Specific Sialochemical and Sialometric Changes and Cariogenic Risk in Patients with Primary Sjogren's Syndrome

CODRINA ANCUTA[1,2], CRISTINA ANGELA GHIORGHE[3]*, RODICA CHIRIEAC[4], ALICE ARINA CIOCAN PENDEFUNDA[3], CRISTINA IORDACHE[3]

[1] Grigore T. Popa University of Medicine and Pharmacy Iasi, Rheumatology Department, 16 Universitatii Str., 700115, Iasi, Romania

[2] Clinical Rehabilitation Hospital, Rheumatology 2 Department, 14, P. Halipa Str., 700661, Iasi, Romania

[3] Grigore T. Popa University of Medicine and Pharmacy Iasi, Faculty of Dental Medicine, 16 Universitatii Str., 700115, Iasi, Romania

[4] SANOCARE Medical Center, 3A Ghica Voda Str., 700469, Iasi, Romania

The main aim of our study was to perform a biochemical analysis of the saliva and to assess potential impact on oral health, particularly cariogenic issues, in patients with salivary secretory dysfunction such as primary Sjogren's syndrome (pSSj). Consecutive pSSj and healthy age-matched controls were prospectively assessed for (i) whole saliva flow rates (unstimulated, RFR, and stimulated, RFS), pH and composition; (ii) cariogenic scores - decayed, missing and filled tooth surfaces (DMFS index), Silnes and Loe bacterial plaque index (PI). Salivary flow rates were significantly lower in pSSj for both unstimulated and stimulated tests ($p<0.05$), with a drop in pH as compared to healthy controls ($p<005$). Abnormal inorganic and organic saliva composition was reported in all cases comprising higher sodium, lower potassium and chloride concentrations, higher amylase activity and lower total protein ($p<0.05$). Statistically significant higher cariogenic scores particularly DMFS, were demonstrated in all pSSj ($p<0.05$). In conclusion, patients with pSSj are at increased risk to develop cariogenic issues due to both quantitative and qualitative salivary changes.

Keywords: saliva, sjogren syndrome, cariogenic issues

Primary Sjögren's syndrome (pSSj) is a complex autoimmune rheumatic disease characterized by lymphocytic infiltration of the exocrine organs, especially salivary and lacrimal glands, with subsequent immune-mediated destruction, reduced lacrimal and salivary secretion [1].

A wide spectrum of clinical and serological manifestation ranging from different degrees of oral and ocular dryness (xerostomia and serophtalmia, respectively) and extra-glandular features (arthralgia or arthritis, peripheral vasculopathy with Raynaud phenomenon, pulmonary, gastrointestinal, neurological and renal pathology, vasculitis, cytopenia) with a background of specific immune abnormalities comprising anti-Ro/SSA and/or anti-La/SSB antibodies are essentially recognized in pSSj and result in significant impaired health-related quality of life [1-3].

Oral health issues are generally identified in pSSj [1-4]; xerostomia, increased susceptibility to develop caries and oral (bacterial and fungal) infections, as well as early dental loss due to caries [4-6] despite good oral hygiene [7] are documented among such patients, requiring a complex diagnostic and management focused on different strategies such as salivary flow stimulation, chemical control of dental plaque, restorative treatment, dental implants [3, 8-13]; however, periodontal disease and periodontal care are still controversial in patients with pSSj [14, 15].

It is widely accepted that oral health impairment secondary to pSSj is multifactorial; immune-mediated salivary secretory dysfunction with chronic xerostomia and hyposalivation, changes in saliva components and quality, changes in oral microbiota (high bacterial count of species such as *Lactobacillus acidophilus, Streptococcus mutans, Candida albicans*) remain major determinants of altered oral health in SSj [8, 12, 13, 16,17]

Furthermore, salivary secretory anomalies, reduced whole saliva flow rates, abnormal *p*H and buffer capacity as well as potentially altered saliva composition with modified concentrations of various electrolytes and organic constituents are characteristically reported in patients with pSSj, result in impaired salivary protective properties and support the occurrence of several oral-health related issues [8, 12, 13, 16, 19,20].

The aim of this study was to evaluate the salivary flow rates and composition and to assess potential impact on oral health, particularly cariogenic issues, in patients with autoimmune salivary secretory dysfunction such as primary Sjogren's syndrome.

Experimental aprt

Material and methods

We performed a prospective observational study in ten consecutive patients with primary Sjogren's syndrome (fulfilling the 2002 American-European consensus classification criteria) [21] attending at least once the outpatient rheumatology department, aiming to assess oral health and to identify potential relation with biochemical salivary parameters. The control group consisted of ten age-matched healthy individuals, without any known diagnosis that could affect their cariogenic risk.

Participants signed the informed consent before enrolment and the study protocol was approved by local Ethics Committee.

All cases were evaluated by the same trained examiner according to a standardized protocol including (i) *resting (unstimulated) (RFR) and stimulated (RFS) whole saliva flow rates, pH and composition (inorganic, organic constituents);* and *(ii)* the number of decayed, missing and

* email: drangycris@yahoo.com: Phone: 0722135113

filled tooth surfaces (*DMFS index*) and the *Silnes & Loe plaque index (PI).*

Salivary flow rates and pH

Saliva was collected only between 09:00 and 10:00 a.m. to elude potential influence of circadian rhythm on saliva secretion and composition. The participants were instructed not to eat or drink for 2 h preceding the procedure and asked to rinse their mouths for 5 s with 10 mL distilled water. Following the spitting out of the water and initial swallow, whole saliva was collected by spitting into a graduated measuring jar every 30 s for six minutes in order to determine the rate of *resting salivary flow (RFR);* finally, the flow rate was calculated as collected volume/ collection time (ml/ min) (normal values 0.5-0.7 mL/min).

Saliva production was stimulated by chewing paraffin wax (mechanical method); participants were asked to chew paraffin wax tablets for 30 s, whole saliva was collected by spitting into a graduated measuring jar for 6 min ; the total salivary volume was divided by six in order to calculate mL of saliva per minute *(paraffin-stimulated salivary flow, RFS)* (normal values 1.00-1.20 mL/min).

Salivary pH was measured using the Saliva-Check BUFFER kit designed for appraising saliva quality, *p*H and buffering capacity; a drop of previously collected saliva was deposited on the enclosed *p*H strip, and the color checked after 10 s (*p*H reading while the paper still moist) using the kit box charts. Highly acidic saliva (*p*H 5.0-5.8) is found in the red section, moderately acidic (*p*H 6.0-6.6) yellow section, while healthy one (*p*H 6.7-7.8) in the green section.

Saliva testing is widely used to identify, measure and assess the caries risk; moreover, based on saliva analysis, dentists can develop preventive care treatment plan strategies and select appropriate dental materials [22,29,30].

Table1

CARIOGENIC INDEXES, SALIVARY FLOW RATE AND SALIVARY *p*H IN STUDY GROUPS

	Group	Average	Std dev
DMFS	**control**	10.61	1.55
	SS	47.17	1.25
PI	**control**	0.38	0.50
	SS	2.17	0.71
RFR (ml/min)	**control**	0.50	0.07
	SS	0.11	0.05
RFS (ml/min)	**control**	0.741	0.214
	SS	0.513	0.387
pH	**control**	6.26	0.15
	SS	4.58	1.05

Salivary composition

Further analysis of saliva components including both *inorganic (anions, cations, gases) and organic*, as well as proteins properties, was made from whole stimulated saliva collected in a glass bead tube and kept at -20°C. Samples were centrifuged at 10000 revolutions per minute and lab examination performed on automated analyser (protocols specific for individual salivary components). Normal amylase activity 11.900- 304.700U/L, total protein 1.1-1.8 g/L, chloride concentrations 5-40 mmol/L, potassium 6.4-37 mmol/L, sodium 2-21 mmol/L.

Carries assessment

The *decayed, missing (due to caries only) and filled (restored) surfaces (DMFS) index* basically counts each affected dental surface in an attempt to assess the prevalence of dental caries prevalence and the requirement of dental treatment based on in-field clinical exam [22,23].

Silness-Loe plaque index measures the status of oral hygiene and is based on recording both soft debris and mineralized deposits on the teeth. Each of the four surfaces of the teeth (buccal, lingual, mesial and distal) is given a score from 0-3 and then used to calculate the plaque index for the tooth [14].

Statistical analysis (descriptive, analytical tests) was done in SPSS package, $p<0.05$. The Newman-Keuls statistical test commonly applied to compare different parameters.

Results and discussions

Demographic data, smoking status, oral hygiene habits, number of annual dental visits were comparable among study groups ($p>0.05$). pSS patients (all woman) were aged between 45 and 62 years, had a mean disease duration of 4.5 years, and were classified as having significant oral dryness.

Cariogenic indexes (DMFS and PI), salivary flow rates (unstimulated and stimulated), salivary *p*H and differences between study groups are summarized in table 1 and 2, respectively, while data about sialochemical salivary analysis in tables 3 and 4.

Table 2

NEWMAN KEULES TEST FOR CARIOGENIC INDICATORS, SALIVARY FLOW RATE AND SALIVARY *p*H IN STUDY GROUPS

Cariogenic indicators	p
DMFS	0.000110
PI	0.000119
RFR	0.000139
RFS	0.000104
pH	0.000055

Table 3

INORGANIC AND ORGANIC SALIVA COMPOSITION IN STUDY GROUPS

	Group	Average	Std dev
Na (mmol/l)	**control**	32.44	10.40
	SS	139.75	4.27
K (mmol/l)	**control**	4.39	0.60
	SS	4.05	0.42
Cl (mmol/l)	**control**	100.12	4.10
	SS	99.75	3.61
Amylases (U/l)	**control**	114456.3	97342.3
	SS	44633.3	16615.1
Total protein (g/l)	**control**	1.506	0.198
	SS	1.233	0.114

Table 4

NEWMAN KEULS TEST FOR INORGANIC AND ORGANIC SALIVA COMPOUNDS IN STUDY GROUPS

Saliva compounds	p
Na	0.000120
K	0.000112
Cl	0.001441
Amylases	0.006539
Total protein	0.298832

Salivary flow rates and pH

Mean salivary flow rates for whole saliva were significantly lower in patients with pSSj-related xerostomia, for both unstimulated and stimulated tests as compared to healthy controls (0.11+0.05 vs.0.50 +0.07 for RFR; 0.513+0.387 vs. 0.741+0.214 for RFS, respectively; $p<0.05$).

Patients with pSSj presented with a drop in salivary *p*H (4.58+1.05) compared to their controls (6.26+0.15), with significant lower *p*H ($p<0.05$).

Sialochemical analysis

A detailed analysis of inorganic and organic saliva composition was done in all pSSj and controls, including data about cations (sodium and potassium concentrations), anions (chloride concentrations), total protein and amylase activity (table 3). Thus, pSSj patients presented with higher sodium levels (139.75U+4.27 mmol/L) versus healthy controls (32.44+10.40 mmol/L), while the other electrolytes tested were lower in pSSj: 4.05+0.42 mmol/L vs. 4.39+0.60 mmol/L for potassium; 99.75+3.61 mmol/L vs. 100.12+4.10 mmol/L for chloride. Differences in inorganic and organic salivary components were statistically significant as shown by Newman-Keuls test ($p<0.05$) (table 4).

Salivary amylase activity was 44633.3+16615.1U/L for pSSj and higher in controls (114456.3+98342.3 U/L), whereas total protein displayed the same trend – higher in healthy controls as compared to pSSj cases (1506+0.198 g/L vs.1233+0.198 g/L) (table 3). Differences in total proteins and amylases concentrations were statistically significant ($p<0.05$) (table 4).

Oral findings

We reported higher DMFS and PI in all studied pSSj as compared to their matched controls (47.17+1.25 vs. 10.61+1.55 for DMSF, 2.17+0.71 vs. 0.38+0.50 for PI respectively). Differences among study groups were statistically significant for all parameters (cariogenic indexes, salivary flow rate and *p*H) as demonstrated by applying the Newman-Keuls test (table 2).

Higher number of missing teeth than the controls as well as higher number of filled (due to carries) tooth surfaces was found in pSSj compared with control group ($p<0.05$).

We could identify a direct relation between DMFS and xerostomia and hyposalivation in our pSSj: patients with significant oral dryness and severely impaired saliva flow rate are characterized by more carries and higher DMFS ($p<0.05$).

Salivary changes may represent the most common oral manifestation of systemic diseases, including autoimmune rheumatic conditions (e.g. primary or secondary SSj). Furthermore, many of the oral changes (increased risk for caries, oral mucosal infections, difficulty in mastication or swallowing) emerged during the course of such systemic disorders can be attributed to salivary dysfunction (xerostomia and hyposalivation, abnormal concentrations of salivary electrolytes, impaired pH and buffer saliva capacity) [8, 13, 16-18].

We performed a detailed sialochemical and sialometric analysis as well as a complex assessment of oral health issues, particularly cariogenic involvement in our pSSj patients and healthy age-matched controls. Firstly, we demonstrated impaired salivary secretion, both unstimulated and stimulated whole salivary flow rates being significantly decreased as compared to healthy individuals ($p<0.05$). Moreover, we revealed abnormal *p*H values, organic (total protein, amylase activity) and inorganic (sodium, potassium, chloride) components of saliva in pSSj versus control group ($p<0.05$).

All these quantitative and qualitative changes are widely recognized in systemic pathologies characterized by saliva secretory dysfunction such as SSj, an autoimmune rheumatic condition, and may alter oral health.

Interestingly, we identified high cariogenic indexes in our pSSj, with significant more injured teeth (number of decayed, missing and filled surfaces, soft debris and mineralized deposits) as reflected by high DMFS and PI ($p<0.05$). We found no other predisposing factor for impaired oral health, particularly carries development, than decreased salivary secretion and abnormal electrolytes concentrations.

The topic of oral manifestations (oral dryness, hiposalivation, caries and/or periodontal disease) and treatment in patients with primary Sjogren's syndrome was largely addressed in the last decade [8, 13, 18, 24-27], indicating an low salivary flow rates, acidic pH and modified saliva composition accompanied by an increased cariogenic risk (as defined by different scores – DMSF, PI) in the specific clinical and immunological settings that characterize pSSj. Moreover, dental status (DMSF] does not correlate with age and oral hygiene habits, but are linked to the severity of xerostomia and hyposalivation (inverse correlation, particularly with the unstimulated whole saliva flow rate) in pSSj [13].

Changes of *p*H with subsequent acidic salivary environment, electrolytes and organic composition together with decreased in salivary flow rates are major determinants of dental caries in pSSj as compared to healthy controls [13].

Furthermore, Pedersen et al [13] suggested that specific sialometric and sialochemical glandular changes in pSSj, particularly modified concentration of two electrolytes – sodium and chloride – could become as oral biomarkers of SSj severity. Higher concentrations of both sodium and chloride were detected in those patients displaying the lowest flow rate, highest lymphocytic glandular deposits and highest levels of specific autoantibodies [13].

We also identified abnormal salivary sodium and chloride, as well as potassium concentrations reflecting an impaired ductal salt reabsorption. However, we reported high sodium concentrations, but low chloride and potassium. The discrepancy should be further evaluated.

Under physiological conditions, acinar cells secrete primary saliva, which has a plasma-like isotonic composition. However, the final saliva discharged into the oral cavity converts into a hypotonic one, with much lower concentrations of sodium and chloride during the ductal passage.

Local lymphocyte infiltration, acinar cell degeneration and ductal damage are three major causes of functional loss and low rate of unstimulated salivary flow in pSSj. Ductal changes are probably the basis of abnormal sodium and chloride levels in RFR and RFS as a result of abnormal electrical channel exchanges [28]. A decrease in salivary cations, particularly sodium and potassium, basically reflects ductal and acinar cells damage, as well as a direct link with decreased salivary amylase concentration. Detailed electrolytic analysis indicates that reabsorption activity in salivary ducts can be diminished, without impaired acinar transport mechanisms [13]. Thus, low levels of different electrolytes including sodium and calcium in saliva can be explained by impaired salivary flow partially related to diminished ductal reabsorption of salivary electrolytes.

Overall, our sialochemical and sialometric results in pSSj and healthy individuals appear to be comparable with data reported by different authors, except the chloride compositional changes. One potential limitation of our study was the limited number of enrolled patients and the fact that all measurements were done only in stimulated whole saliva.

Conclusions

Patients with primary SSj are at increased risk to develop cariogenic issues due to both quantitative and qualitative salivary changes. Abnormal saliva secretion commonly described in SSj is widely responsible for the increased susceptibility of oral mucosa to various physical, chemical as well as microbial aggressions, secondary to altered physical barrier role. A multidisciplinary approach including dentist- rheumatologist-primary care physician is suitable for the complex management of such patients.

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