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Evaluation of Circulating Nitric Oxide Levels in Patients with Complex Regional Pain Syndrome Type 1

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Abstract

Objectives: Proinflammatory mediators play an important role in the pathophysiology and development of complex regional pain syndrome type 1. Elevated cytokine production is suggested to increase nitric oxide production by activating the inducible nitric oxide synthase pathway. In this study, we aimed to determine the circulating nitric oxide concentrations in complex regional pain syndrome type 1 patients, and to compare them with those of healthy controls. **Methods:** Serum circulating nitric oxide concentrations were measured in twenty-five patients (15 female and 10 male) with complex regional pain syndrome type 1 who fulfillied the criteria of the modified International Association for the Study of Pain (IASP) and compared those of twenty-five (15 female and 10 male) age, gender matched healthy subjects. Nitric oxide concentration is estimated indirectly based on Greiss method by measuring the combined oxidation products of nitric oxide (total nitrites and nitrates). **Results:** There was no significant difference in the demographic data among two groups ($p>0.05$). The mean serum nitric oxide concentration (39.50±13.26 µmol) was significantly higher in the complex regional pain syndrome type 1 patients group compared to those of controls (27.15±11.90 µmol) ($p<0.001$). **Conclusions:** We suggest that nitric oxide levels play an important role on inflammatory reactions in CPRS 1 patients.

Keywords: complex regional pain syndrome type 1, free radical, inflammation, nitric oxide, pathophysiology.

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1. Introduction

Complex regional pain syndrome (CRPS) is a neurophatic pain syndrome characterized with pain; temparature altitudes, oedema, sweating and trophic changes in a local zone of skin, mostly on limbs. More than fifty synonyms have been used by researchers for identifiying the disease during a long period of time. International Association for the Study of Pain (IASP) has named these clinical conditions as CRPS Type I referring to the reflex sympathic dystropy, CRPS Type II referring to causalgia and CRPS Type III referring to undefined type (Bruehl et al. 2002; Harden et al. 2007). CRPS often occurs secondary to surgery or local trauma and limb extremities are primarily affacted. The exact pathophysiology is not fully understood. Both central and peripheral changes are presented in patients with CRPS. Central nervous system (CNS) representations include somatosensory, somatomotor, and sympathetic changes. Altered peripheral changes such as oedema, inflammation, and trophic changes accompanies to central changes (Baron and Janig 2004; Janig and Baron 2003). Involvement of exaggerated inflammatory processes have been proposed to play a prominent role in the pathophysiology of CPRS.

Nitric oxide (NO) is one of the main modulator of vascular tone relaxation (Napoli and Ignarro 2009). Altered NO levels have been shown in many inflammatory conditions including CRPS (Akdeniz et al. 2004; Bruch-Gerharz et al. 1998; Kröncke et al. 1998; Moshage 1997) and there is a relationship between NO and pain pathways (Koch et al. 2007).

This study was designed to investigate the possible relationship between CRPS and nitric oxide.

1. Materials and Methods

Twenty-five patients diagnosed as CRPS 1 according to the criteria of the modified International Association for the Study of Pain (IASP) were included to the study. Participants who had chronic diseases, other rheumatic diseases, infections or malignant tumors and an ovarage dietary intake of nitrite-nitrate were excluded from the study. The control group was composed of twenty-five age and gender matched healthy subjects who had normal physical examination and routine test results with no history of any chronic diseases, chronic pain or neuropathy. Demographic, clinical and laboratory findings of patients and controls were recorded.

Venous blood samples were collected in the morning hours after overnight fasting at ice-cooled conditions. After separating serum, samples were stored at -80 °C until analyse day. Serum samples were deproteinized by Smogyi reagent (2.0 ml 55 mmol/L NaOH+ 2.5 ml 75 mmol/L $ZnSO_4$) (Somogyi 1930) and then ultrafiltered (Millipore, no: 42421) in order to prevent bacterial contamination.

Since NO is a very labile molecule and it is converted to nitrates and nitrites in seconds, circulating NO levels are indirectly estimated by measuring nitrates and nitrites, the last stable oxidation products of NO (Akyol et al. 2002). We used a colorimetric nitric oxide synthase assay kit which is commercially available. In this two-step analyse method; the first step is reducing nitrate to nitrite by nitrate reductase. In the second step the whole nitrite formed with Griess reagent (sulfanilamide and naphthalene-ethylene diamine dihydrochloride) is measured spectrophotometrically. Results are given as µmol.

Data were analyzed using the SPSS/PC statistical software package (SPSS, v.20.0 for Windows, SPSS Inc. Chicago). All results were expressed as mean±standard deviation. The Student's t test was used to evaluate the significance of differences between groups since they distrubuted normally. P values less than 0.05 were considered significant, at 95% confidence interval. Ethics approval was obtained for this study, informed consent was obtained from each participant and patient anonymity was preserved. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

2. Results

Demographical characteriscs and serum NO levels of groups are given in Table 1. There was no statististically significant differences between patient and control groups in terms of age and gender. The study showed significantly elevated serum levels of NO in patients with CRPS I (39.50±13.26 µM) compared to healthy controls (27.15±11.90 µM) with $p<0.001$.

Table 1. Demographic Data and Serum NO levels in the Patient Group and the Control Group

	Patient Group (n=25)	Control Group (n=25)	Test statistic p value
Age (years, mean±SD)	55.4±10.8	56.8±11.2	0.72
Gender (no, female/male)	15/10	15/10	0.85
Disease duration (months, mean±SD)	3.2±1.4	-	-
VAS	7.8±0.7	-	-
Serum NO (µmol)	39.50±13.26	27.15±11.90	<0.001

NO: Nitric oxide, SD: standard deviation, VAS: Visual analog scale (VAS) pain score

3. Discussion

The pathophysiology of CPRS has not been fully understood. Both peripheral and central mechanisms are proposed to play key role in the pathophysiology (Mrabet et al. 2012). In a comprehensive review written by Goebel A, eight major concepts for the etiology of CRPS are stated (Goebel 2011). A combination of abnormalities including an inflammatory process, sympathetic dysregulation, nerve damage, serum autoantibodies, central sensitization, ischaemia or ischaemia reperfusion injury and cortical reorganization are thought to have role in the occurence of CRPS.

The existence of an inflammatory process in the occurence of CRPS is certain. In fact it has been suggested by Sudeck P. a long time ago and the condition presenting the five signs of inflammation was called as Sudeck athropy (Rather 1971). Cytokines, neuropeptides and other inflammatory mediators are released throughout the neurogenic inflammation process (Birklein 2005;Huygen et al. 2002). Elevated levels of inflammatory mediators including TNF-a are reported in blister fluids of affected limbs of CRPS patients (Huygen et al. 2002; Rather 1971) and these mediators have been suggested to be responsible in the initation and development of CRPS. Despite the concentration of these mediators are not related to the density of pain, there are animal model studies in accordance with these findings (Kingery 2010).

NO is a key regulatory element for endothelial function and vascular tone relaxation (Napoli and Ignarro 2009). It is well known that nitric oxide involves in numerous inflammatory and autoimmune diseases (Akdeniz et al. 2004; Bruch-Gerharz et al. 1998; Kröncke et al. 1998; Moshage 1997). Elevated NO production is supposed to be related with inflammatory processes particularly in the acute stages (Akdeniz et al. 2004). Evidences suggest that endothelial dysfunction results in reduction of blood flow in CRPS (Schattschneider et al. 2006).

Cytokine release after a stimulus triggers a cascade of biological events. Following cytokine release NO production is induced and the cascade proceeds (Goris 1998). It is notable that NO generation is altered in inflammatory (Yamamoto et al. 1998) and neuropathic (Sarchielli et al. 1992) conditions, which have similar clinical symptoms with CRPS. Many studies have reported elevated NO levels in CRPS. In a study comparing cerebrospinal fluid (CSF) levels of the nitric oxide metabolites (nitrate and nitrite) on twenty-two patients with CRPS, CSF levels of the nitric oxide metabolites were significantly higher compared to the levels of patients with other diseases (Alexander et al. 2007) as well as compared to those of two studies which were conducted on individuals with no neurological symptoms (Yamada et al. 1997; Yumite et al. 2001). Altered NO production was obserbed from interferon-γ stimulated peripheral blood monocytes of CRPS patients compared to controls (Hartrick 2002).

It is known that clinical presentation of CRPS are increased skin temperature, redness, and a shiny skin with loss of function and pain. While some of these signs lessens or disappears over the progression of syndrome, pain usually lasts unrelieved and CRPS is a highly painful condition. As the symptoms are associated with regional inflammation in the acute stage, the chronic stage is more likely to a neuropathic disorder. Besides the significant role of NO in inflammatory diseases, the role of NO in pain pathways is remarkable. Increased levels of NO have been evaluated in chronic pain (Koch et al. 2007) and many painful disorders (Anbar 1998; Fan 2012; Hosseini et al. 2004). Growing evidence suggests that NO plays role in the modulation of nociception and can contribute to peripheral and central sensitization (Cury et al., 2011; Meller et al., 1994; Wu et al., 1998).

In our study NO levels of CRPS 1 patients who were in the acute stage of their disease were significantly higher compared to healthy controls. Considering elevated NO levels and high VAS levels of our patients together, we can conclude that altered NO levels may be related to inflammation and pain in CRPS patients. Additionally, it must be kept in mind that inflammatory profiles differ between acute and chronic stages of CRPS.

Evaluating NO levels together with other inflammatory cytokines in studies including large series and different stages of CRPS patients may clearly evaluate the role of NO in CRPS pathophysiology.

Conflict of interest

The authors declare no conflict of interest.

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