



Thiol-disulphide homeostasis in chronic sinusitis without polyposis

Kazım Bozdemir¹, Arife Sezgin², Ahmet Akkoz¹, Özcan Erel³, Mehmet Hakan Korkmaz¹

¹Department of Otolaryngology, Medicine Faculty of Yıldırım Beyazıt University, Ankara, Turkey

²Department of Otolaryngology, Ankara Atatürk Training and Research Hospital, Ankara, Turkey

³Department of Biochemistry, Medicine Faculty of Yıldırım Beyazıt University, Ankara, Turkey

ABSTRACT

Objectives: Oxidative stress plays a role in the pathogenesis of chronic mucosal inflammation in chronic sinusitis and we aimed to investigate a novel oxidative stress marker, thiol/disulphide homeostasis.

Patients and Methods: A total of 60 subjects (30 chronic sinusitis patients and 30 healthy volunteers) were included in the study. Thiol/disulphide levels were analyzed with a newly developed method by Erel and Neselioglu.

Results: The average native thiol value of the chronic sinusitis group was 475.6 $\mu\text{mol/L}$ and 515.8 $\mu\text{mol/L}$ in the control group. The total thiol was 509.4 and 552.2 $\mu\text{mol/L}$ respectively for the chronic sinusitis and control groups. Chronic sinusitis patients had significantly lower native and thiol value than control group ($p < .001$ and $p = .001$). There was no significant difference between chronic sinusitis and control groups with respect to disulphide levels.

Conclusion: Serum thiol and disulphide measurements can be used as a novel method for the reflection of inflammation for chronic rhinosinusitis patients.

Keywords: Chronic sinusitis; homesostasis; thiol-disulphide.

Chronic rhinosinusitis (CRS) is a broad clinical syndrome that is defined by mucosal inflammation of the nose and paranasal sinuses. The inflammatory condition is commonly divided into three phenotypes CRS with nasal polyps (CRSwNP), CRS without nasal polyps (CRS sine NP, CRSsNP), and allergic fungal sinusitis. The coexistence of rhinitis and sinusitis is called rhinosinusitis defined as the inflammation of nose and paranasal sinuses with two or more of the following symptoms: nasal congestion or blockage, anterior or

posterior nasal discharge, facial pain or pressure, reduction or loss of smell, and complementary endoscopic signs and computed tomography (CT) changes. The ostiomeatal complex plays a fundamental role in the pathogenesis of rhinosinusitis.^[1] If rhinosinusitis persists for more than 12 weeks, it would be classified as CRS,^[2] which can be a consequence of epithelial damage and activation of immunity.^[3]

Since it is not possible to differentiate NP and CRS, they are often taken as one disease entity,^[4] and NP is considered as a

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Correspondence: Arife Sezgin, MD. Yıldırım Beyazıt Üniversitesi Tıp Fakültesi Kulak Burun Boğaz Anabilim Dalı, 06760 Çubuk, Ankara, Turkey.

e-mail: drarife@gmail.com

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subgroup of CRS. Many factors have been held responsible for the chronicity of inflammation in rhinosinusitis such as genetics, alterations in the barrier mechanism of mucosal lining, infections, allergy and eosinophilic activity. These factors cause an inflammatory process and cytokines, endotoxins and mediators cause chronic oxidative stress.^[5]

Thiols are a class of organic compounds, also known as mercaptans, which include the sulfhydryl (-SH) group that has a critical role in preventing the formation of any oxidative stress situation in cells.^[6] Thiol (R-SH) groups may be converted into reversible disulphide (SH) bond structures by being oxidized by oxidant molecules in the environment.^[7] The disulphide bond structures thus formed can be reduced into thiol (R-SH) groups again, and thus a thiol-disulphide homeostasis is maintained. This thiol-disulphide homeostasis, which is a recently defined oxidative stress indicator, is of vital importance.^[8] The contribution of the dynamic thiol-disulphide homeostasis to antioxidant protection, detoxification,^[9] apoptosis,^[10] regulation of enzymatic activity and cellular signal mechanisms,^[11] pathogenesis of various chronic diseases such as diabetes,^[12] cancer,^[13] chronic renal disease, liver disorders^[14] and cardiovascular diseases^[15] has also been shown. Currently, there is no method that simultaneously measures the dynamic plasma thiol-disulphide balance by colorimetry.^[16] The double-sided thiol-disulphide balance can only be measured unilaterally since 1979,^[17] however it can now be fully assessed with a new colorimetric method recently developed by Erel and Neselioglu.^[11] It is an easy, reliable, sensitive, cheap, fast, highly accurate, and repeatable method and that can be operated both manually and automatically.^[11]

In this study, we hypothesized that oxidative stress plays a role in the pathogenesis of chronic mucosal inflammation in chronic sinusitis and we aimed to investigate a novel oxidative stress marker, thiol-disulphide homeostasis.

PATIENTS AND METHODS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or

national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All participants provided written informed consent. The principles of good clinical practices were followed during the study period.

A total of 60 subjects (30 chronic sinusitis patients and 30 healthy volunteers) who applied to Ankara Atatürk Research and Training Hospital between April 2016 and September 2016 were included in this study. None of the chronic sinusitis patients had nasal polyposis. All patients had a duration of symptoms more than 12 weeks and radiological findings confirmed the diagnosis of chronic sinusitis.

Venous blood samples were taken to measure thiol-disulphide homeostasis parameters of all participants who were included in the study. Blood samples were taken before surgical intervention. In healthy volunteers, blood samples were taken when they were admitted to our clinic. After blood samples were quickly centrifuged at 1500 rpm for 10 minutes, plasma and serum samples were separated. Serum samples have been stored at -80°C until all samples were obtained.

Thiol/disulphide levels were analyzed with a newly developed method by Erel and Neselioglu.^[11] In summary, reducible disulphide bonds were first reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde, and all thiol groups including reduced and native ones were detected after reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid. Half of the difference between total and native thiols provided the dynamic disulphide amount (R-SH). After the determination of native thiol (R-SH) and disulphide (-SH) amount, native thiol/disulphide ratio (R-SH/-SH) was calculated.

IBM SPSS for Windows version 21.0 (IBM Corp., Armonk, NY, USA) software was used for statistical analyses. Kolmogorov-Smirnov test was used to determine the distribution of data. Continuous variables with normal distribution were given as mean \pm standard deviation and continuous variables without normal distribution were given as median interquartile range [IQR]. Categorical variables were expressed as

Table 1. Thiol-disulphide and age variation

Variables	Chronic sinusitis			Control			Test statistics	
	Mean±SD	Median	Min-Max	Mean±SD	Median	Min-Max	Z; t	p
Age	43.0±11.8	45.0	19.0-64.0	31.2±9.9	30.0	18.0-56.0	3.676	<0.001*
Native thiol (µmol/L)	475.6±28.2	471.6	391.9-535.0	515.8±46.1	505.6	453.2-634.0	3.733	<0.001**
Disulphide µmol/L	16.9±9.3	18.3	1.8-32.6	18.5±9.4	17.1	5.4-39.2	0.466	0.641
Total thiol µmol/L	509.4±33.0	508.7	422.4-583.0	552.2±52.6	541.9	484.3-672.0	3.186	0.001*
Disulphide/total (%)	3.28±1.70	3.75	0.36-6.12	3.30±1.57	3.09	1.03-7.29	0.059	0.953
Native thiol/total thiol (%)	93.44±3.41	92.49	87.76-99.29	93.51±3.28	93.91	85.42-97.94	t= 0.072	0.943
Disulphide/ Native thiol (%)	3.57±1.94	4.06	0.36-6.97	3.59±1.85	3.29	1.05-8.53	0.059	0.953

SD: Standard deviation; * Mann-Whitney U test; ** Independent sample t-test.

numbers and percentage. Continuous variables were compared with independent sample t-test, ANOVA, Mann-Whitney U test, and Kruskal-Wallis test where appropriate. Chi-square test and Fisher exact chi-square test were used to compare categorical variables. The relationship between the numeric parameters was analyzed by Pearson and Spearman correlation analysis.

RESULTS

The sex distribution in both groups was similar (8 females and 22 males). A significant difference was observed between groups with respect to the age. The mean age was 43 (range, 19-64) in the chronic sinusitis group and 31 (range, 18-56) in the control group ($p<.001$).

The average native thiol values were measured as 475.6 µmol/L and 515.8 µmol/L in the chronic sinusitis group and control group, respectively. The total thiol level was 509.4 µmol/L and 552.2 µmol/L in the chronic sinusitis and control groups respectively (as shown in Table 1). Chronic sinusitis patients had significantly lower native and thiol values than controls ($p<.001$ and $p=.001$). There was no significant difference between chronic sinusitis and control groups with respect to disulphide levels (16.9 µmol/L in chronic sinusitis group and 18.5 µmol/L in the control group; $p=0.641$).

DISCUSSION

Thiols are a class of organic compounds that contain a sulphidryl group (-SH), which is composed of a hydrogen and a sulphur atom attached to a carbon atom.^[6] Those disulphide bonds can be reduced back to thiol groups; therefore, thiol/disulphide homeostasis is maintained.^[8] Thiols contribute the major portion of the total antioxidants present in the body and play an important role in defense against reactive oxygen species and also play critical roles in programmed cell death, detoxification, antioxidant protection, and regulation of cellular enzymatic activity.^[10,11] Recently, it became that an abnormal thiol/disulphide homeostasis state is involved in the pathogenesis of various acute and chronic diseases.^[11] Measuring thiols in serum provides an indirect reflection of the antioxidative defense. The measurement of dynamic thiol/disulphide ratio was first started by a new automated method developed by Erel and Neselioglu.^[11] Under oxidative stress, the disulphide level is expected to increase as thiol level decreases.

Chronic rhinosinusitis is a clinical syndrome defined by persistent symptomatic inflammation of the mucosa of the nasal cavities and paranasal sinuses, with a prevalence estimated to be

approximately 10% of the adult population in the Western world.^[1,18,19] The syndrome is defined by the subjective presence of at least two of the following symptoms for a minimum of 12 weeks: nasal obstruction, nasal discharge, facial pain, or olfactory dysfunction. Due to symptomatic overlap with other common conditions, confirmation of sinonasal mucosal inflammation is required using nasal endoscopy and/or diagnostic imaging.^[1] This definition of CRS is purposely broad, encompassing a spectrum of clinical variants, inflammatory profiles, histologic features, and associated comorbidities. The overwhelming majority of CRS cases are idiopathic however, with classification systems commonly dividing the disorder into three subgroups based on nasal endoscopy and etiology; CRSsNP, polyps CRSwNP, and allergic fungal sinusitis. Although quite simplistic, the separation remains useful in many clinical settings, since the vast majority of nasal polyps in Western countries are eosinophilic and steroid responsive, which helps to guide therapy.

Historically, CRSsNP was considered to be the result of an incompletely treated or unresolved bacterial infection, while CRSwNP was regarded as a noninfectious disorder linked to atopy.^[1] There is still strong evidence supporting an important role for both host and environmental factors in CRS pathophysiology, but we have a very incomplete understanding of the molecular pathways that lead to the tissue manifestations and clinical symptomatology that define CRS.

Many studies were conducted to show the elevation of inflammatory and oxidative mediators and decreased levels of antioxidant enzymes. Mrowicka et al.^[20] found significantly increased serum levels of nitric oxide and decreased levels of antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase in chronic sinusitis patients, mainly in those with chronic sinusitis with nasal polyposis. But the decrease in these antioxidant enzymes was not statistically significant.

Serum thiol and disulphide measurements can be used as a novel method for the reflection of inflammation. In a recently study, Şimşek et al.^[21] found significantly lower levels of native

and total thiol and higher disulphide values in nasal polyposis patients compared to controls. However patients in our study did not have polyposis. To the best of our knowledge ours is the first study considering thiol/disulphide homeostasis in chronic sinusitis patients without nasal polyposis. We obtained lower native and total thiol values in the chronic sinusitis group than the control group. These results suggest that chronic sinusitis is a condition that lowers the native thiol amount, so that homeostasis is altered in the direction of oxidation and this is probably the result of the inflammatory process of the disease.

There are several limitations in this study that should be taken into consideration. First, this was a pilot study representing an initial investigation into the relationship between chronic sinusitis and thiol-disulphide homeostasis parameters. Second is the inclusion of a relatively small number of patients who were admitted to a single center and inclusion criteria were not strict so groups were not homogenous.

Conclusion

Low native thiol values of chronic sinusitis patients demonstrated that dynamic thiol-disulphide homeostasis shifted towards disulphide formation as a result of thiol oxidation in patients with chronic sinusitis. This result suggests that if antioxidative defense mechanisms such as native thiols decrease, inflammatory diseases causing mucosal damage can appear. However more prospective and randomized controlled trials are necessary to confirm the accurate pathophysiologic role of thiol/disulphide homeostasis in chronic sinusitis. Further studies are also required to show the results of the usage of antioxidative treatment modalities to prevent the development of mucosal epithelial damage. We did not compare patients' postoperative and preoperative results so we did not compare the effect of surgical intervention on thiol-disulphide homeostasis.

Declaration of conflicting interests

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