Pain 138 (2008) 226–232

PAIN

www.elsevier.com/locate/pain

Serum and salivary oxidative analysis in Complex Regional Pain Syndrome

Elon Eisenberg [a,e], Shalom Shtahl [b,e], Rimma Geller [a], Abraham Z. Reznick [c,e], Ordi Sharf [a,b,e], Meirav Ravbinovich [a,b], Adam Erenreich [a,b], Rafael M. Nagler [d,e,*]

[a] *Pain Relief Unit, Rambam Medical Center, Haifa, Israel*
[b] *Department of Hand Surgery, Rambam Medical Center, Haifa, Israel*
[c] *Department of Anatomy and Cell Biology, Rambam Medical Center, Haifa, Israel*
[d] *Oral and Maxillofacial Surgery Department and Oral Biochemistry Laboratory, Rambam Medical Center, Bat Galim, 31096 Haifa, Israel*
[e] *Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

Received 13 November 2007; received in revised form 9 April 2008; accepted 15 April 2008

Abstract

Although both inflammatory and neural mechanisms have been suggested as potential contributors to Complex Regional Pain Syndrome type I (CRPS-I), the pathogenesis of the syndrome is still unclear. Clinical trials have shown that free radical scavengers can reduce signs and symptoms of CRPS-I, indirectly suggesting that free radicals and increased oxidative stress are involved in the pathogenesis of CRPS-I. This study investigated this premise by determining the levels of antioxidants in the serum and saliva of 31 patients with CRPS-I and in a control group of 21 healthy volunteers. Serum lipid peroxidation products (MDA) and all antioxidative parameters analyzed were significantly elevated in CRPS-I patients: median salivary peroxidase and superoxide dismutase (SOD) activity values, uric acid (UA) concentration and total antioxidant status (TAS) values were higher in CRPS-I patients by 150% ($p = 0.01$), 280% ($p = 0.04$), 60% ($p = 0.0001$), and 200% ($p = 0.0003$), respectively, as compared with controls. Similar although not as extensive pattern of oxidative changes were found in the serum: mean serum UA and MDA concentrations and TAS value in the CRPS-I patients were higher by 16% ($p = 0.04$), 25% ($p = 0.02$), and 22% ($p = 0.05$), respectively, than in the controls. Additionally, median salivary albumin concentration and median salivary LDH activities in the patients were 2.5 times ($p = 0.001$) and 3.1 ($p = 0.004$) times higher than in the controls. The accumulated data show that free radicals are involved in the pathophysiology of CRPS-I, which is reflected both in serum and salivary analyses. These data could be used for both diagnostic and therapeutic purposes in CRPS-I patients.

Keywords: Complex Regional Pain Syndrome type I (CRPS-I); Neuropathic pain; Inflammation; Ischemia; Oxidative stress; Antioxidants; Free radicals

1. Introduction

Complex Regional Pain Syndrome type I (CRPS-I) is a disabling neuropathic pain syndrome characterized by spontaneous and stimulus-evoked pain, edema, vasomotor, and sudomotor abnormalities, motor dysfunction, and trophic changes, but with no clear evidence for peripheral nerve injury [12,21,25,28,29,35]. Though varying suggestions have been made, such as inflammation, altered sympathetic activity, ischemia, and reperfusion injury and central sensitization, the underlying mechanism of CRPS-I is as yet unclear. Moreover, the therapeutic interventions for the management of this disease are both controversial and limited. These thera-

* Corresponding author. Address: Oral and Maxillofacial Surgery Department and Oral Biochemistry Laboratory, Rambam Medical Center, Bat Galim, 31096 Haifa, Israel. Tel.: +972 4 854 2234; fax: +972 4 854 3505.
E-mail address: nagler@tx.technion.ac.il (R.M. Nagler).

0304-3959/$34.00
doi:10.1016/j.pain.2008.04.019

peutic strategies include pharmacologic pain relief, sympatholytic interventions, and rehabilitation [2,25,29,36].

Furthermore, to date there is no specific laboratory test for CRPS-I; diagnosis of the disease is based on clinical observations of signs and symptoms and on tests such as the WHO analgesic ladder, quantitative sensory testing (QST), autonomic testing that include quantitative sudomotor axon reflex test (QSART) for sweating abnormalities, the cold pressor test in conjunction with thermographic imaging to observe the vasoconstrictor response, and laser Doppler flowmetry to monitor background vasomotor control. Until a better understanding of mechanistic overtones helps to put in place mechanism-based therapeutic strategies, management will continue to be built around a rehabilitation model [29].

An exaggerated inflammatory response to tissue injury, neurogenic inflammation, ischemia and reperfusion injuries can all result in excessive production of free radicals [6,40]. Free radicals, in turn, can increase vascular permeability, release neuropeptides (i.e. substance P), enhance inflammation and cause further tissue damage [33,38]. However, the involvement of free radicals in the pathogenesis of CRPS has never been proved.

Our working hypothesis was that if indeed increased production of free radicals in patients with CRPS-I could be demonstrated, this should facilitate the utilization of antioxidants and free radical scavengers in these patients as efficient therapeutic agents. Such an increase is expected to result in a change in their serum and saliva concentrations, which could also be a monitor for diagnostic purposes [18,19]. In the past, we have found oxidative changes in saliva in various pathologies to be a highly beneficial finding as saliva testing is non-invasive, inexpensive and easily performed [2,40]. Accordingly, in the current study we measured various serum and salivary oxidative parameters and antioxidants as well as saliva composition in CRPS-I patients and in healthy volunteers.

2. Patients and methods

2.1. Subjects and CRPS diagnosis

The study protocol was approved by the Rambam Medical Center Ethics Committee, and written informed consent was obtained from each participant. Patients who were referred to the Department of Hand Surgery or to the Pain Relief Unit at Rambam Medical Center in Haifa, Israel, with a possible diagnosis of CRPS-I, were considered for the study. The diagnosis of CRPS-I was based upon the criteria established by Bruehl et al. [4] and the following evaluation: (a) a study questionnaire on demographic data; the circumstances and date of pain onset; (b) evaluation of the history of changes in skin temperature or color, sudomotor abnormalities, swelling, motor dysfunction, or trophic changes in the skin, hair or nails of the affected limb; (c) measurement of pain intensity on a 10-cm blank visual analog scale (VAS); (d) inspection of the affected (and the contralateral) limb with special attention to the presence of changes in skin color, sweating, swelling, and limb posture; (e) examination of motor power (whenever possible) and range of motion; (f) application of paintbrush strokes (3×) and a single pinprick (3×) to the painful site on the affected limb and the contralateral limb to determine the presence of mechanical allodynia and hyperalgesia; (g) measurements of skin temperature with a surface thermometer. Patients who had any other unrelated medical problems were excluded from the study. Healthy subjects served as a control group, with age and gender matched to the patient group.

2.2. Serum and saliva collections

Serum and saliva samples were collected following at least a 1 h fast. For serum samples, a 21-gauge cannula was inserted into the antecubital vein in the unaffected limb. In the case of lower limb pain, the contralateral upper extremity was used. Ten milliliters of blood was drawn and immediately transferred to EDTA-containing tubes that were kept for 1 h at 8 °C and then centrifuged at 3000 rpm for 7 min at 4 °C. The serum was carefully transferred to polypropylene tubes for storage at −20 °C. For saliva collection, subjects were requested to drink 100 ml of water that was followed by mouth-wash with fresh water. The subjects were then asked to collect saliva in a tube over a period of up to 10 min without making any effort to increase their saliva secretion (i.e. no sucking). The saliva was transferred to polypropylene tubes for storage at −20 °C.

2.3. Biochemical analysis

2.3.1. Sialochemistry analysis

The sialochemistry analysis was performed as previously described [18] and included pH values and potassium (K), calcium (Ca), magnesium (Mg), and phosphate (P) concentrations as well as amylase and lactic dehydrogenase (LDH) activities and total protein (TP) and total albumin (Alb) concentrations. Total protein and albumin as well as sodium (Na), chloride (Cl), K, and LDH were analyzed in the serum, using similar procedures. C-reactive protein (CRP) was also analyzed in the serum using the BN ProSpec System (Dade Behring GmbH, Marburg, Germany).

2.3.2. Oxidative analysis

MDA (malondialdehyde) serum levels were assessed with the use of the Slater–Sawyer method [27]. Briefly, thiobarbituric acid was used during the reaction to assess the level of induced lipid peroxidation, as there is a direct correlation between the two [16].

Peroxidase activity was measured in the patients' saliva according to the NBS assay as previously described [24]. Briefly, the calorimetric change induced by the reaction between the enzyme and the substrate, Dithiobis 2-Nitrobensoic Acid (DTNB) in the presence of mercapto-ethanol, was read at a wavelength of 412 nm for 20 s.

Superoxide dismutase (SOD) activity: Total salivary activity of the SOD enzyme (Cu/Zn- and Mn-SOD) was measured using the xanthine oxidase/XTT method as previously

described [32]. The method is a modification of the NBT assay in which XTT is reduced by superoxide anion (SO) that is generated by xanthine oxidase. The formazan is read at 470 nm. The SOD enzyme inhibits the reaction by scavenging the SO. One unit of the enzyme is defined as the amount of enzyme needed for 50% inhibition of the absorption.

Uric acid concentration was measured both in the serum and saliva of the patients using a kit supplied by Sentinel CH (Milan, Italy), as previously described [18]. UA is transformed by uricase into allantoin and hydrogen peroxide which, under the catalytic influence of peroxidase, oxidizes the chromogen (4-aminophenazone/*N*-ethyl-methylanilin propan-sulphonate sodic) to form a red compound whose intensity of color is proportional to the amount of UA present in the sample and is read at a wavelength of 546 nm.

Total antioxidant status was assessed both in the saliva and serum samples as previously described [19]. Briefly, this assay is based on a commercial kit supplied by Randox (USA) in which metmyoglobin in the presence of iron is turned into ferrylmyoglobin. Incubation of the latter with the Randox reagent ABTS results in the formation of a radical colored blue–green which can be detected at 600 nm.

2.4. Statistical analysis

Data concerning the various parameters of plasma and saliva were compared between the two groups (CRPS-I patients and healthy controls). Due to the large in-born variability of parameters in saliva, median values were used for saliva comparisons, while mean values (and standard error) were calculated for plasma parameters. The comparisons between the two groups of saliva parameters were analyzed using "Wilcoxon rank-sum test" whereas the comparisons of the plasma parameters were done with the use of "One-way analysis of variance".

3. Results

3.1. Subjects and CRPS-I diagnosis

Thirty-one patients who met CRPS criteria according to Bruehl et al. [4] and provided written informed consent were included in the study. Twenty-one patients served as a control group. All patients were classified as CRPS type I. The median age of the CRPS-I patients was 39 years (range 21–73) and was comparable to that of the controls (median 37; range 29–51). Seventeen patients were males and 14 were females. The upper limb was affected in 15 patients and the lower limb in 16. The time elapsed since injury was greater than 1 year in 25 patients. Of these, 11 patients had their symptoms for more than 3 years (maximum 16.5 years). Only four patients had CRPS-I for less than 6 months (Table 1). None of the patients had any symptoms or signs of CRPS-I in more than one limb. Notably, many of the patients consumed various medications at the time examined (Table 1); however, none of these medications is known to affect any of the salivary or serum parameters analyzed.

Table 1
Demographic, CRPS characteristics and treatment ($n = 31$)

Demographic and injury		Current signs and symptoms		Previous treatments		Current medications	
Age, median (range): 39 (21–73)[a]		Spontaneous pain	31	NSAIDs[c]	31	*For pain control*	
				Opioids/tramadol	13	NSAIDs	7
Gender		Mechanical allodynia	31	Antidepressants	11	Opioids/tramadol	12
Male	17			Anticonvulsants	6	Antidepressants	10
Female	14	Color changes	31	Epidural/nerve blocks	16	Anticonvulsants	4
		Edema	29	Topical agents	3	Topical agents	1
Affected limb		Hyperhydrosis	14	P.T/O.T[d]	31	Calcitonin	2
Upper	15	Temperature changes	26	Acupuncture	15	Dipyrone	2
Lower	16	Motor changes	31	Biofeedback	3	*For other conditions*	
		Trophic changes	24	Spinal cord stimulation	1	Diuretics	2
Type of injury				I.V. ketamine	1	Low dose aspirin	2
Trauma[b]	28					ACE inhibitors	1
Surgery	2						
Overuse injury	1						
Symptoms duration							
<6 months	4						
6–12 months	2						
>12 months	25						

[a] Median (range) age of the controls = 37 (29–51) years.
[b] Trauma included: bone fracture – 12; crush injury – 4; ankle sprain – 2; gun-shot – 2; fall – 5; amputation of phalang – 1.
[c] NSAIDs, non-steroidal anti-inflammatory drugs.
[d] P.T/O.T, physiotherapy/occupational therapy.

Table 2
Salivary biochemical analysis of CRPS-I vs healthy controls

Saliva parameters		CRPS	Controls	*p* value
pH	Range	(5.5–7.3)	(5.2–7.3)	0.68
	Median	6.70	6.55	
Ca (mg/dL)	Range	(0.7–5.3)	(0.5–4.7)	0.48
	Median	1.40	1.80	
P (mg/dL)	Range	(5.2–16.7)	(5.5–11.8)	0.45
	Median	10.70	9.40	
Mg (mg/dL)	Range	(0.3–1.9)	(0.3–1.1)	0.85
	Median	0.70	0.85	
Fe (ug/dL)	Range	(0–5)	(1–4)	0.54
	Median	2.0	2.0	
TP (mg/dL)	Range	(10–244)	(13–183)	0.23
	Median	72.0	52.0	

$p > 0.05$ (NS); (Wilcoxon rank-sum test).

3.2. Biochemical analysis of saliva and serum

The median salivary values of pH and amylase activity as well as the median salivary concentrations of Ca, P, Mg, Fe, and total protein were not statistically different between the CRPS-I and healthy control groups (Table 2). In contrast, there were significant differences in the median salivary albumin concentration and LDH enzymatic activity values between the two groups. The median salivary albumin concentration of the CRPS-I group was 50 mg/L, 2.5 times higher than that of controls ($p = 0.001$) and the median salivary LDH activity in the CRPS-I group was 54 IU/L, 3.1 times higher than that of controls ($p = 0.004$) (Fig. 1).

The mean serum values of the amylase and the LDH activity and the concentrations of Na, K, Cl, total protein and albumin were not significantly different between the CRPS-I and control groups (Fig. 2 and Table 3).

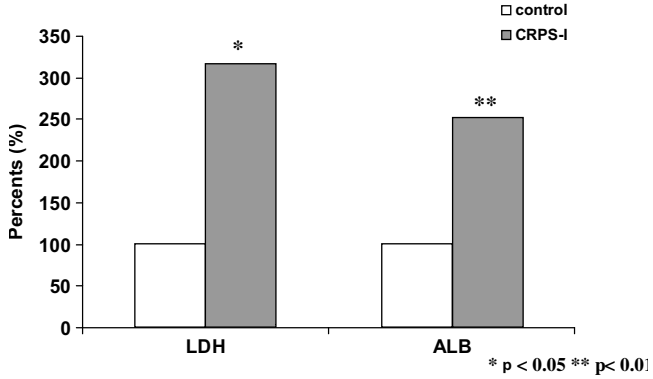

Fig. 1. LDH activity and albumin concentrations in the saliva of CRPS-I patients and healthy controls. * and ** depict $p < 0.05$ and $p < 0.01$, respectively.

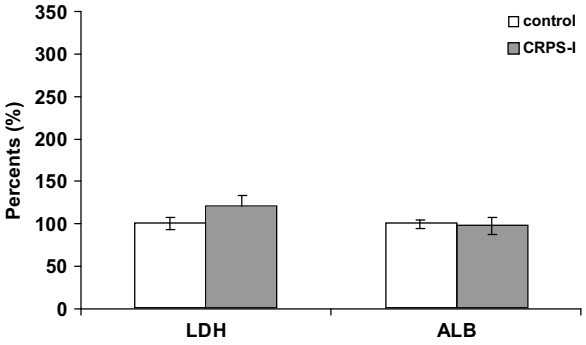

Fig. 2. LDH activity and albumin concentrations in the serum of CRPS-I patients and healthy controls.

3.3. Oxidative analysis of saliva and serum

All oxidative parameters analyzed demonstrated the same pattern when compared between the CRPS-I and healthy control groups, i.e. increased levels of antioxidants in the saliva of the CRPS-I patients. Thus, the median salivary peroxidase, SOD activity, uric acid concentration, and TAS values in the CRPS-I patients were 451 mU/ml, 2.3 U/ml, 2.5 mg/dL, and 0.9 mmol/L, respectively. These were higher by 150% ($p = 0.01$), 180% ($p = 0.04$), 60% ($p = 0.0001$), and 100% ($p = 0.0003$), respectively, than those of controls (Fig. 3). This pattern of oxidative changes was quite similar though not as extensive when we analyzed the serum; i.e. the median serum uric acid, MDA concentrations and TAS value in the CRPS-I patients were 5.7 mg/dL, 10.3 nmol/ml, and 0.9 mmol/L, respectively,

Table 3
Serum biochemical analysis of CRPS vs healthy controls

Serum parameters		CRPS-I	Controls	*p* value
Na (mmol/L)	Range	(131–144)	(136–144)	0.23
	Mean	138	139	
	SER	0.94	0.82	
K (mmol/L)	Range	(3.9–5.1)	(3.8–5.2)	0.31
	Mean	4.38	4.56	
	SER	0.10	0.14	
Cl (mmol/L)	Range	(100–109)	(103–112)	0.92
	Mean	105	105	
	SER	0.80	0.84	
LDH (U/L)	Range	(58–1155)	(46–363)	0.44
	Mean	330	265	
	SER	78	24	
Amy (10^2 IU/L)	Range	(27–92)	(36–75)	0.45
	Mean	58.5	53.5	
	SER	5.5	3.1	
TP (mg/dL)	Range	(6.5–8.0)	(6.7–8.3)	0.09
	Mean	7.1	7.5	
	SER	0.15	0.14	

*$p < 0.05$ (Sig); $p > 0.05$ (NS); (one-way analysis of variance test).

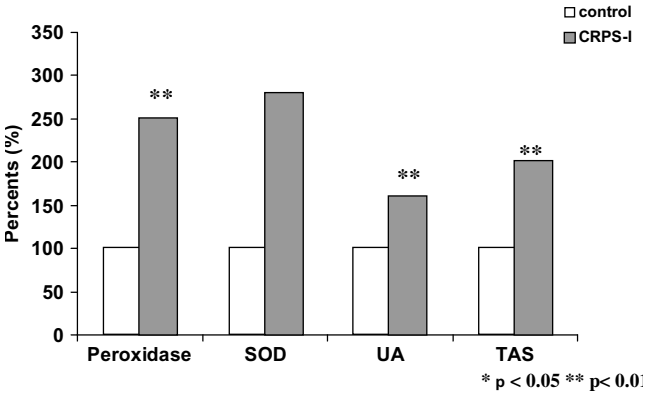

Fig. 3. Salivary oxidative profile in CRPS-I. The peroxidase and SOD activities, UA concentrations and TAS values are compared between CRPS-I patients and healthy controls. * and ** depict $p < 0.05$ and $p < 0.01$, respectively.

higher by 16% ($p = 0.04$), 25% ($p = 0.02$), and 22% ($p = 0.05$) than in the controls (Fig. 4).

4. Discussion

The novel oxidative analysis currently performed both in the saliva and in the serum of the CRPS-I patients revealed significantly elevated MDA levels which directly prove an oxidative inflicted damage in these patients [14]. Moreover, an increase of all antioxidants examined in the CRPS-I patients was found, especially extensive in the saliva. Notably, the importance of detecting serum and saliva antioxidant levels has already been stressed [18,19]. This demonstration of the salivary substantial oxidative alterations in CRPS-I, besides being novel, is mechanistically interesting and may be of special clinical importance, as it is a potential clinical marker of this syndrome. The rise in the levels of antioxidants indicates a general increase in the oxidative stress in CRPS-I, an oxidative stress which induces a compensative response of producing higher levels of antioxidants [18]. Thus, our study showed a profound increase (2.5–2.8 times) of the peroxidase and SOD enzymes in the saliva of CRPS-I patients. Additionally, there was a 1.6-fold increase of the salivary major antioxidant molecule, the UA, and a 2-fold increase of the salivary total antioxidant status. These results were further supported by the increase of serum MDA (a lipid peroxidation product) levels previously mentioned as well as by the significant increase of serum antioxidants: UA and total antioxidant status.

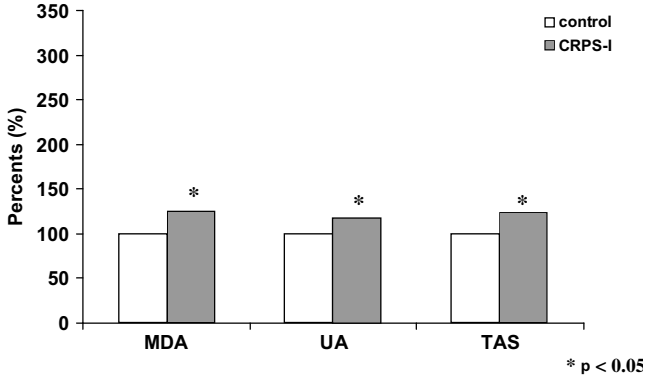

Fig. 4. Serum oxidative profile. MDA and UA concentrations and TAS values are compared between CRPS-I patients and healthy controls. * depicts $p < 0.05$.

The oxidative changes demonstrated in our CRPS-I patients are both novel and important with respect to the pathogenesis of this syndrome whose underlying mechanism is considered enigmatic. In addition to peripheral and central neural, autonomic and possibly ischemic components [1,6–8,14,15,20], inflammatory processes were also suggested as being involved in the pathogenesis of CRPS-I [21,26,30,37]. Experimental and clinical data suggest that tissue injury, neurogenic inflammation and ischemia-reperfusion injury can all lead to excessive production of free radicals [6,40]. Free radicals, in turn, can increase vascular permeability, release neuropeptides (i.e. substance P), enhance inflammation and cause further tissue damage [33,38]. Furthermore, a number of clinical trials have shown that the free radical scavengers, dimethylsulfoxide (DMSO), *N*-acetylcysteine (NAC), vitamin C, and mannitol, can reduce signs and symptoms of CRPS-I [9,10,22,42–44]. It is important to note the recent report of diminished nitric oxide levels in fluids aspirated from artificial suction blisters, which occured in the affected limb of patients with CRPS-I, as compared to blisters in the contralateral healthy limb [11]. Taken together with the results of our study, this may further support our suggestion that the increased oxidative stress taking place in the affected limb leads to a compensatory overproduction of antioxidants in other body tissues.

We believe that the pain as well as the other symptoms found in CRPS-I is, at least in part the result of the effects of free radicals, as the injurious effects of free radicals of various sorts (as nitric oxide, aldehydes, hydroxyl radicals) on neurons resulting in a neuropathic pain and other neuropathies are well established. So is the pivotal role of oxidative stress in the pathogenesis of different other neurological pathologies. For example, nitric oxide (NO) participates in critical actions involving several aspects of peripheral nerve function and disease. It offers important roles in "normal" afferent signaling of pain through the dorsal horn of the spinal cord and in autonomic control through nitrergic innervation. NO is generated during the fundamental processes of Wallerian degeneration of peripheral nerves following injury that bear on subsequent regenerative events. Through its actions on vasa nervorum, the blood supply to nerves, NO participates in microvascular

changes following injury but also has direct roles in axon and myelin breakdown and "clearance" prior to regeneration. During such processes, NO contributes to the development of neuropathic pain. Excessive local levels of NO during inflammation may damage axons and growth cones. Low-grade chronic rises in NO may also contribute toward peripheral nerve damage, or neuropathy in diabetes [3,13,17,23,31,41]. Other mechanisms which relate neuronal pathologies and free radicals induced damage have been suggested. Free radicals and oxidative stress can be elicited from mitochondrial dysfunction. This may be the case for example in ALS, in Alzheimer's disease or in Friedreich's ataxia [5,34,39].

In summary, the current study shows that saliva and serum antioxidants and serum MDA levels are elevated in patients with CRPS-I. These results may be considered direct proof for the involvement of free radicals in the pathophysiology of CRPS-I. However, due to the relatively small number of patients in our study, the high intensity of the pain (>7/10) in most of the patients and the fact that the majority of patients had had the syndrome for more than 12 months, we could not correlate the levels of antioxidants with the duration or the severity of the syndrome. Future studies with larger numbers of patients and various levels of pain intensity and prolongation of the disease are warranted. In any case, most important is that MDA and the various antioxidants examined may be used as laboratory tests for diagnosing the disease while using systemic antioxidants for CRPS-I patients may be considered. The fact that the oxidative related alterations could be found in saliva is espacially encouraging, as saliva monitoring is non-invasive and easily performed (even by the patient himself).

Acknowledgement

All the authors declare that they have no conflict of interest, financial or otherwise, concerning this manuscript.

References

[1] Albrecht PJ, Hines S, Eisenberg E, Pud D, Finlay DR, Connolly MK, et al. Alterations in cutaneous innervation and vasculature of affected limbs in patients with complex regional pain syndrome. Pain 2006;120:244–66.

[2] Bahar G, Feinmesser R, Shpitzer T, Popovtzer A, Nagler RM. Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. Cancer 2007;109:54–9.

[3] Barzilai A. The contribution of the DNA damage response to neuronal viability. Antioxid Redox Signal 2007;9:211–8.

[4] Bruehl S, Harden RN, Galer BS, Saltz S, Bertram M, Backonja M, et al. External validation of IASP diagnostic criteria for complex regional pain syndrome and proposed research diagnostic criteria. Pain 1999;81:147–54.

[5] Calabrese V, Lodi R, Tonon C, D'Agata V, Sapienza M, Scapagnini G, et al. Oxidative stress, mitochondrial dysfunction and cellular stress response in Friedreich's ataxia. J Neurol Sci 2005;233:145–62.

[6] Coderre TJ, Xanthos DN, Francis L, Bennett GJ. Chronic post-ischemia pain (CPIP): a novel animal model of complex regional pain syndrome-type I (CRPS-I; reflex sympathetic dystrophy) produced by prolonged hindpaw ischemia and reperfusion in the rat. Pain 2005;112:94–105.

[7] Eisenberg E, Chistyakov AV, Yudashkin M, Kaplan B, Hafner H, Feinsod M. Evidence for cortical hyperexcitability of the affected limb representation area in CRPS: a psychophysical and transcranial magnetic stimulation study. Pain 2005;113:99–105.

[8] Galer B, Schwartz L, Allen R. Complex regional pain syndromes – type I: reflex sympathetic dystrophy, and type II: Causalgia. In: Loeser J, editor. Bonica's management of pain. Philadelphia: Lippincott Williams & Williams; 2001. p. 388–411.

[9] Geertzen JHB, de Bruijn H, de Bruijn-Kofman AT, Arendzen JH. Reflex sympathetic dystrophy: early treatment and psychological aspects. Arch Phys Med Rehabil 1994;75:442–6.

[10] Goris RJA, Dongen LMV, Winters HAH. Are toxic oxygen radicals involved in the pathogenesis of reflex sympathetic dystrophy? Free Radic Res Commun 1987;3:13–21.

[11] Groeneweg JG, Huygen FJ, Heijmans-Antonissen C, Niehof S, Zijlstra FJ. Increased endothelin-1 and diminished nitric oxide levels in blister fluids of patients with intermediate cold type complex regional pain syndrome type 1. BMC Musculoskelet Disord 2006;7:91–8.

[12] Harden RN, Bruehl S, Stanton-Hicks M, Wilson PR. Proposed new diagnostic criteria for complex regional pain syndrome. Pain Med 2007;8:326–31.

[13] Hidalgo C, Carrasco MA, Muñoz P, Núñez MT. A role for reactive oxygen/nitrogen species and iron on neuronal synaptic plasticity. Antioxid Redox Signal 2007;9:245–55.

[14] Janig W, Baron R. Complex regional pain syndrome: mystery explained? Lancet Neurol 2003;2:687–97.

[15] Ludwig J, Gorodetskaya N, Schattschneider J, Janig W, Baron R. Behavioral and sensory changes after direct ischemia-reperfusion injury in rats. Eur J Pain 2007;11:677–84.

[16] Lykkesfeldt J. Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin Chim Acta 2007;380:50–8.

[17] Marchitti SA, Deitrich RA, Vasiliou V. Neurotoxicity and metabolism of the catecholamine-derived 3,4-dihydroxyphenylacetaldehyde and 3,4-dihydroxyphenylglycolaldehyde: the role of aldehyde dehydrogenase. Pharmacol Rev 2007;59:125–50.

[18] Nagler RM, Klein I, Zarzhevsky N, Drigues N, Reznick AZ. Characterization of the differentiated antioxidant profile of human saliva. Free Radic Biol Med 2000;32:268–77.

[19] Nagler RM, Hershkovich O, Lischinsky S, Diamond E, Reznick AZ. Saliva analysis in the clinical setting: revisiting an underused diagnostic tool. J Investig Med 2002;50:214–25.

[20] Oaklander AL, Rissmiller JG, Gelman LB, Zheng L, Chang Y, Gott R. Evidence of focal small-fiber axonal degeneration in complex regional pain syndrome-I (reflex sympathetic dystrophy). Pain 2006;120:235–43.

[21] Oyen WJG, Arntz IE, Claessen RAMJ, van der Meer JWM, Corstens FHM, Goris RJA. Reflex sympathetic dystrophy of the hand: an excessive inflammatory response? Pain 1993;55:151–7.

[22] Perez RS, Zuurmond WW, Bezemer PD, Kuik DJ, van Loenen AC, de Lange JJ, et al. The treatment of complex regional pain syndrome type I with free radical scavengers: a randomized controlled study. Pain 2003;102:297–307.

[23] Peterson JR, Sharma RV, Davisson RL. Reactive oxygen species in the neuropathogenesis of hypertension. Curr Hypertens Rep 2006;8:232–41.

[24] Reznick AZ, Klein I, Eiserich JP, Cross CE, Nagler RM. Inhibition of oral peroxidase activity by cigarette smoke: in vivo and in vitro studies. Free Radic Biol Med 2003;34:377–84.
[25] Ribbers GM, Geurts AC, Stam HJ, Mulder T. Pharmacologic treatment of complex regional pain syndrome I: a conceptual framework. Arch Phys Med Rehabil 2003;84:141–6.
[26] Schinkel C, Gaertner A, Zaspel J, Zedler S, Faist E, Schuermann M. Inflammatory mediators are altered in the acute phase of posttraumatic complex regional pain syndrome. Clin J Pain 2006;22:235–9.
[27] Slater TF, Sawyer BC. The stimulatory effect of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vivo. Biochem J 1971;123:805–14.
[28] Stanton-Hicks M, Janig W, Hassenbusvh S, Haddox JD, Boas R, Wilson P. Reflex sympathetic dystrophy: chancing concepts and taxonomy. Pain 1995;63:127–33.
[29] Stanton-Hicks M. Complex regional pain syndrome. Anesthesiol Clin North America 2003;21:733–44.
[30] Sudeck P. Über die akute (reflektorische) Knochenatrophie nach Entzündungen und Verletzungen in den Extremitäten und ihre klinischen Erscheinungen. Fortschr Röntgenstr 1991;5:227–93.
[31] Trushina E, McMurray CT. Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases. Neuroscience 2007;145:1233–48.
[32] Ukeda H, Maeda S, Ishii T, Sawamura M. Spectrophotometric assay for superoxide dismutase based on tetrazolium salt. 3-1-(Phenylamino)carbonyl-3,4-tetrazolium-bis(4-methoxyl-6-nitro)-benzenesulfonic acid hydrate reduction by xanthine oxidase. Anal Biochem 1997;251:206–9.
[33] Van der Laan L, Kapitein PJC, Oyen WJG, Verhofstad AAJ, Hendriks T, Goris RJA. A novel animal model to evaluate oxygen derived free radical damage in soft tissue. Free Radic Res 1997;26:363–72.
[34] Vande Velde C, Miller TM, Cashman NR, Cleveland DW. Selective association of misfolded ALS-linked mutant SOD1 with the cytoplasmic face of mitochondria. Proc Natl Acad Sci USA 2008;105:4022–7.
[35] Vaneker M, Wilder-Smith OH, Schrombges P, Oerlemans HM. Impairments as measured by ISS do not greatly change between one and eight years after CRPS 1 diagnosis. Eur J Pain 2006;10:639–44.
[36] Wasner G, Backonja MM, Baron R. Traumatic neuralgias: complex regional pain syndromes (reflex sympathetic dystrophy and causalgia): clinical characteristics, pathophysiological mechanisms and therapy. Neurol Clin 1998;16:851–68.
[37] Weber M, Birklein F, Neundorfer B, Schmelz M. Facilitated neurogenic inflammation in complex regional pain syndrome. Pain 2001;91:251–7.
[38] Yonehara N, Yoshimura M. Effect of nitric oxide on substance P release from the peripheral endings of primary afferent neurons. Neurosci Lett 1999;271:199–201.
[39] Zhu X, Smith MA, Perry G, Aliev G. Mitochondrial failures in Alzheimer's disease. Am J Alzheimer's Dis Other Demen 2004;19:345–52.
[40] Zloczower M, Reznick AZ, Zouby RO, Nagler RM. Relationship of flow rate, uric acid, peroxidase and superoxide dismutase activity levels with complications in diabetic patients: can saliva be used to diagnose diabetes? Antioxid Redox Signal 2007;9:765–73.
[41] Zochodne DW, Levy D. Nitric oxide in damage, disease and repair of the peripheral nervous system. Cell Mol Biol (Noisy-le-grand) 2005;51:255–67.
[42] Zollinger PE, Tuinebreijer WE, Kreis RW, Breederveld RS. Effect of vitamin C on frequency of reflex sympathetic dystrophy in wrist fractures: a randomized trial. Lancet 1999;345:2025–8.
[43] Zuurmond WWA, Langendijk PHJ, Bezemer PD, Brink HEJ, de Lange JJ, van Loenen AC. Treatment of acute reflex sympathetic dystrophy with DMSO 50% in a fatty cream. Acta Anaesthesiol Scand 1996;40:364–637.
[44] Zyluk A. The reasons for poor response to treatment of posttraumatic reflex sympathetic dystrophy. Acta Orthop Belg 1998;64:309–13.