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Oral health status and trace elements in saliva of children and teenagers with intellectual disabilities: A preliminary study

Ruiz Roca Juna Antonio[1], Lorca Larrosa Maria[1], Ruiz Roca MI[2], Tvarijonaviciute Asta[3], Pons-Fuster Eduardo[1] and López- Jornet Pia[4*]

[1]Department of Medicine and Dentistry, University of Murcia, Spain

[2]Head of studies at Public Special Education Center (c.p.e.e. primitiva lópez), Cartagena, Spain

[3]Regional campus of international excellence "mare nostrum", university of Murcia, ed.16, 4a planta, Espinardo, Murcia, Spain

[4]Oral Medicine, Faculty of Medicine and Dentistry, University of Murcia, Spain

Abstract

Aim: The aim of this study was to assess oral health status and analyze trace elements present in the saliva of children with intellectual disabilities and compare them with a control group.

Methods: This prospective case-control study included 30 patients diagnosed with intellectual disability attending the *Primitiva López* Special Education Center (Cartagena, Spain) and 22 healthy control subjects. Medical histories were prepared for each subject who then underwent oral examination. Unstimulated whole saliva was collected and the following elements were measured using inductively coupled plasma mass spectrometry (ICP-MS): Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, P, Rb, S and Zn.

Results: The oral and dental health status of patients with intellectual disability was similar to that of control group subjects. Similar values were found in both the study group and the control group for Ca, K, Mg, Na, Pb, P and S. Significantly higher values were found in the study group for Fe and Mn ($p<0.01$).

Conclusion: The present finding that Fe and Mn are present at higher levels in the saliva of study group patients poses the question of whether these differences could be a reflection of intellectual disability.

Clinical significance

Trace elements or oligoelements play a vital role in maintaining the integrity of a range of physiological and metabolic processes. In this context determining the concentrations of trace elements is important for a better understanding of their impact on oral health.

Introduction

Oral health is an important component of general health and promoting oral health can reduce the overall burden of illness. Patients with intellectual disability are often a forgotten group when it comes to oral healthcare although they may require complex multidisciplinary care [1-3]. The evidence shows that persons with intellectual disability suffer worse oral health, periodontal status, and present a higher risk of caries [1-3]. In addition to compromised oral health status, these patients may also have greater difficulty in accessing oral healthcare services [1-6]. Routine oral healthcare and dental treatment is often erratic, leading to poor oral health and the rapid progression of any oral diseases present [2,7].

Trace elements or oligoelements play a vital role in maintaining the integrity of a range of physiological and metabolic processes that take place in living tissue. As each trace element is related to enzymatic systems, the inadequate ingress of trace elements into the organism may have deleterious effects on different tissue functions and may lead to disease [8]. For this reason, analyzing changes to the concentrations of oligoelements in saliva could lead to better understanding of any oral functional abnormalities present [9-10].

Hedge, *et al.* [11] found significant increases in Cu and Zn in the saliva of patients with caries in comparison with a caries-free control group. Watanabe, *et al.* [12] also reported an increase in salival Cu among subjects with caries. But these findings contradict Borella, *et al.* [13] who reported reductions in Zn and Cu proportions in patients with more than three carious teeth. Gaur and Agnihotri [14] found that micronutrient oligoelements such as Fe, Zn, Cu and Se are essential for regulating immuno-inflammatory pathways and increased levels can be a reflection of chronic periodontitis. In this way, identifying and measuring biomarkers can help assess oral health status and it is for this reason many researchers have set out to identify the specific markers of each oral disease [15,16].

Correspondence to: López- Jornet Pia, senior lecturer and professor of oral medicine, faculty of medicine and dentistry, university of murcia. Hospital morales meseguer. clínica odontológica universitaria. Medicina oral adv. Marques de los velez s/n murcia 30008. Spain, Tel: 00 34 968 398588; E-mail: majornet@um.es

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The aim of this study was to analyze the oral health status and presence of trace elements in children with intellectual disability, comparing data with a control group.

Materials and methods

Design, area, study population, and ethical considerations

This prospective, observational case-control study was designed to fulfill regulations established in the Helsinki Declaration and was approved by the Research Ethics Committee of the University of Murcia (16th October 2014). Informed consent was obtained from the parents/guardians of the subjects. The study followed the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines

The study group was made up of children attending a state-run Special Education Center (CPEE *Primitiva López*, Cartagena, Murcia, Spain). The center has 143 students aged between 4 and 21 years with different levels of intellectual disability (slight, moderate, or severe). A total of 30 subjects were recruited into the study participants are consecutively selected in order of apperance according to their convenient accessibility. All underwent oral examination and saliva sampling. The control group consisted of children without any disability (between 7years and -15 years age group) attending the *Santa María de la Paz* primary school in Murcia (Spain), matched to the study group

Inclusion, and exclusion criteria

Study group inclusion criteria were: subjects with intellectual disability defined according to WHO criteria. Exclusion criteria were: the presence of dental restorations, prostheses, orthodontic apparatus, or treatment by anticolinergics, acute infection, decompensated systemic disease, cancer, radiotherapy, or unwillingness to grant consent to take part. Medical notes were prepared for each subject registering socio-demographic information and the data obtained in oral examination. A single researcher (MLL) performed all intraoral examinations of subjects in both groups, registering the following: presence of caries (according to criteria proposed by the WHO in Oral Health Survey Basic Methods); the presence or absence of oral mucosa lesions, soft and hard deposits evaluated using the simplified Greene and Vermillion oral hygiene index (OHI-S).

Saliva collection

Unstimulated mixed saliva was collected in repose using the draining method, always in the morning. The patient was instructed not to eat or chew gum for 2 hours before collection. Patients remained seated in a comfortable position with the head slightly inclined forwards and the lips half open. They were asked to hold the saliva collecting in the mouth for a minute, allowing it to fall into a funnel placed in a calibrated test tube at the end of each minute; the process was continued for 5 minutes.

Trace element analysis was performed at the Science and Applied Biology Center (CEBAS-CSIC) of the University of Murcia (Spain). Inductively coupled plasma mass spectrometry (ICP-MS) was used to analyze levels in saliva of Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, P, Rb, S and Zn.

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics v21 software (SPSS Inc., Chicago, IL, USA). Homogeneity of variance was analyzed using Levene's test. Comparison of means was performed applying Student's t-test for independent samples for two factors. Statistical significance was set at $p<0.05$.

Results

The general characteristics of the study population are shown in Table 1. A relatively low percentage (13.3%) of the study group presented slight intellectual disability. With regard to medication, subjects were taking a mean of 1.20 ± 1.15 drugs. Of all patients with disability, 66.7% did not speak or had problems communicating; only 33.3% were able to communicate without difficulty. Oral exploration found that of the 30 subjects in the study group, 11 (36.7%) presented caries. Only one presented an oral lesion (queilitis exfoliative).

When soft deposits on teeth were examined, no significant differences were found between the study and control groups ($p>0.05$). But for hard deposits on dental surfaces, significant differences were found ($p<0.01$) between the groups, hard deposits having greater presence in the control group. In the analysis of trace elements present in saliva, similar values were found between the study and control groups for Ca, K, Mg, Na, Pb, P y S. In the study group values for Cr, Cu, Fe, Mn, Ni, Rb and Zn were higher, although statistically significant differences were only identified for Fe and Mn ($p<0.01$) (Table 2).

Table 1. The general characteristics of the study population

		Disability (n = 30)	Control group (n = 22)	P-value
Age years		15.03 ± 4.61	12 ± 0.5	< 0.05
Sex	Male: n (%)	20 (66.7%)	13 (59.1%)	0.575
	Female: n (%)	10 (33.3%)	9 (40.9%)	
Intellectual disability	Slight moderate severe	4(13.3%) 14(46.7%) 10(40.0%)		
Caries	Yes: n (%)	11 (36.7%)	7 (31.8%)	0.717
	No: n (%)	19 (63.3%)	15 (68.2%)	
Oral lesions	Yes: n (%)	1 (3.4%)	0	0.387
	No: n (%)	29 (96.6%)	0	
Soft deposits on dental surfaces	Yes	9 (30%)	10 (45%)	0.253
	No	21 (70%)	12 (55%)	
Hard deposits on dental surfaces	Yes	4 (13.3%)	10(45%)	0.010
	No	26 (86.7%)	12 (55%)	

Table 2. Analysis of trace elements present in saliva Mean and standard deviation (SD), and p value

Element	Group study Mean ± SD (N = 30)	Control Mean ± SD (N = 22)	
Ca (g/100gr)	0.0092 ± 0.0133	0.0079 ± 0.0024	0.501
Cr (mg/kg)	1.309 ± 3.8494	0.0360 ± 0.1684	0.128
Cu (mg/kg)	0.0932 ± 0.1286	0.1114 ± 0.4407	0.831
Fe (mg/kg)	4.6626 ± 9.0565	0.0387 ± 0.1811	**0.009**
K (g/100gr)	0.0698 ± 0.03001	0.0715 ± 0.01441	0.795
Mg (g/100gr)	0.0002 ± 0.0004	0.0004 ± 0.0002	0.160
Mn (mg/kg)	0.0610 ± 0.0957	0.0005 ± 0.0021	**< 0.01**
Na (g/100gr)	0.0272 ± 0.0307	0.0175 ± 0.0153	0.139
Ni (mg/kg)	0.3978 ± 0.8731	0.0866 ± 0.1809	0.107
Pb (mg/kg)	0.1933 ± 0.3286	0.1597 ± 0.1687	0.664
P (g/100gr)	0.0169 ± 0.0073	0.0178 ± 0.0061	0.662
Rb (mg/kg)	0.8031 ± 1.1977	0.3921 ± 0.1556	0.1171
S (g/100gr)	0.0044 ± 0.0034	0.0046 ± 0.0024	0.822
Zn (mg/kg)	3.3857 ± 9.9561	1.7242 ± 4.6543	0.472

Table 3. Analysis of trace elements present in unstimulated saliva with caries and soft deposits on dental surfaces

	Group With Caries			Soft Deposits On Dental Surfaces		
Element	**STUDY (N = 11)**	**Control (N = 7)**	**Pvalue**	**Study (N = 9)**	**Control (N = 10)**	**P value**
Ca (g/100gr)	0.008 ± 0.006	0.008 ± 0.003	0.888	0.007 ± 0.003	0.008 ± 0.003	0.661
Cu (mg/kg)	0.068 ± 0.092	0.305 ± 0.782	0.326	0.046 ± 0.063	0.221 ± 0.653	0.438
Cr (mg/kg)	2.510 ± 6.294	0.112 ± 0.298	0.334	**0.480 ± 0.525**	**0.079 ± 0.249**	**0.045**
Fe (mg/kg)	6.829 ± 13.293	0.121 ± 0.321	0.205	0.934 ± 1.392	0.085 ± 0.268	0.075
K (g/100gr)	0.066 ± 0.037	0.069 ± 0.0161	0.844	0.066 ± 0.020	0.072 ± 0.014	0.442
Mg (g/100gr)	0.0001 ± 0.0003	0.0001 ± 0.0003	0.065	0.0002 ± 0.0004	0.0005 ± 0.0002	0.111
Mn (mg/kg)	0.0313 ± 0.0697	0.001 ± 0.003	0.280	0.036 ± 0.073	0.001 ± 0.003	0.142
Na (g/100gr)	0.027 ± 0.034	0.021 ± 0.025	0.743	0.017 ± 0.008	0.022 ± 0.021	0.461
Ni (mg/kg)	0.583 ± 1.389	0.133 ± 0.303	0.416	0.143 ± 0.079	0.121 ± 0.250	0.805
Pb (mg/kg)	0.125 ± 0.174	0.192 ± 0.249	0.511	**0.069 ± 0.071**	**0.226 ± 0.207**	**0.045**
P (g/100gr)	0.018 ± 0.007	0.017 ± 0.006	0.778	0.015 ± 0.007	0.017 ± 0.006	0.565
Rb(mg/kg)	1.108 ± 1.859	0.346 ± 0.185	0.301	0.457 ± 0.124	0.351 ± 0.131	0.092
S (g/100gr)	0.004 ± 0.001	0.004 ± 0.002	0.747	0.004 ± 0.002	0.005 ± 0.002	0.311
Zn (mg/kg)	5.808 ± 15.948	3.370 ± 7.799	0.713	1.032 ± 1.104	2.571 ± 6.496	0.494

Among subjects with caries, mean values of trace elements Ca, K, Mg, Na, P and S were very similar, while Cr, Fe, Mn, Ni, Rb and Zn levels were much higher in the study group, while and Cu and Pb were higher in the control group, although no element showed statistically significant difference ($p>0.05$) between the two groups (Table 3). Analyzing subjects showing presence of soft deposits on dental surfaces, Cr, Fe, Mn obtained higher values in the study group, with statistically significant differences for Cr ($p = 0.045$) and Pb ($p = 0.045$) (Table3). For subjects showing presence of hard deposits, Cr, Fe, Mn, Ni and Rb obtained higher mean values in the study group, while Ca, Cu, Na, Pb and Zn were higher in the control group, although statistically significant differences were not found between study and control subjects.

Discussion

Healthcare for patients with intellectual disabilities is of increasing interest to healthcare professionals as many of these patients have a limited capacity for cooperation, communication, and oral self-care and so constitute a risk group requiring specialized dental care from an early age [5,6].

Lakshmi and Geetha [17] studied the levels of certain trace elements in the hair and nails of children with autism, finding higher concentrations of Cu, Pb and Hg than among healthy children, as well as decreased levels of Mg, Zn and Se. It is known that the presence of Cu is essential to health but excessive levels have neurotoxic effects such as irritability, depression, nervousness and learning and behavioral disorders. Kalra, *et al.* [18] found lower blood levels of Fe, Cu and Mg among children with cerebral paralysis compared with a group of healthy children. In the present study, mean Fe and Mn values were significantly higher in subjects with intellectual disability than control subjects. Manganese (Mn) is an essential nutrient that protects against oxidative damage; but exposure to excessive levels of Mn can cause toxic effects, especially on the central nervous system; neurotoxic effects have been detected in children exposed to high concentrations of Mn in drinking water. Neurotoxicity deriving from Mn produces subtle early effects that may be present prior to the appearance of any clinical signs or symptoms [18,19].

The main oral health problems among children with intellectual disabilities are dental caries, periodontal disease, and malocclusion [5-8]. Among the various factors involved in the appearance of these diseases are poor oral hygiene maintenance, a cariogenic diet, oral respiration, bruxism and thumb sucking. A cariogenic diet is common among all children, as many eat between meals, often in the form of refined carbohydrates and it is difficult to manage or prevent this behavior [3-5]. They are also affected by the medications manufactured for children with high sugar content, which have secondary effects such as gingival hyperplasia or increased risk of caries. The present study did not find significant differences in oral health status between the study and control groups.

Poor oral hygiene is common among persons with intellectual disability who often suffer high levels of dental plaque, gingivitis, intense halitosis with food remnants left on the teeth and mucosa [20]. Gingival inflammation can develop extensively and rapidly at an early age among children with intellectual disability, more so than among children without this disability, increasing in severity with age and according to the degree of disability [14,21]. In the present work, 30% of the subjects with intellectual disability presented bacterial plaque, a similar percentage to the control group. Perhaps this can be explained by the fact that this particular group was under the supervision of a multidisciplinary group of healthcare professionals.

Concentrations of oligoelements in saliva have been associated with the incidence of oral disease in different population groups [14]. In this context, determining the concentrations of trace elements is important for a better understanding of their impact on oral health. In subjects presenting good oral health, mean Ca, Cu, Cr, Fe, Na, Mn, Ni, Pb, Rb and Zn concentrations were higher in the study group, with statistically significant differences for Cu, Fe, Mn and Na ($p < 0,05$). Disequilibrium in Fe levels affects the periodontal, bone metabolism, and host response, Fe being essential to life as it plays a role in almost all oxidation/reduction (redox) processes and is present in many enzymes involved in the maintenance of cell integrity [14].

When the trace elements present in subjects with caries in both groups were analyzed, it was found that mean Cr, Fe, Mn, Ni, Rb and Zn levels were higher in the study group. Increased Cu and Pb could be related to dental caries, as stated by Zhair, *et al.* [22]. Although the mechanism of interrelation between Cu in saliva and caries remains unknown, a possible explanation is that the fracture of hydroxyapatite crystals releases Cu ions into saliva [12]. Decreases in other trace elements such as Ca and Zn have been associated with caries and would appear to play a protective role, whereby increasing amounts of these trace elements in diet could improve oral and dental health status. However, Bhandary, *et al.* [23] obtained contradictory results,

with lower levels of Cu and Zn in patients with caries. Zinc (Zn) is naturally present in plaque, saliva and teeth and is essential to the remineralization process, while its deficit increases the frequency of infection and degenerative pathology.

The saliva collection procedure is easy, inexpensive, and non-invasive. Furthermore, the samples are readily accessible (24). The study suffered several limitations, in particular its small sample size and transversal design. Further research is necessary through studies of prospective design. Furthermore, gingival inflammation was measured using the simplified version of the Greene and Vermillion oral hygiene index (GVOHI-S); as an oral hygiene index, this is not the appropriate means of measuring periodontal disease activity. However, the index could be a significant parameter for oral health analysis and in particular for assessing the oral health of persons with disability.

In conclusion, both the group of subjects with intellectual disability and the control group presented similar oral healthcare needs. Concentrations of Ca, K, Mg, Na, Pb, P and S in saliva were similar in both groups. But mean Fe and Mn values were significantly higher among subjects with intellectual disability and control subjects. This poses the question of whether these differences could be a reflection of intellectual disability.

Conflict of interest

The authors declare that they have no conflict of interest.

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