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The association of metal exposure and Sjögren's disease: a comparative study

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Abstract

Background

Sjögren's disease (SjD) affects the ocular surface and oral health and has systemic manifestations. We investigated metals as potential triggers and diagnostic markers in the pathophysiology of SjD.

Methods

The comparison made between SjD (n = 57), in accordance with the guidelines of the American College of Rheumatology and European League for Rheumatism, and healthy control volunteers (n = 21). They were compared with demographic data, clinical evaluation, and the dosage of metals in the saliva and urine by the Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Results

SjD patients exhibited higher urinary excretion of Ag, Hg, Mn, Pt, Sb, Sn, and U, while Al, Se, and Mg were lower ($p < 0.05$). In saliva, 16 metals had higher levels in SjD: Al, As, Be, Ca, Cd, Co, Cu, Fe, Hg, Mn, Mo, Pb, Sb, U, W, and Zn ($p < 0.05$). Correlational analysis showed that Cu and Sb in saliva correlated with higher OSDI score ($r = 0.55$, $p = 0.0002$, 95%CI 0.28–0.75 and $r = 0.34$, $p = 0.026$, 95%CI 0.04–0.59, respectively), As and Cu in saliva with lower salivary flow ($r=-0.56$, $p < 0.0001$, 95%CI -0.74– -0.30 and $r=-0.41$, $p = 0.007$, 95%CI – 0.64– -0.11, respectively) and urinary Sn and salivary As and Zn were associated with higher corneal staining score ($r = 0.40$, $p = 0.010$, 95%CI 0.095–0.635, $r = 0.35$, $p = 0.0025$, 95%CI 0.37–0.6 and $r = 0.33$, $p = 0.038$, 95%CI 0.01–0.58, respectively).

Conclusions

Metal levels in saliva and urine were significantly different between SjD and healthy individuals. Antimony, arsenic, copper, tin, and zinc presented correlations with SjD clinical findings, suggesting that environmental metal exposure may influence disease manifestations in SjD.

1. Introduction

Sjögren's disease (SjD) is a multisystemic autoimmune pathology that can affect multiple organs and cause hypofunction of the lacrimal and salivary glands [1]. The cause is unknown, and due to the variable presentations, it is plausible to consider that environmental factors interfere with the clinical presentations [2;3;4]. In recent years, the growing evidence of the influence of metals on disease presentations has motivated the investigation of the relationship between metals and SjD [5].

The prevalence of SjD is between 0.3 to 1.5% of the population, and it is predominantly in women, with a mean age of the beginning of symptoms of 45 years [6]. SjD affects exocrine glands, but extraglandular manifestations are common, and the mechanisms that modulate the inflammatory pathways, including the kynurenine/tryptophan and oxidative stress pathways, could be influenced by environmental contaminants, triggering dysfunctions on the immune system [7–9].

Trace metals are associated with essential metabolic pathways, including catalytic, structural, and regulatory actions in the cellular actions [10]. Cobalt, copper, iron, manganese, molybdenum and zinc are integral parts of metaloenzymes and metalloproteinases, associated with redox processes, protein, DNA, and RNA, synthesis, gene expression, cell replication in addition to carbohydrate and lipid metabolism [11, 12].

In recent years, those inorganic elements have also been associated with disease mechanisms. Although tolerable in the environment and in the body in small concentrations, human activities, including mining and industrial waste, may expose the soil, water, air, and food to toxic levels of several metals and their intake through dermal, lung, and gastrointestinal contact [13–15].

Metals are biomarkers of connective tissue diseases (CTD) and associated with disease mechanisms [5].

In rodent models of autoimmune disease, excess of iron (Fe) in the diet induced pro-inflammatory manifestations, including autoantibodies production, T follicular helper (Tfh) cell expansion and cytokines secretion [16]. Zinc (Zn) and Cadmium (Cd) deficiency have been associated with rheumatoid arthritis (RA) [17, 18]. In a comparative study in psoriasis, the serum levels of Cooper (Cu) was higher, and the levels of Selenium (Se) and Zn was lower in patients compared to healthy controls [19].

Moreover, in a comparative study with RA and healthy controls, the serum levels of Cu were also higher and the Zn levels were lower than in healthy controls [20].

Although the causes and the mechanisms are not fully understood, the high frequency of SjD in the population and the complexity in the diagnosis and in the treatment of refractory SjD cases, more information about the environmental influence, including trace metals and their correlation with clinical manifestations justify this study.

Our hypothesis is thatenvironmental contaminants, including trace metals, are higher in SjD individuals and they can trigger epigenetic or metabolic and functional processes associated with the disease.

The present study aims to investigate whether SjD individuals and healthy controls present different concentrations of metals potentially involved in the pathophysiology of SjD. To answer that, the levels of 25 metals in the saliva and 29 metals in the urine were evaluated in SjD and healthy controls, and their correlation with clinical signs and symptoms was analyzed.

2. Material and Methods

2.1. Study population and design

Recruit of Individuals with SjD from the outpatient clinic of Ophthalmology of the Clinical Hospital of Ribeirão Preto Medical School at University of São Paulo (FMRP-USP), Brazil, ocurred from March to June 2023. For the control group (n = 21), healthy volunteers recruited from the hospital staff, without any other remarkable medical history and negative for Dry Eye Disease and blepharitis, were recruited to match the age and sex profile of the SjD group individuals. All participants gave informed consent, and the Research Ethics Committee of HCFMRP – CEP/HCFMRP– approved the study (CAAE: 37209120.3.0000.5440, File number: 4.347.216). This study was performed in accordance with relevant guidelines and regulations of the Declaration of Helsinki.

The design is a cross-sectional case-control study. The participants were recruited by spontaneous demand among SjD patients (n = 57) of the outpatient clinic and healthy volunteer health workers (n = 21) of the institution. The inclusion criteria for the SjD group followed the guidelines of the American College of Rheumatology and European League for Rheumatism 2016 [1].

2.2. Clinical Evaluation

Evaluation of the exposure to metals by the application of questionnaires about lifestyle habits, like the frequency of use of cosmetic products, such as sunscreen, hair gel or spray, perfume, body lotion, plastic to heat food, use of hygiene products such as antiseptic soap, shampoo/conditioner, consumption of canned food, and source of drinkable water, among others.

All participants answered a structured questionnaire regarding demographic data, chronic and recent medication, lifestyle, and medical history.

The questionnaire contained yes/no questions and questions investigating the frequency of several habits (never, rarely, < once a week, once a week, 2–3 times a week, more than 3 times a week, or every day), and the answers were converted in score from 0 to 7 [10].

All the data collected in the clinical, ocular, and laboratory exams were stored in the RedCap, and the data available in the data bank or patient record were used for analysis and comparisons described below. The same tests for the control group. The ocular exam included biomicroscopy, tonometry, and fundus examination. Ocular surface evaluation followed the sequence of the least invasive to the most invasive tests. The evaluation of the ocular surface consisted of:

1. Application of The Ocular Surface Disease Index (OSDI) validated Portuguese version, and the scores compared among the groups [23].
2. Tear film break-up time (TFBUT) measured after instilling fluorescein 1% eye drops (Eye Pharma, São Paulo, Brazil), into the conjunctival sac, the individual is instructed to blink once and keep his eye open for analysis of the tear film breakage at the slit lamp with cobalt blue light.
3. Cornea staining test with fluorescein eye drops was examined with the slit lamp with cobalt blue light filter, and the changes were graded from 0 to 15. The classification of the cornea alterations

was done by the intensity and position of the staining in 5 different areas. The staining of these areas is quantified by a numeric scale in each region, varying from 0–3, in which 0 means no staining, 1 is punctate staining, 2 is confluent points, and 3 is staining in plates. The score is measured by the sum of the values of each of the 5 areas.

4. Conjunctiva staining with lisamin green 1% eye drops (Eye Pharma, São Paulo, Brazil) instilled in the conjunctival sac, the conjunctiva and cornea were examined at the slit lamp, and the staining was classified from 0 to 18, considering the intensity of each area from 0 to 3.
5. Schirmer Test measured the tear flow in both eyes of each volunteer, of all groups, using standard strips of filter paper of 5x60mm (Eye Pharma, São Paulo, Brazil), with the conjunctival cul-de-sac for 5 minutes. The reading of the length of humidity registered in mm, and the values compared between the two groups.

The diagnostic methods and scores applied according to the criteria established in “Tear Film Ocular Surface Dry Eye Workshop” (TFOS DEWS), and the data for comparison considered in the eye with the worst results for each indicator [22].

The salivary flow evaluated by measuring the complete unstimulated flow of secretion in rest conditions for 15 min as previously described [8].

2.3 Analysis of the dosage of metals in urine and saliva

For the analysis of metals in urine samples, the method of inductively coupled plasma mass spectrometry (Nexlon 200, Perkin Elmer, Norwalk, CT, USA), operating with high purity argon (99.999%, AirLiquid, Brazil), was used. All the reagents used in this study were analytical grade (Sigma-Aldrich, St Louis, USA). Nitric acid (HNO3) was purified by distillation under the boiling temperature (sub-boiling), using a quartz distillator (Kürner Analysentechnik).

The determination of the metals in saliva samples, calibration curves, and patterns prepared in concentrations varying from 0 to 200µg/L, with a diluting solution containing HNO3 2%. Following, each sample was diluted 10x in a diluent solution and injected directly into the equipment, with readings in triplicate and the results expressed in µg/L.

To determine the metals in urine samples, calibration curves and patterns made with matrix adjustments, and the patterns were prepared in concentrations varying from 0 to 200µg/L, with a diluent solution containing HNO3, 0.5% Triton X-100, 0.01%. To verify the accuracy and safety of the results, it was utilized as a reference material for the urine of the Institut National de Sante Publique du Quebec (Quebec, Canada) (INSPQ). Following it, each sample diluted 20 times in a diluent solution and injected directly into the equipment, with readings in triplicate and the results expressed in µg/L [9].

For saliva, the dilution was 200 µL/4 mL 2% HNO3. Analyses performed by using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) equipped with a Quadrupole Ion Deflector (NexION ® 2000, PerkinElmer, Shelton, CT, USA).

In order to verify the accuracy of the data, several reference biological samples obtained from the INSPQ were used as part of the external quality assessment Schemes (EQAS), and were analyzed. All the analyses were made in the Analytical Toxicology Laboratory (ASTox) of the Clinical Analysis, Toxicological and Bromatological, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo.

2.4 Statistical analysis of the results

Descriptive statistics were applied for demographic, clinical, and laboratory tests. The urinary and salivary values of metals were compared with the Shapiro-Wilk test and were proven non-parametric. For the comparison of clinical, salivary, and urinary metal dosage results, the Mann-Whitney test was used. Categorical data were analyzed by the Fisher test. The Spearman test was applied to compare the levels of trace metals and clinical findings in the SjD group (GraphPad Prism, Boston, MA, USA).

The correlation between clinical data and metal dosage in urine (Fig. 1A) and saliva (Fig. 1B) was described using heat maps.

3. Results

The present work compared demographic, clinical, and metal levels data in urine and saliva between SjD and control individuals and correlated the metal levels with the clinical data.

The demographic data between the two groups are similar (Table 1). The clinical parameters revealed the marked signs and symptoms of SjD in this group, including a high score of OSDi, for frequency of ocular surface symptoms, reduced salivary and tear flow (Schirmer test), and scores of corneal and conjunctival staining (Table 1).

Table 1
Comparative demographic and clinical data between SjD and the Control Group (C). Med = median. The tests for parametric data were the Student, and for non-parametric data, the Mann-Whitney U Test; $p < 0.05$ was considered significant.

Parameters	**Control**				**SjD**				**p-value**
Demography									
Sex ratio	21	21 F: 0 M			57	54 F: 0 M			-
	n	**Mean ± SD**			**n**	**Mean ± SD**			
Age (years)	21	50.1±11.6			57	54.4±13.2			0.18
Body Weight (kg)	14	69.2±10.9			45	69.9±12.1			0.84
Height (m)	14	1.62±0.05			45	1.62±0.06			0.88
BMI (kg/m^2)	14	26.3±4.1			45	26.9±4.6			0.66
Clinical Data	**n**	**Med**	**P25**	**P75**	**n**	**Med**	**P25**	**P75**	
OSDI		-			44	30.9	20.1	49.3	-
Salivary flow (g/min)	16	0.15	0.08	0.17	44	0.05	0.1	0.08	0.0008
Corneal Staining score (OD)					43	3.0	2.0	6.0	-
Corneal Staining score (OS)					43	4.0	1.0	6.0	-
Conjunctival Staining Score (OD)	14	4.5	1.75	7.75	45	6.0	2.0	9.5	0.54
Conjunctival Staining Score (OS)	14	5.0	2.0	6.25	45	6.0	2	8	0.54
Schirmer Test (OD)	17	17.0	8.0	24.0	48	5.0	1.0	16.25	0.0046
Schirmer Test (OS)	17	20.0	8.5	26	48	5.0	1.25	15.0	0.0050

Med: Median, OSDI: ocular surface disease index, P25: percentile 25, P75: percentile 75, SD: standard deviation.

Table 1. Comparative demographic and clinical data between SjD and the Control Group (C). Med = median. The tests for parametric data were the Student, and for non-parametric data, the Mann-Whitney U Test; $p < 0.05$ was considered significant.

The questionnaire to explore the frequency of potential exposure to environmental contaminants to investigate possible contact with trace metals did not identify a significant difference in habits between SjD and control individuals, $p > 0.05$ for all comparisons (Table 2).

Table 2
Questionnaire about frequency of exposure to potential environmental contaminants of SjD (n = 44) and Controls (n = 14). The test for non-parametric data used was the Mann-Whitney U Test, $p < 0.05$, were considered significant.

Variable	C				SjD				p-value
	N	Med	P25	P75	N	Med	P25	P75	
Water in Plastic Bottles	14	6	1.5	7	44	7	7	7	0.0127
Cigarettes	14	0	0	0	44	0	0	0	0.7224
Deodorant	14	7	7	7	44	7	0	7	0.0157
Canned food	14	1	0	1	44	1	0	1	0.5623
Mouthwash	14	0	0	3.25	44	2	0	7	0.2034
Body sunscreen	14	0	0	7	44	0	0	7	0.5714
Facial sunscreen	14	7	0	7	44	6.5	0	7	0.7234
Hair gel or spray	14	0	0	3.75	44	0	0	0	0.1191
Moisturizing Lotion	14	3	2	7	44	7	6.25	7	0.0135
Perfume	14	3.5	0	7	44	3	0	7	0.7834
Plastic to heat food	14	1	0	5.5	44	0	0	6.75	0.4590
Antisseptic soap	14	0	0	1.75	44	0	0	0	0.7994
Shampoo/conditioner	14	3	2	3.25	44	3	1.25	4	0.5254
Total	14	33	29	42.75	44	36.5	25.25	46.5	0.9565
Med: median, P25: percentile 25, P75: percentile 75.									

Table 2. Questionnaire about frequency of exposure to potential environmental contaminants of SjD (n = 44) and Controls (n = 14). The test for non-parametric data used was the Mann-Whitney U Test, $p < 0.05$, were considered significant.

Comparative analysis of 29 metals in saliva and 29 in urine of individuals with SjD and Controls revealed that 16 were significantly higher in the saliva of SjD individuals (Al, As, Be, Ca, Cd, Co, Cu, Fe, Hg, Mn, Mo, Pb, Sb, U, W, Zn) (Table 3) and 07 (Ag, Hg, Mn, Pt, Sb, Sn, U) in the urine of individuals with SjD (Table 4). Among those, Hg, Mn, Sb, and U were higher in both secretions of the SjD individual compared to the controls. In addition, Al, which was significantly higher in the saliva of SjD individuals and Se, which was similar in the saliva of both groups, presented significantly higher concentrations in the urine of Control individuals (Tables 3 and 4).

Table 3. Dosage of 25 Metals in the Saliva of Individuals With Sjögren Disease and Controls

Variable	Controls			Sjögren disease				P value
	N	Median	P25	P75	N	Median	P25	P75	
Ag 107 (µg/L)	21	2.83	1.90	3.28	54	1.47	0.96	2.09	**0.0003**
Al (µg/L)	21	1.12	1.03	1.20	54	1.28	1.15	1.57	**0.0001**
As 75 (µg/L)	21	0.93	0.74	1.11	54	1.11	0.85	1.73	**0.0304**
Ba 138 (µg/L)	21	33.01	28.69	64.69	54	39.48	23.46	86.85	0.9812
Be 9 (µg/L)	21	0.22	0.17	0.38	54	0.34	0.26	0.46	**0.0410**
Ca 43 (mg/L)	21	102.73	80.87	130.12	54	133.56	105.85	190.11	**0.0087**
Cd 111 (µg/L)	21	0.09	0.09	0.10	54	0.11	0.10	0.15	**0.0005**
Co 59 (µg/L)	21	1.12	0.97	1.31	54	1.56	1.19	1.96	**0.0005**
Cu 63 (µg/L)	21	111.15	91.94	151.84	54	240.74	151.74	372.44	**<.0001**
Fe 57 (mg/L)	21	51.57	45.75	62.82	54	74.62	50.38	115.75	**0.0042**
Hg 202 (µg/L)	21	0.64	0.01	1.61	54	2.98	0.01	9.31	**0.0071**
Mg 24 (mg/L)	21	4.59	3.51	8.81	54	7.30	4.18	11.70	0.0849
Mn 55 (µg/L)	21	29.78	25.00	42.51	54	45.62	34.40	82.78	**0.0017**
Mo 98 (µg/L)	21	1.64	1.37	2.28	54	2.67	1.76	3.21	**0.0010**
Ni 60 (µg/L)	21	12.77	12.17	19.06	54	15.64	12.29	18.68	0.3482
Pb (µg/L)	21	7.35	6.81	7.82	54	8.66	7.58	12.83	**<.0001**
Pt 195 (µg/L)	21	0.40	0.35	0.48	54	0.41	0.35	0.46	0.7184
Rb 85 (mg/L)	21	9.56	7.94	19.33	54	11.85	7.84	18.89	0.7725
Sb 121 (µg/L)	21	4.26	4.16	4.41	54	4.96	4.52	5.21	**<.0001**
Se 82 (µg/L)	21	101.92	67.11	146.31	54	111.01	73.06	174.62	0.5050
Sn 118 (µg/L)	21	106.66	96.22	110.94	54	101.12	82.74	122.44	0.9061
Tl 205 (µg/L)	21	0.82	0.53	1.39	54	0.80	0.34	1.29	0.6538
U 238 (µg/L)	21	0.04	0.02	0.06	54	0.09	0.04	0.23	**0.0027**
W 184 (µg/L)	21	0.95	0.92	1.06	54	1.21	1.01	1.51	**0.0004**

Zn 66 (mg/L)	21	0.56	0.43	0.79	54	1.11	0.76	1.79	**<.0001**

Med = median; P25 = 25th percentile; P75 = 75th percentile; Sjögren disease = SjD.

Mann–Whitney U test. P < .05 was considered statistically significant. Bold indicates statistically significant differences.

Concentrations are reported in µg/L or mg/L, as indicated.

Table 4. Urinary metal concentrations in individuals with SjD and controls

Variable	Control				SjD				P value
	N	Median	P25	P75	N	Median	P25	P75	
Ag 107 (µg/L)	18	0.68	0.51	0.80	49	1.16	0.80	1.75	**0.0001**
Al 27 (µg/L)	18	67.62	54.05	106.81	49	39.78	19.46	63.32	**0.0044**
As 75 (µg/L)	18	1.56	1.15	2.16	49	1.31	1.01	2.27	0.4201
Ba 138 (µg/L)	18	1.56	0.93	3.75	49	2.37	0.94	5.58	0.2578
Be 9 (µg/L)	18	0.09	0.07	0.17	49	0.13	0.11	0.21	0.1477
Ca 43 (mg/L)	18	61.83	35.05	88.07	49	40.72	18.09	87.07	0.2490
Cd 111 (µg/L)	18	0.08	0.01	0.12	49	0.09	0.04	0.15	0.1116
Co 59 (µg/L)	18	0.71	0.63	0.88	49	0.71	0.61	0.89	0.9718
Cs 133 (µg/L)	18	37.93	22.64	54.32	49	29.02	18.73	50.67	0.2730
Cu 63 (µg/L)	18	101.72	64.05	174.35	49	89.82	63.41	123.27	0.4577
Fe 57 (mg/L)	18	15.91	12.40	19.98	49	13.67	12.17	17.76	0.3361
Hg 202 (µg/L)	18	2.40	1.70	2.67	49	3.87	2.91	5.58	**<0.0001**
Li 7 (µg/L)	18	3.75	2.46	5.65	49	3.12	2.36	4.84	0.2211
Mg 24 (mg/L)	18	17.12	11.02	23.97	49	12.81	5.86	21.48	0.0965
Mn 55 (µg/L)	18	0.54	0.37	1.04	49	1.68	0.76	2.91	**0.0004**
Mo 98 (µg/L)	18	34.23	13.55	54.50	49	22.27	7.24	47.82	0.2792
Ni 60 (µg/L)	18	16.69	5.30	37.17	49	16.76	7.39	22.90	0.5572
Pb 208 (µg/L)	18	1.77	1.72	1.86	49	1.81	1.75	1.92	0.0646
Pt 195 (µg/L)	18	0.01	0.01	0.04	49	0.06	0.02	0.11	**0.0023**
Rb 85 (mg/L)	18	3.48	2.31	5.38	49	2.89	1.81	5.53	0.6406
Sb 121 (µg/L)	18	0.33	0.12	0.48	49	0.37	0.29	0.65	**0.0356**
Se 82 (µg/L)	18	40.15	24.04	47.38	49	22.82	15.07	46.75	**0.0376**
Sn 118 (µg/L)	18	5.81	5.41	6.89	49	7.14	6.09	8.27	**0.0102**
Sr 88 (µg/L)	18	175.30	136.01	295.16	49	144.18	75.86	284.28	0.1284
Te 130 (µg/L)	18	0.47	0.13	0.62	49	0.27	0.01	0.57	0.1872
Tl 205 (µg/L)	18	1.79	0.95	2.13	49	1.09	0.62	1.98	0.1678
U 238 (µg/L)	18	0.03	0.03	0.03	49	0.04	0.04	0.07	**<0.0001**

W 184 (µg/L)	18	0.36	0.29	0.55	49	0.46	0.33	0.71	0.1612
Zn 66 (µg/L)	18	157.70	125.55	199.11	49	189.57	147.81	274.85	0.0713

Note. Values are presented as median and interquartile range (P25–P75). C, controls; SjD, Sjögren disease; P25, 25th percentile; P75, 75th percentile. The Mann–Whitney U test was used for comparisons between groups. Bold values indicate statistically significant differences (P < 0.05).

Table 3. Dosage of 25 Metals in the saliva of individuals with SjD and Controls. The test for non-parametric data used was the Mann-Whitney U Test, p < 0.05, which was considered significant (elements in bold indicate significant differences, p < 0,05).

Table 4. Metals in the urine of individuals with SjD and Controls. The test for non-parametric data used was the Mann-Whitney U Test, p < 0.05, which was considered significant (elements in bold indicate significant differences, p < 0,05).

The correlations between levels of metals in the saliva and symptoms of dry eye in both groups revealed that Cu and Sb levels in the saliva positively correlated with higher scores of OSDI (r = 0.55, CI 95%: 0.28–0.73, p = 0.0002, and r = 0.34, CI 95%: 0.04-0,59, p = 0.0257, respectively). (Spearman test).

The levels of As and Cu in the saliva correlated with lower salivary flow rate (r: -0.56, CI 95% -0.74- -0.30, p < 0.0001 and r: -0.41, CI 95% -0.64- -0.11, p = 0.007, respectively). The salivary As and Zn levels correlate with punctate keratitis score tested by fluorescein corneal staining (r = 0.35, CI 95%: 0.04–0.60, p = 0.025 and r = 0.33, CI 95%: 0.01–0.58, p = 0.038, respectively). Curiously, the salivary levels of Ca, Cd, and Pb correlated with higher salivary flow in this group (r = 0.46, CI 95% 0.17–0.67, p = 0.002, r = 0.48, CI 95% 0.20–0.69, p = 0.001, and r = 0.46, CI 95% 0.17–0.67, p = 0.002, respectively).

There was no correlation between salivary or urine levels of trace metals and tear flow measured by the Schirmer test.

There was a correlation between the urine levels of Sn and the punctate keratitis score tested by fluorescein corneal staining in the SjD group (r = 0.4, CI 95% 0.09–0.64, p = 0.01). In addition, there was a correlation between the levels of Ag in urine and higher salivary flow (r = 0.33, CI 95% 0.01-0,59, p = 0,038) and Se in urine and lower punctate keratitis score tested by fluorescein corneal staining (r=-0.32, CI 95%: -0,58 - -0.01, p = 0.043).

Figure 1A. Correlation between clinical data and levels of metals in the Urine

Figure 1B.Correlation between clinical data and levels of metals in the Saliva

4. Discussion

The present work compared the levels of metals in the urine and saliva of SjD and age-matched controls. The major findings were the significantly higher levels of some of those metals in both the urine and

saliva of SjD, where Hg, Mn, Sb, and U were higher in both secretions, and As, Cu, Sb, Sn, and Zn correlated with deterioration of the clinical findings of SjD.

The concentration of Ag, Ba, Fe, Mn, Pb, Pt, Sb, Se, and Sn were higher in saliva. In other hand Al, BE, Hg, Mg, Mo, Ti and Zn was higher in urine, which may reflect distinct excretory mechanisms. The only surprise was Se, which was present in higher concentrations in urine than in saliva in a previous study, and the opposite occurred in our work, suggesting variations in the assay and the population samples [21].

A large population study was conducted in the same region of southeast Brazil, dosing metal levels in the urine of 996 volunteers, and the results were compared with similar data from France and USA [22].

Considering the intragroup large variability in both studies, it is notable the much higher concentration of Sn and much lower levels of Rb in the urine of SjD in our study. However, their study adjusted the concentration or urine metals for creatinine levels, and our work analyzed the sample concentration.

The causes of higher levels of those trace metals in the urine and saliva from the SjD group were not identified, since the questionnaire of exposure to contaminants detected a similar lifestyle in both controls and SjD groups. The causes could be associated with the presence of some metals in the chronic medication, health supplements or impaired excretory mechanisms.

The metals more suspicious to be involved in pathological conditions due to their higher levels compared to the levels observed in the controls and in the healthy population and their correlation with clinical findings were As, Cu, Zn and Sn. The mechanisms of how heavy metals would interfere and respond to the clinical findings are related to their actions in the cellular metabolism, oxidative stress and proinflammatory outcomes [23, 24].

Copper (Cu), Arsenium (As) and other metals have been correlated with autoimmune diseases but not with Sjogren's disease so far. In a large cohort study, searching the fish contaminated with heavy metals and the onset of juvenile idiophatic arthritis (JIA), the authors examined more than 15 thousands individuals. They compared the levels of heavy metals in the early life and the diagnosis of JIA and concluded that fish in the meal more than once a week was associated with higher risk and the metals Al, Cd and Li correlated with the cord blood levels of those individuals [25].

In a recent review of the correlation of heavy metals and autoimmunity, the authors observed that although several trace metals are essential for the normal metabolism, the excess or imbalance among them are associated with distinct manifestations of rheumatoid arthritis (RA). Cu, Se and Zn are relevant for the immune system regulation and excessive exposure to Cd, Hg, Ni and Pb is associated with joint inflammation in RA [23].

In our study Se was significantly lower in the urine of the SjD group compared to controls. A recent review, including 32 original articles addressing the topic, found low levels of Selenium in patients with

autoimmune diseases, supporting the role for this trace metal for autoimmunity homeostasis in these patients. Which may be due to the anti-inflammatory effects of selenium [26].

An original article comparing Se serum levels in SjD and controls observed that lower levels of Se are more frequent in SjD group and correlated with polyneuropathy, in particular motor neuropathy, suggesting the supplementation to address this manifestation [27].

Our study has limitations. We were unable to find the sources or the habits that caused the distinct exposure to those trace metals with the demographic and questionnaire analysis. Moreover, it is largely known that excretory mechanisms form lacrimal and salivary glands of SjD individuals are impaired, including the kidney function, due to renal tubular acidosis, among others may cause bias [28].

Those points are certainly fragilities of our exploratory study; the much higher concentrations of Cu and Hg in the saliva, and Mn and Pt in the urine of SjD individuals and the very lower concentrations of Se in SjD urine samples deserves future investigation.

In conclusion, the present work identified trace and heavy metals distinct levels in the saliva and urine of SjD individuals and observed the correlations of Copper, Arsenic, Manganese and Zinc with major signs and symptoms of SjD. The present work opens the opportunity for future investigation of the metals and other environmental contaminants in physiopathology of SjD. It also can lead to improve the preventive and therapeutic measures for this disease.

Declarations

Declaration of competing interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data are available from Harvard Dataverse Repository entitled: “The association of metal exposure and Sjögren´s Disease: a comparative study” https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/7MQCCB. Harvard Dataverse, DRAFT VERSION, UNF:6:gTgOd/FThs7p6x3Olqyuvg== [fileUNF]

Author Contribution

L.P.B wrote the main manuscriptL.P.B, D.R.G., I.H.P.S., A.L.C.A., M.F., D.M.G., M.M., M.Z.F. helped with the collection of data.T.C.M. prepared figures 1 and 2L.P.B. and E.M.R. prepared tables 1 and 2L.P.B and T.C.M did the statistical analysisB.A.R and F.C.B did the data analysisF.R.O, B.A.R., A.A.B.M.and E.M.R. reviewed the manuscript

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Figures

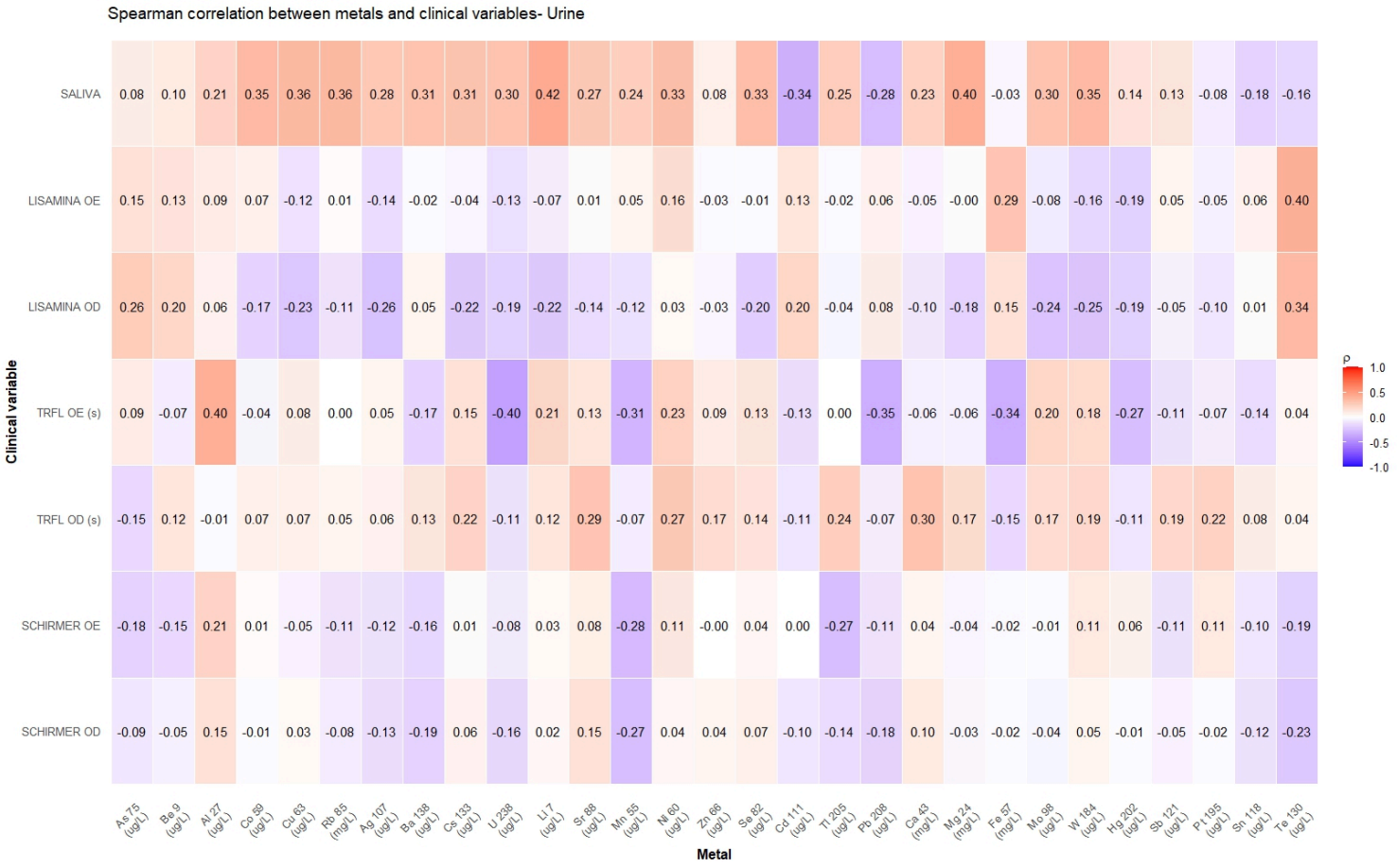


Figure 1

Figure 1A. Correlation between clinical data and levels of metals in the Urine

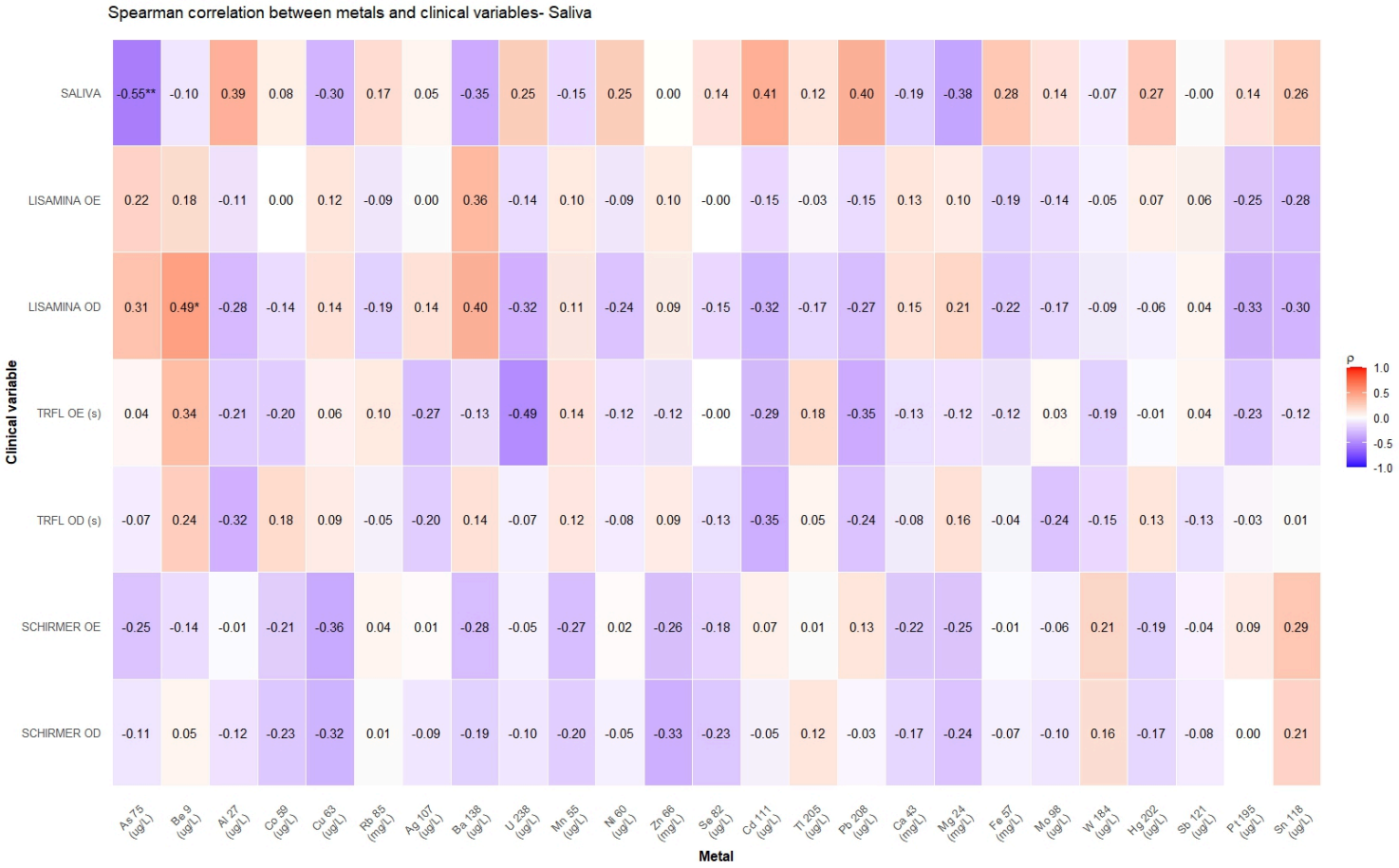


Figure 2

Figure 1B.Correlation between clinical data and levels of metals in the Saliva