Salivary Gland Involvement and Oxidative Stress in Juvenile Idiopathic Arthritis: Novel Observation in Oligoarticular-type Patients

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ABSTRACT. ***Objective.*** The salivary glands may become affected in various collagen diseases, but their involvement in juvenile idiopathic arthritis (JIA) has received little attention. We studied the salivary composition and the antioxidant profile in patients with JIA, as well as their serum antioxidant status.

Methods. Twenty-two children with JIA according to the American College of Rheumatology criteria (10 oligoarticular, 7 polyarticular, and 5 systemic-type) and 15 healthy controls were studied. Serum and saliva samples were obtained simultaneously and analyzed.

Results. Significantly raised levels of antioxidant enzyme activity were observed in the patients with JIA, in both saliva and serum. The salivary peroxidase activity was significantly higher in the total group of patients with JIA by 8.5% ($p < 0.01$) as compared to controls (0.76 vs 0.70 mU/ml). Salivary superoxide dismutase was found to be significantly increased mainly in the patients with systemic JIA (by 74%; $p < 0.02$). Significantly higher levels of peroxidase activity were observed in serum of patients with JIA, particularly of the polyarticular group, by 17% ($p < 0.05$). Major changes in saliva composition were observed in patients with oligoarticular disease compared to controls: the patients had a lower salivary flow by 33%, less acidic saliva, and significantly lower salivary levels of magnesium by 44% ($p < 0.01$), total protein by 44% ($p < 0.02$), amylase by 34% ($p < 0.02$), and lactate dehydrogenase by 62% ($p < 0.02$).

Conclusion. Children with JIA exhibited a major increase in antioxidant enzyme activity, both in serum and in saliva. Patients with oligoarticular JIA displayed indications of significant and specific damage to the salivary glands, a novel observation. (J Rheumatol 2006;33:2532–7)

Key Indexing Terms:
JUVENILE ARTHRITIS | OLIGOARTICULAR | SALIVA
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Juvenile idiopathic arthritis (JIA) is one of the most common rheumatic diseases of childhood, with at least 3 primary modes of onset, namely the systemic, the polyarticular, and the oligoarticular types[1]. The pathogenesis of JIA is characterized by prolonged chronic inflammation of the synovial membranes, accompanied by recruitment of mononuclear cells and phagocytes into the synovial fluid. The accompanying T cell abnormalities and the pathological characteristics of the chronic synovitis suggest an immune cell-mediated pathogenesis[2]. Although there are undoubtedly genetic predispositions and putative environmental triggers, no single factor can explain the pathogenesis of the disease and the diversity of its clinical patterns. In recent years, oxidative stress is increasingly recognized as one of the major factors contributing to the chronic inflammatory process within the inflamed joint; in effect, a 2-fold increase in the level of free-radical-induced damage to proteins has been demonstrated in the synovial fluid of adult patients with rheumatoid arthritis (RA)[3,4]. Further, an increase of *in vivo* generation of oxidants and lipid peroxidation products with a concomitant rise in antioxidant enzyme levels in the serum of patients with RA has been described[5]. Suggested mechanisms for the increased activity of free radicals in the synovia of patients with RA include the production of various free radicals (such as superoxide, hydroxyl, and hypochlorous) by the invading phagocytes[6], along with intraarticular hypoxia caused by an increase of intraarticular pressure to above the synovial capillary perfusion pressure[7]. Involvement of the salivary glands in adults with RA has been recognized for quite a long time, and minor salivary glands of patients with RA were shown to be markedly infiltrated with lymphocytes, with B cells predominating over T cells[8]. Other studies showed that the typical minor sali-

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vary gland alterations in RA included fibrosis, acinar atrophy, and lympho-plasma cell sialadenitis[9]. A study of the antioxidant profile of the secreted saliva of patients with RA showed remarkably increased levels of antioxidant enzymes both in saliva and in serum, especially in severely affected patients, and also a decrease in salivary rate[10].

In view of these data, we considered that saliva might be an appropriate representative of serum composition while its collection for analysis is less invasive and friendlier to children. We undertook to analyze the antioxidant status in serum and in saliva of children with JIA. We also evaluated the occurrence of salivary gland involvement in JIA, addressing specifically its oligoarticular, polyarticular, and systemic subtypes.

MATERIALS AND METHODS

Patients. This study was approved by the Human Studies Ethics Committee of Rambam Medical Center, Haifa, and each participant/parent signed an informed consent form.

Twenty-two patients with JIA according to the International League of Associations for Rheumatology criteria[11] agreed to participate. Ten had oligoarticular, 7 had polyarticular, and 5 had systemic course of the disease. All the patients were followed by the same pediatric rheumatologist (RB) in the Pediatric Rheumatology Clinic at the Meyer Children's Hospital. Of the 22 patients, 15 were girls. The mean age of the patients in the study was 12.2 ± 1.07 years (range 4–19 yrs). The duration of followup was 5.8 ± 0.89 years (range 0.5–16 yrs) and duration of disease activity was 5.1 ± 3.3 years. Fourteen patients had active disease at the time of study as defined by established criteria[12]. Fifteen healthy age-matched children (mean age 12 ± 1.03 yrs) constituted the control group. Serum and saliva samples were obtained concomitantly from each participant and were immediately stored at –70°C for up to 3 months until analyzed.

Saliva analysis. Sialometry — flow rate (FR) measurement. Unstimulated whole saliva specimens were obtained in the morning (between 9:00 AM and noon), and no oral stimulus was permitted for 60 min prior to collection, as described[10]. Following collection on ice, the salivary specimens were frozen and stored at –70°C until analysis. Salivary flow rates were expressed as volume of saliva (ml) secreted per minute.

Sialochemistry. Sialochemistry analysis included examination of the following: pH, calcium (Ca), phosphate (P), magnesium (Mg), total protein, albumin (ALB), lactate dehydrogenase (LDH), and amylase (AMY). All these were evaluated as described[13].

Salivary antioxidant status analysis. Salivary peroxidase (SPO): SPO activity was measured according to the NBS assay, as described[14]. Briefly, the colorimetric change induced by the reaction between the enzyme and the substrate, dithiobis 2-nitrobenzoic acid (DTNB) in the presence of mercapto-ethanol, was read by spectrophotometry at 412 nm wavelength for 20 seconds.

Salivary superoxide dismutase (SOD): The total activity of the SOD enzyme (Cu/Zn- and Mn-SOD) was measured using the xanthine oxidase/XTT method described by Nagler, *et al*[15]. This is a spectrophotometric assay for SOD, based on tetrazolium salt 3'-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro) benzenesulfonic acid hydrate reduction by xanthine-xanthine oxidase. The method is a modification of the NBT assay. XTT is reduced by superoxide anion (SO) that is generated by xanthine oxidase. The formazan is read at 470 nm. SOD inhibits this reaction by scavenging the SO. One unit of the enzyme is defined as the amount of enzyme needed for 50% inhibition of the absorption.

Salivary total antioxidant status (TAS): TAS was assessed as described[15]. Briefly, this assay is based on a commercial kit supplied by Randox (San Francisco, CA, USA) in which metmyoglobin is turned into ferrylmyoglobin in the presence of iron. Incubation of ferrylmyoglobin with the Randox reagent ABTS results in the formation of a blue-green colored radical that can be detected by spectrophotometry at 600 nm.

Salivary uric acid (UA): UA concentration was measured with a kit supplied by Sentinel CH (Milan, Italy) as described[15]. In this assay, UA is transformed by uricase into allantoin and hydrogen peroxide, which, under the catalytic influence of peroxidase, oxidizes the chromogen (4-amniophenazone/N-ethyl-methylalnin propan-sulfonate sodic) to form a red compound whose intensity of color is proportional to the amount of uric acid present in the sample, and is read at a wavelength of 546 nm.

Serum analysis. Serum samples were analyzed for total antioxidant status, peroxidase activity (both methods are described above), uric acid, total protein, and albumin.

Immunological studies. Erythrocyte sedimentation rates (ESR), antinuclear antibodies (ANA), rheumatoid factor (RF), anti-SSA and anti-SSB antibodies were measured in serum employing routine procedures. ANA, anti-SSA, and anti-SSB were measured in saliva using the same methods.

Statistical analysis. We used 2-sample T test for differences in means analysis to compare patients and controls. Spearman's rank coefficient of correlation was used for analyzing saliva and serum results of the various parameters tested. A p value < 0.05 was regarded as statistically significant.

RESULTS

Salivary analysis. Sialometry. The median salivary flow rate value of the whole group of patients with JIA was within the normal range and did not differ from that of healthy controls. However, the median flow rate in the oligoarticular group was lower than in the controls by 33%. This reduction did not reach statistical significance due to the large standard error values (Table 1).

Sialochemistry. No significant differences were found between the total JIA group and controls regarding salivary calcium and albumin levels. However, evaluation of each JIA subgroup separately showed major alterations in the saliva composition of the patients with oligoarticular type compared to controls and to the systemic and polyarticular groups. The oligoarticular group had significantly lower salivary levels of Mg ($p < 0.01$), total protein ($p < 0.02$), amylase ($p < 0.02$), and LDH ($p < 0.02$). Salivary calcium and phosphate were lower in the oligoarticular group by 25%, which was borderline significant. Compared to controls, the patients with systemic type showed increased levels of salivary total protein ($p < 0.03$), while in the polyarticular group the only significant change in saliva was increased levels of amylase ($p < 0.03$). Salivary pH was higher in all the patients with JIA than in controls (6.82 ± 0.05 vs 6.68 ± 0.06, respectively); the highest pH was noted in the oligoarticular group (6.94 ± 0.04; $p < 0.04$; Tables 1 and 2).

Salivary antioxidant analysis. The salivary peroxidase activity was higher in all 3 subgroups of JIA patients. In the total group of JIA patients, the salivary peroxidase activity was significantly higher by 8.5% ($p < 0.01$) compared to controls (0.76 vs 0.70 mU/ml). The other salivary antioxidant enzyme analyzed, SOD, was found to be raised in patients with JIA, mainly in those with the systemic type, in whom it was increased by 74% ($p < 0.02$). Salivary UA and TAS values in patients with JIA were not statistically different from controls (Table 3).

Table 1. Salivary flow rate (FR), pH, calcium (Ca), potassium (P), and magnesium (Mg) in total JIA, systemic, polyarticular, and oligoarticular-type patients.

Characteristic	Healthy Controls (n = 15)	JIA Total (n = 22)	JIA Systemic (n = 5)	JIA Poly (n = 7)	JIA Oligo (n = 10)	Controls vs Total JIA, p	Controls vs Systemic, p	Controls vs Poly, p	Controls vs Oligo, p
FR, ml/min									
Range	(0.05–3.94)	(0.04–1.6)	(0.28–0.67)	(0.11–0.66)	(0.04–1.6)				
Median	0.33	0.26	0.38	0.24	0.22	0.90	0.38	0.83	0.62
Mean	0.54	0.40	0.45	0.32	0.42				
SE	0.25	0.08	0.08	0.08	0.17				
pH									
Range	(6.1–7.0)	(6.1–7.0)	(6.7–7.0)	(6.1–7.0)	(6.7–7.0)				
Median	6.70	7.00	6.70	7.00	7.00	0.04	0.53	0.65	0.01
Mean	6.68	6.82	6.76	6.70	6.94				
SE	0.06	0.05	0.06	0.15	0.04				
Ca, mg/dl									
Range	(3.0–15.0)	(3.1–15)	(6.7–11.8)	(3.7–15.0)	(3.1–13.5)				
Median	9.10	8.10	10.30	7.80	6.85	0.31	0.90	0.67	0.12
Mean	9.50	8.53	9.82	3.75	7.43				
SE	0.80	0.68	0.98	1.42	0.98				
P, mg/dl									
Range	(9.1–23.1)	(8.8–20)	(11.7–20.0)	(9.2–20.0)	(8.8–15.2)				
Median	14.50	13.70	16.40	16.30	10.80	0.70	0.29	0.60	0.08
Mean	14.56	14.00	16.62	15.55	11.58				
SE	1.09	0.80	1.55	1.50	0.73				
Mg, mg/dl									
Range	(0.3–1.1)	(0.2–1.2)	(0.4–1.0)	(0.2–1.2)	(0.2–0.8)				
Median	0.80	0.50	0.60	0.80	0.45	0.12	0.53	0.83	0.01
Mean	0.70	0.58	0.64	7.23	0.45				
SE	0.06	0.05	0.10	0.12	0.05				

Statistically significant: $p \leq 0.05$

Table 2. Salivary total protein (TP), albumin (ALB), lactate dehydrogenase (LDH), and amylase (AMY) in total JIA, systemic, polyarticular, and oligoarticular.

Characteristic	Healthy Controls (n = 15)	JIA Total (n = 22)	JIA Systemic (n = 5)	JIA Poly (n = 7)	JIA Oligo (n = 10)	Controls vs Total JIA, p	Controls vs Systemic, p	Controls vs Poly, p	Controls vs Oligo, p
TP, mg/dl									
Range	(42.9–123.7)	(10.8–198.4)	(73.4–170)	(36.6–119)	(10.8–198.4)				
Median	90.20	68.10	118.0	92.90	51.15	0.65	0.02	0.97	0.028
Mean	84.97	82.96	118.0	84.84	63.95				
SE	7.12	9.74	15.60	12.73	15.70				
ALB, mg/l									
Range	(16.6–226.3)	(14.7–146.1)	(41.1–146.1)	(14.7–134.1)	(16.1–105.5)				
Median	45.80	42.50	72.40	51.30	33.10	0.48	0.10	0.80	0.10
Mean	73.76	57.41	88.44	60.40	39.80				
SE	16.55	8.87	22.90	16.22	8.73				
LDH, IU/l									
Range	(86–1000)	(25–1000)	(168–1000)	(69–434)	(25–696)				
Median	511	300	720	259	193	0.07	0.02	0.148	0.026
Mean	568	352	610	287	268				
SE	94	57	155	48	76				
Amy, IU/l									
Range	(16–2147)	(17–5720)	(361–2314)	(919–5720)	(17–918)				
Median	956	824	794	2320	632	0.52	0.02	0.03	0.02
Mean	1101	1236	996	2375	559				
SE	150	273	340	640	107				

Statistically significant: $p \leq 0.05$.

Table 3. Salivary peroxidase, superoxide dismutase (SOD), total antioxidant status (TAS), and uric acid in total JIA, systemic, polyarticular, and oligoarticular.

Characteristic	Healthy Controls (n = 15)	JIA Total (n = 22)	JIA Systemic (n = 5)	JIA Poly (n = 7)	JIA Oligo (n = 10)	Controls vs Total JIA, p	Controls vs Systemic, p	Controls vs Poly, p	Controls vs Oligo, p
Peroxidase, mU/ml									
Range	(0.63–0.84)	(0.60–0.93)	(0.60–0.86)	(0.73–0.87)	(0.63–0.93)				
Median	0.70	0.76	0.73	0.77	0.77	0.01	0.31	0.01	0.04
Mean	0.70	0.76	0.74	0.79	0.76				
SE	0.02	0.02	0.61	0.02	0.02				
SOD, U/ml									
Range	(026–0.87)	(0.22–1.95)	(0.46–1.95)	(0.33–0.90)	(0.22–0.72)				
Median	0.47	0.50	0.82	0.47	0.46	0.25	0.026	0.48	0.93
Mean	0.47	0.61	1.00	0.53	0.46				
SE	0.05	0.08	0.25	0.08	0.05				
TAS, mmol/l									
Range	(022–0.69)	(0.24–0.81)	(0.31–0.81)	(0.24–0.75)	(0.25–0.48)				
Median	0.39	0.34	0.44	0.29	0.32	0.37	0.60	0.36	0.22
Mean	0.42	0.38	0.48	0.37	0.34				
SE	0.04	0.03	0.08	0.06	0.03				
Uric acid, mg/dl									
Range	(0.99–6.79)	(1.26–5.98)	(1.67–3.62)	(1.26–5.98)	(1.47–3.81)				
Median	2.75	2.40	2.45	2.22	2.45	0.44	0.93	0.19	0.76
Mean	2.76	2.51	2.50	2.50	2.53				
SE	0.35	0.23	0.37	0.61	0.28				

Statistically significant: $p \leq 0.05$.

Serum analysis. Compared to the controls, significantly higher level of serum peroxidase activity was observed in the patients with JIA, particularly of the polyarticular group but also in the systemic group. The maximal increase in the patients with polyarticular type disease over the controls was by 17%, from 0.76 to 0.89 mU/ml ($p < 0.05$). All 3 JIA subgroups had a reduction in the UA concentrations (by approximately 10%) compared to controls, which did not achieve statistical significance. The differences in TAS values among the 3 groups were also not significant. Total serum protein levels were similar in the patients and controls. However, an increase in serum albumin concentrations was observed in all 3 subgroups of patients with JIA. The mean serum albumin in the total group of patients was higher by 9.3% ($p < 0.01$) over the mean control value (4.3 mg/dl). The increased serum albumin concentrations were directly correlated with increased saliva albumin concentrations ($r = 0.45$). The results of the serum analysis are detailed in Table 4.

Immunological studies. The mean ESR of the total JIA group was 22.28 ± 4.75 mm/h, while the control group had a mean ESR of 18.2 ± 4.7. The highest ESR was observed in the patients with systemic type disease (median 44.4 ± 13.88). RF was negative in all the patients. ANA was positive in the serum of 5 of the patients with oligoarticular type disease; in 2 of them it was also positive in the saliva. Anti-SSA and Anti-SSB were negative in all patients, both in serum and in saliva.

DISCUSSION

Sjögren's syndrome in children has only seldom been reported in association with JIA, and very little is known about the involvement of the salivary glands as a component of JIA[16]. We therefore studied salivary gland functions and assessed the antioxidant status in the serum and saliva of children with JIA. Further, we chose to specifically examine salivary peroxidase, SOD, UA, and TAS, since these have been shown to represent the enzymatic, molecular, and overall salivary antioxidant systems in a meaningful manner[13-15]. In this respect, it is worthwhile to mention that salivary NO_2 has been shown to be increased in patients with Sjögren's syndrome[17], although its role is considered more relevant as a promoter of oral inflammatory and carcinogenic conditions than as a salivary antioxidant.

Our main finding was significant alterations in the saliva of patients with oligoarticular JIA, compared to controls and also to other patients with JIA. Whereas the total group of patients showed increased levels of antioxidant enzyme activity both in serum and in saliva, presumably as a result of the ongoing inflammatory process, only the patients with oligoarticular-type disease showed evidence of specific injury to the salivary glands, as reflected by a reduced salivary flow rate, low levels of amylase and LDH activity, and lower concentrations of total proteins. The raised levels of the antioxidant enzymes, salivary-peroxidase, and SOD in the saliva of oligoarticular-type JIA may designate the salivary glands (in similarity to joints) as target organs of inflammatory, oxidative stress-mediated injury. Whereas raised salivary albumin and LDH were observed also in the systemic subgroup, supporting the idea of disease-induced injury to salivary glands in JIA, salivary gland involvement in the oligoarticular patients appeared to be the most pronounced.

Table 4. Serum peroxidase, total antioxidant status (TAS), uric acid, total protein (TP), and albumin (ALB) in total JIA, systemic, polyarticular, and oligoarticular.

Characteristic	Healthy Controls (n = 15)	JIA Total (n = 22)	JIA Poly (n = 7)	JIA Systemic (n = 5)	JIA Oligo (n = 10)	Controls vs Total JIA, p	Controls vs Poly, p	Controls vs Systemic, p	Controls vs Oligo, p
TAS, mmol/l									
Range	(0.81–1.64)	(0.91–2.36)	(0.97–2.36)	(0.91–2.26)	(0.93–2.21)				
Median	1.01	1.00	1.06	1.0	0.99	0.50	0.19	0.90	0.72
Mean	1.05	1.19	1.23	1.1	1.22				
SE	0.05	0.10	0.19	0.16	0.25				
Peroxidase, mU/ml									
Range	(0.58–0.83)	(0.44–1.20)	(0.77–1.20)	(0.44–1.13)	(0.60–0.85)				
Median	0.76	0.78	0.89	0.8	0.73	0.21	0.033	0.21	0.80
Mean	0.74	0.80	0.91	0.8	0.73				
SE	0.17	0.04	0.08	0.07	0.05				
Uric acid, mg/dl									
Range	(3.36–6.82)	(2.46–10.4)	(2.46–10.41)	(3.62–6.88)	(3.38–8.97)				
Median	4.85	4.37	4.56	4.3	4.22	0.99	0.62	0.21	0.90
Mean	4.80	5.00	5.43	4.6	5.08				
SE	0.31	0.44	0.99	0.40	1.00				
TP, mg/dl									
Range	(6.8–8.6)	(6.6–8.4)	(7.2–8.4)	(6.6–8.2)	(7–8.1)				
Median	7.60	7.40	7.60	7.20	7.60	0.95	0.45	0.38	0.82
Mean	7.50	7.46	7.60	7.26	7.52				
SE	7.50	0.10	0.16	0.17	0.20				
ALB, mg/dl									
Range	(3.6–4.8)	(4–5.3)	(4.3–5.3)	(4–4.9)	(4–5.2)				
Median	4.30	4.70	4.90	4.50	4.80	0.014	0.01	0.31	0.07
Mean	4.30	4.66	4.80	4.50	4.70				
SE	0.10	0.08	0.12	0.10	0.20				

Oligoarticular JIA is a unique subtype of the disease, since it does not bear the characteristics of systemic inflammatory disease and the inflammatory process is mostly limited to the joints[18]. Nevertheless, up to 20% of children with oligoarticular JIA suffer also from chronic uveitis[19]. The pathogenesis of chronic uveitis and the nature of its association with JIA are not known. Whereas ANA positivity is the laboratory finding that chronic uveitis and oligoarticular JIA may often have in common, there are no studies implicating ANA antibody as a pathogenetic factor for this disease combination[20,21]. On the other hand, there is fairly convincing evidence that free radical oxidative mechanisms are involved in the pathogenesis of both arthritis and uveitis, and that antioxidant therapy might be protective[22,23]. In autoimmune uveitis that was induced in rats by human S-antigen, increased free radicals and evidence of lipid peroxidation were found in the anterior segment of the eye[24]. Further, increased antioxidant activity has been demonstrated by chemiluminescence in children with uveitis, both in the lacrimal fluid and in the serum, along with a positive correlation between remission and relapses of uveitis and antioxidant activity in tears and in serum[25].

Several reports on antioxidant activity in the serum of children with JIA[26,27] have shown evidence of increased oxidative stress and reduced antioxidant levels, but only a few studies addressed each JIA subgroup separately[28]. Similarly to previously reported data, our results show a major increase in serum antioxidant enzyme activity in children with systemic-type and polyarticular JIA. On the other hand, we observed novel, significantly specific damage to the salivary glands in patients with oligoarticular JIA. We also showed that saliva is a reliable representative of serum composition and can be used for pertinent testing in children. Interestingly, our results gain support from the data of Renke, *et al*, who in 2000 described the oxidative stress induced by the disease in children with JIA and in 2005 reported an increase of the blood SOD activity[29,30].

Our results are unique regarding salivary findings in children with JIA in general, and those with oligoarticular JIA in particular. Further investigation is required of the implications of these observations on the longterm repercussions of salivary gland involvement, and on the possible role of antioxidant therapy in children with JIA.

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