications, the element pairs of Al–Sr, Cu–Li, and Mn–Mo were in strong positive correlation of statistical significance ($r = 0.849$, $p = 0.002$; $p < 0.01$, $r = 0.834$, $p = 0.01$; $p < 0.05$, $r = 0.726$, $p = 0.027$; $p < 0.05$, respectively), the Sr–Pb pair was in strong negative correlation of statistical significance ($r = -0.802$, $p = 0.017$; $p < 0.05$, respectively), and the Mn–Zr pair was in moderate negative correlation of statistical significance ($r = -0.661$, $p = 0.038$; $p < 0.05$, respectively) (Table 3).

Discussion

The results of our study showed that there were significant differences in the levels of elements evaluated in carious and non-carious teeth between children using asthma medication and healthy children, and therefore, the null hypotheses were rejected.

Compared with other analysis techniques, ICP-MS and Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) is more advantageous since they have appropriate limits of detection, allows simplicity in preparing samples for analysis, provides ease of injection, requires only small quantities of samples for analysis, allows the simultaneous analysis of multiple elements, achieve high accuracy and sensitivity in analysis results, and facilitate easy data processing [16]. In this study, ICP-MS was preferred because it has a wider detection limit range.

As a result of the data we obtained, it was found that the Pb level of NCAM was higher than NCH, and the Pb level of CAM was higher than CH, which was not statistically significant. Yalçın et al., [17] on the other hand, studied the dental and blood samples collected from healthy children and children with asthma. They reported that blood and tooth Pb levels were similar in the asthma group and the control group, and no significant difference was reported between the two groups. Increased Pb levels in tissues and body fluids affect IL-4 synthesis and result in increased IgE synthesis by B lymphocyte cells, thereby indirectly affecting the mechanism of asthma [18]. This further explains the relationship between asthma and high Pb levels in tissues and body fluids. In the present study, on evaluating Pb levels in carious and non-carious teeth, they were found to be higher in the carious teeth of both the healthy children and children of asthma medication, with no statistically significant difference among the groups ($p > 0.05$). Overall, above mentioned possible positive correlations between Pb and dental caries can be explained by Pb being a divalent cation (Pb^{+2}) and the fact that it replaces the isovalent fields of Ca in hydroxyapatite crystals, which are among the basic structures of dentine tissue [19].

In a study where Yalçın et al. [17] analyzed the levels of elements in the blood and teeth samples collected from healthy children and children with asthma. The Mn levels in the samples were only slightly higher in healthy children than in those with asthma, and no statistically significant differences were found among the groups ($p>0.05$). In a study where Soutar et al. [20] focused on the role of Mn in asthma, it was suggested that Mn intake was inversely proportional to bronchial reactivity. However, as per the results of the current study, the Mn levels were found to be slightly higher in the NCAM group than in the NCH group, with no significant difference between them ($p>0.05$). Considering the involvement of Mn in dental caries, it has been suggested that Mn stimulates the metabolism of dental caries-causing bacteria, such as Streptococcus mutans, facilitates sucrose-associated biofilm development, and protects against the oxidative stresses that occur during dental plaque formation [21]. According to the results of this study, the Mn level of CAM was found to be statistically significantly higher than the Mn level of NCAM ($p<0.001$), in the presence of caries, Riyat et al. [9] and Poletto et al. [14] also detected statistically significantly higher levels of Mn in the teeth and saliva, respectively. These findings are consistent and may also indicate the medication-based increase for the present study. Possibly, caries existence could elevate the absorbed amount of Mn in primary teeth tissue.

Considering that increased Cu levels can incapacitate the antioxidant system and indirectly cause inflammation, which is a primary symptom of asthma [22], a positive correlation between asthma and Cu can be suggested. In the present study, the Cu levels were statistically significantly higher in the CAM group than in the CH group ($p = 0.002$), while the Cu levels were only slightly higher in the NCAM group than in the NCH group, with no statistical difference between the groups ($p>0.05$). These findings are consistent with another study [23], in which asthmatic patients were found to have higher serum Cu levels. Furthermore, in the present study, Cu levels were found to be increased in the presence of dental caries in both healthy children and children on asthma medication. The literature presents different findings regarding Cu's involvement in dental caries. Additionally, there is one study that found higher levels of Cu in carious teeth [12] and another study that reported lower levels of Cu in carious teeth [13].

In the present study, the Sr levels were also found to be statistically significantly higher in the NCAM group than in the NCH group ($p=0.002$). Regarding the association between Sr and dental caries, when used in specific concentrations, it is known that the combination of Sr and fluoride enhances enamel remineralization in vitro experiments [8]. In the current study, The Sr level of NCH was higher than CH, and the Sr level of NCAM was higher than CAM. Based on the correlation coefficients of the non-carious teeth of children using asthma medication, a statistically strong negative correlation was found between Sr and Pb, which, to the best of our knowledge, are considered to be cariostatic and cariogenic, respectively, according to the literature. ($r=-0.802, p=0.017; p<0.05$, respectively). Both Li et al. [10] and Riyat et al. [9] has reported higher Sr levels in non-carious teeth than in carious teeth. This negative correlation between Sr and the presence of dental caries can be explained by the fact that Sr shrinks the hydroxyapatite crystal surface, making it harder to dissolve, thereby reducing its solubility as Sr covers the crystal surfaces and inhibits bacterial growth that could be considered as consistent to our findings including non-carious teeth samples [10].

The present study reported slightly higher levels of Mo in the CAM group than in the CH group; however, no statistical difference was observed between the two groups ($p>0.05$). A study in Hungary that investigated the relationship between dental caries and Mo found that children living in an area where the drinking water contained Mo had lower rates of dental caries than those living in an area with no Mo content in the drinking water [7]. In the present study, Mo levels were higher in the CAM. However, no significant difference was found between the CAM and NCAM groups in terms of Mo levels ($p>0.05$). Since the amount of previously published articles is very limited, this finding may also require further investigation.

Tanaka et al. also found that Al levels in the enamel of non-carious teeth were significantly higher than that in the enamel of carious teeth and that the Al levels were significantly higher in the dentin of non-carious teeth than in that of carious teeth. It has been said to inhibit bacterial growth and reduce bacterial growth and reduce bacterial colonization on enamel surfaces [24]. In the present study, Despite Al levels being significantly lower in the NCH group than in the NCAM group ($p=0.007$), no significant differences were found between CH-NCH and CAM-NCAM. These contradictory findings may be related to the sample size inadequacy that should be further investigated. However, a statistically significant positive correlation was observed between the Al–Sr levels in the carious and non-carious teeth of children on asthma medication ($r = 0.778$, $p = 0.008$; $p < 0.01$, $r = 0.849$, $p = 0.002$; $p < 0.01$, respectively) which may indicate the possible cariostatic effect of Al and Sr irrespective of the asthma medication usage.

Lithium has been reported to reduce the incidence of dental caries [7]; Riyat et al. [9] found higher levels of Li in non-carious teeth. In the current study as well, the NCAM group showed higher levels of Li than the CAM group, but there was no statistically significant difference between the groups ($p>0.05$). The results of the current study may also support the cariostatic effect of Li.

In the current study, Zr levels were found to be statistically significantly higher in the NCAM group than in the NCH group ($p=0.009$). To the best of our knowledge, there is only one study that has investigated the relationship of Zr with dental caries and asthma, and these studies have been unable to detect this element in teeth and blood [11].

To the best of our knowledge, the effect of S on cariogenicity has not been investigated adequately. According to the results of the current study, the S level of CAM was statistically significantly higher than NCAM, and the S level of CH was found to be statistically significantly higher than NCH ($p=0.004$, $p=0.003$, respectively). In their study, Yamaguchi et al. found that hydroxyapatite in the enamel structure had a smaller and more ambiguous crystal configuration in teeth exposed to hydrogen sulfide [25]. These changes generated by an S-containing compound in the enamel may be closely associated with the increase in its level in the presence of dental caries, as consistent with our findings.

For the other paired correlation coefficients obtained in this study, to the best of our knowledge, no relationship was found to form a basis in the literature. This issue should also be investigated in future studies.

There are some limitations of this study. Increasing the total number of teeth can help attain higher clarity and precision in the results. Furthermore, there is no complete standardization on the duration of the medications used by the children with asthma and possible rehabilitation conditions. Recall collected from the patients with asthma and their parents were obtained verbally and then confirmed with the information available from the electronic database in our clinic. Despite detailed recall, there may still be errors because of potentially incorrect information reported by the parents. The depth and extent of the carious lesions on the collected carious teeth from both healthy children and children on asthma medication have not been standardized. The above issues may affect the results of the analyses.

In conclusion, regardless of the presence of dental caries, the teeth of children who were on asthma medication had higher Sr, Zr, Cu, and Al elements compared to healthy children's teeth. This finding is one of the most noteworthy results of this study. The current study reported higher levels of Cu and S in the carious teeth of healthy children and children on asthma medications compared with non-carious teeth. In addition, children on asthma medications were found to have higher levels of Mn in their carious teeth than in their non-carious teeth. These results also provide important data regarding the relationship between caries, elemental composition, and the use of asthma medication.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article. This study was produced from the thesis number YOKTEZ-725654 prepared by Yakup Dogru, under the supervision of Tamer Tüzüner.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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