Ann Med Res 2020;27(12):3076-80

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.04.313

Original Article

Dynamic thiol-disulfide balance in patients with chronic sinusitis with and without nasal polyposis

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Abstract

Aim: Reactive oxygen species (ROS) have a crucial role in the pathogenesis of chronic rhinosinusitis (CRS). The current study investigates a novel oxidative stress marker (thiol (SH)/disulfide (SS) homeostasis) in patients with CRS with and without nasal polyposis (NPs).

Materials and Methods: A total of 167 subjects, including 94 patients with CRS and 73 healthy controls, were included in the study. The patients were subdivided into two groups those with CRS with NP and those with CRS without NP. Comparisons were made between the groups in respect of serum native SH, total thiol (TT), and SS levels and the SS/SH and SS/TT ratios.

Results: There was no significant difference in TT between the CRS with the NP group and the CRS without the NP group ($p > 0.05$). There was a significant difference ($p < 0.05$) between the CRS with the NP group and the CRS without the NP group in %SH/TT, %SS/TT, %SS/SH, SS, and SH. SH was higher in the CRS without the NP group while the CRS with the NP group reported significantly higher SS. There was no significant difference ($p > 0.05$) between the CRS without the NP group and the control group in %SH/TT, %SS/TT, %SS/SH, SS, and SH. A significant difference ($p < 0.05$) was found between the CRS with the NP group and the control group in %SH/TT, %SS/TT, %SS/SH, SS and SH values.

Conclusion: Dynamic SH/SS homeostasis shifts towards SS formation as a result of SH oxidation in patients with CRS.

Keywords: Chronic sinusitis; nasal polyp; oxidative stress; sulfhydryl compounds

INTRODUCTION

One of the most common chronic diseases is chronic rhinosinusitis (CRS) of which pathophysiology has not been completely known. Generally, CRS is categorized based on whether the nasal polyps (NPs) are present or absent (1,2).

Several reports have indicated that there is a close relationship between nicotinamide adenine dinucleotide phosphate (NADPH) infiltration which mainly causes biologically reactive oxygen species (ROS) to produce free radicals (FRs) during phagocytosis and CRS histopathological abnormalities including inflammation and edema (3).

Oxidative stress (OS) expresses high amounts of reactive oxygen species ROS and deoxidation systems of cells that remove these radical species (4). The cells are not harmed by ROS production under the physiological conditions and ROS production is restricted by the antioxidants.

When ROS becomes higher than a specified amount, the harmful effects cannot be reduced by the antioxidants (5). Thiols (SH) which are also known as mercaptan are made of sulfhydryl groups (6). The albumin SHs and protein SHs mainly produce the blood SH pool and the low molecular weight SHs which contain glutamylcysteine, homocysteine, glutathione, cysteine, and cysteinyl glycine partially form the blood SH pool (7).

Several crucial functions including detoxification, antioxidant protection, programmed cell death and regulation of cellular enzymatic activity are performed through disulfide/ thiol homeostasis.

Therefore, the transformation of disulfide/ thiol homeostasis to disulfide formation has an adverse effect on these crucial events, leading to the development of pathologies in the functions and structure of many organs. Failure to keep the balance between ROS production and antioxidant defense activity will lead to oxidative stress which plays a major role in CRS pathogenesis in which NP

Received: 06.04.2020 **Accepted:** 23.11.2020 **Available online:** 22.12.2020
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causes injury or death of cell, chronic disease and then, tissue damage (7,8).

Erel and Neselioglu developed in 2014 a colorimetric method that has been completely automated to show the general balance between oxide thiol reserve and thiol in the body (6). Thus, the aim of this study as the first study in the related literature was to specify homeostasis of disulfide/ thiol with or without the nasal polyp.

MATERIALS and METHODS

Ninety-four patients (34 females and 60 males; mean age (standard deviation) age of 33.9 ± 9.5 years were included in the prospective randomized controlled study. The studied patients were diagnosed to have CRS when they referred to the Otorhinolaryngology-Head and Neck Surgery outpatient department of our tertiary reference hospital from December 2014 to November 2017. Seventy-three patients (30 females and 43 males with a mean age (standard deviation) of 34.1 ± 9.6 years along with the healthy subjects who didn't have CRS were included in the study as the control group for comparison (Table 1). Based on the paranasal sinus computed tomography (CT) and endoscopic examination, the studied patients were divided into two groups with CRS having NP and those with CRS not having NP.

Table 1. Clinical characteristics and laboratory findings of the study and control

	Study group (n = 94)	Control group (n =73)	P value
Age	33.9 9.5 years	34.1 9.6 years	0.334
Gender (n, %)			
Female	34 (%36.2)	30 (%41.1)	
Male	60 (%63.8)	43 (%58.9)	
Thiol-disulfide homeostasis			
-SH, mol l^{-1}	440.08 63.76	475.78 ± 39.75	**<0.001**
-SH+-S-S-, mol l^{-1}	484.8 62.08	509.27 ± 42.71	**<0.001**
-S-S-, mol l^{-1}	22.35 8.18	22.74 ± 6.3	**<0.001**
-S-S-/-SH, %	5.27 2.37	6.2± 2.1	**<0.001***
-S-S-/-SH+-S-S-, %	4.69 1.84	5.3 ± 1.51	**<0.001***
-SH/-SH+-S-S-, %	90.6 3.69	89.39 ± 3.02	**<0.001***

-SH: Native Thiol, -SH+-S-S-; Total Thiol, -S-S-; Disulfide
***The values calculated by Mann Whitney U, and the others by independent student t test. Results were given as mean sd. P value <0.05 considered significant. Bolds are statistically significant**

Informed consent was received from all patients and the control group. The study was approved by the Institutional Review Board at the School of Medicine (Ethics Committee Decision no: 03/12/2014-224).

The criteria including any evidence of respiratory, liver or kidney disease, malignancy, diabetes mellitus, an infiltrative or inflammatory disorder, acute infectious disease, use of any drug such as local or systemic drug which has antioxidant properties, using the drug before surgery such as antibiotics, parenteral or oral corticosteroids for at least 4 weeks before sampling, smoking and drinking alcohol regularly in the last 48 hours were excluded from the study. The studied patients didn't have any acetylsalicylic acid sensitivity or asthma.

Sinus CT scan and endoscopy were performed on all of the patients. CT imaging, endoscopy, and anterior rhinoscopy were used to diagnose CRS with or without NP.

Biochemical analysis

A 19-gauge butterfly needle that was connected to a plastic syringe was used to draw all blood samples following fasting during the night from a big-sized antecubital vein for 12 hours without interrupting the venous circulation. The first few amounts of the total drawn blood (20 ml) was discarded. 10 ml of each blood sample was used for performing baseline tests in laboratory and the remaining of the syringe was immediately transferred to the polypropylene tubes, centrifuged at 3,000 rpm for a term of 10 min at 10 -18^0 C. The plastic tubes were used for the storage of supernatant plasma samples at a temperature of at -80^0 C until they were assayed. A novel and automatic measurement method recently developed, was used to determine homeostasis of the serum thiol/ disulfide based on the automated clinical analyzer (Cobas 501, Mannheim, Roche, Germany). Native thiol (-SH) and also total thiol (TT; -SH+-S-S-) were directly measured and the amounts of disulfide/native thiol ratio (-S-S-/-SH), the disulfide (-S-S-) level and disulfide/total thiol ratio (-S-S-/TT) were determined.

Statistical analyses

SPSS software, Version 20.0 for Macintosh was used to analyze the obtained data. Mean ± SD for continuous variables was obtained as the results of this study. To compare the parameters between the studied groups, normal distribution of the data was done to perform the independent sample t-tests. The Mann Whitney-U test was used to compare the non-parametric data. Based on the distribution type of the parameters, Pearson's or Spearman's correlation analysis was used to analyze the relationship between variables. To specify the concentrations of native and total thiol, disulfide/total thiol, native/total thiol, native and total thiol, disulfide/ native thiol, disulfide, which best defined CRS with NPs, receiver-operating characteristic (ROC) curves were used to study the functional properties of thiol-disulfide variables. The statistically significant value of <0.05 was accepted.

RESULTS

Table 1 shows the laboratory findings of the study, the clinical characteristics and the control groups. There was no significant difference in TT between the CRS with the NP group and the CRS without the NP group (p> 0.05). There was a significant difference (p< 0.05) between the CRS with the NP group and the CRS without the NP group in %SH/TT, %SS/TT, %SS/SH, SS, and SH. Table 2 shows that SH was higher in the CRS without the NP group while the CRS with the NP group reported significantly higher SS.

Table 2. Comparison of CRS with NP and those with CRS without NP based on SH, SS, TT, % SS/SH, % SS/TT, and % SH/TT

n = 94	CRS with NP (n=66)	CRS without NP (n=28)	P-value
	Mean ± SD	Mean ± SD	
-SH, mol l^{-1}	435.49 ± 49.2	442.91 ± 55.3	0.018
-SH+-S-S-, mol l^{1}	478.06 ± 53.32	480.71 ± 58.84	0.286
-S-S-, mol l^{-1}	23.89 ± 5.2	19.28 ± 5.8	0.017
-S-S-/-SH, %	6.8 ± 1.6	5.4 ± 1.4	0.001
-S-S-/-SH+-S-S-%	6 ± 1.2	5 ± 1.2	0.001
-SH/-SH+-S-S-, %	88.96 ± 2.4	90.97 ± 2.4	0.001

-SH: Native Thiol, -SH+-S-S-; Total Thiol, -S-S-; Disulfide, CRS: Chronic rhinosinusitis, NP: Nasal Polyp. Results were given as mean sd. P value <0.05 considered significant

There was no significant difference (p> 0.05) between the CRS without the NP group and the control group in %SH/TT, %SS/TT, %SS/SH, SS, and SH. A significant difference (p< 0.05) was found between the CRS with the NP group and the control group in %SH/TT, %SS/TT, %SS/SH, SS and SH values.

The CRS with NP group had significantly lower TT and SH levels (p< 0.05) than the control group had. Table 3 shows no significant difference (p> 0.05) between the CRS with NP group and the control group in reporting SS value.

Binary logistic regression analysis was performed to predict the values of the total and native thiol, indicating a significant difference between the patients and the control group. Table 4 shows that the significantly created model could explain 45.3% of the variation. The cut-off value of the disulfide level was specified to predict nasal polyp patients based on the ROC analysis.

Table 3. Comparison of the control and case group based on SH, SS, TT, % SS/SH, % SS/TT and % SH/TT

	Control group	Study group			
		CRS with NP		CRS without NP	
	Mean ± SD	Mean ± SD	P	Mean ± SD	P
Native SH	475.78 ± 39.75	435.49 ± 49.2	0.009	442.91 ± 55.3	0.116
TT	509.27 ± 42.71	478.06 ± 53.32	0.034	480.71 ± 58.84	0.093
SS	22.74 ± 6.3	23.89 ± 5.2	0.210	19.28 ± 5.8	0.186
%SS/SH	6.2± 2.1	6.8 ± 1.6	0.005	5.4 ± 1.4	0.837
%SS/TT	5.3 ± 1.51	6 ± 1.2	0.005	5 ±1.2	0.837
%SH/TT	89.39 ± 3.02	88.96 ± 2.4	0.005	90.97 ± 2.4	0.834

-SH: Native Thiol, TT: Total Thiol, -S-S-; Disulfide, CRS: Chronic Rhinosinusitis, NP: Nasal Polyp

Table 4. Summary ROC curve analyses of blood parameters for predicting occurence of nasal polyp

			95% Confidence Interval				
	AUC	P Value	Lower	Upper	Cut-off point	Sensitivity %	Specifity %
-SH, □mol l^{-1}	0.871	<0.001	0.828	0.931	488.55	81.9	81.8
-SH+-S-S-, □mol l^{1}	0.862	<0.001	0.809	0.918	529.65	79.8	79.5
-S-S-, □mol l^{-1}	0.657	0.001	0.574	0.739	20.57	61.7	53.1
-S-S-/-SH, %	0.751	<0.001	0.684	0.832	4.09	69.1	63.2
-S-S-/-SH+-S-S-,%	0.754	<0.001	0.681	0.828	3.68	70.2	62.1
-SH/-SH+-S-S-, %	0.757	<0.001	0.674	0.831	92.38	68.1	65.8

-SH: Native Thiol, -SH+-S-S-; Total Thiol, -S-S-; Disulfide, AUC; Area Under Curve, *P value <0.05 considered significant

The cut-off value of disulfide level with the specificity of 72.1% and sensitivity of 74.3% was determined to be 17.94. The area under the curve was 0.80. To predict NP in all CRS patients, the cut-off value of the native thiol level was 386.29. Its specificity was 81.8% and sensitivity was 81.9%.

DISCUSSION

Lack of balance between antioxidant defense activity and ROS production causes oxidative stress which plays a major role in the pathophysiology of CRS. The accurate regulation of organism functions such as transcription activation, intracellular signaling, inflammation, cell

proliferation, and apoptosis depends on the definite physiological achievement of reactive oxygen species (9).

The balance between levels of reactive oxygen species and endogenous enzymes such as superoxide dismutase (SOD), catalase, thiol buffers, GSHPX, and particularly thioredoxin and GSH will result in the reduction-oxidation state of the organism (10). Potentially cytotoxic oxidative stress will result from the amounts of oxidizing equivalents that exceed the enzymatic capabilities and cell-buffering capacity (11).

Nasal polyp development causes some problems such as nasal obstruction, the most important of which is chronic hypoxia. There is a belief that ROS plays a major role in the pathogenesis of CRS with NP (12,13). Deterioration of the antioxidant scavenging systems and the increase of the oxidative stress markers are highly found in the recent literature. It has been strongly shown that CRS with NP pathogenesis and presentation is caused by oxidative stress (14). To study the behavior of ROS and antioxidants in CRS with NPs, 31 patients and 19 controls were evaluated by Dagli et al. showing higher malondialdehyde-thiobarbituric acid (MDA) in the NP tissue and the serum level of patients with NPS, as compared with the control group (15). Dogru et al. and Uneri et al. recently published an article that elaborated on the effect of ROS on the development of NPs, emphasizing that pathogenesis of NPs was highly affected by ROS (12,13). They report that the pathogenesis of CRS with NP is affected by ROS (13).

The organism is affected by the dynamic homeostasis of thiol-disulfide. Changes in the disulfide -thiol balance will affect the adjustment of enzymatic activity, detoxification, antioxidant protection and cellular signaling mechanisms (16).

Thiol groups decrease the toxic harmful effects of oxygen-activated processes, significantly affecting the organism. There is a relationship between the sulfhydryl groups and proteins and a reduction of the thiol level in the serum will decrease its antioxidant power.

The studies done on the healthy subjects show that 52.9% of total serum antioxidant activity is shown by the SH protein types (5). Besides, it is strongly shown that lack of balance between SH and SS affects the pathogenesis of many health problems (17). A recent study demonstrates that patients with chronic rhinosinusitis had significantly lower native and thiol values than the control group had (18). A study showed that patients with nasal polyposis showed higher disulfide values and lower natural and total thiol levels than the healthy control subjects did (19).

In some studies, a close relationship between polyp and oxidative stress was reported but some others didn't report any significant results (20). The current study found some significant differences between the control group and CRS in terms of thiol/disulfide homeostasis. The redox balance system of disulfide and thiol was directed to the formation of disulfide bond due to the decrease in the native thiol to total thiol ratio (SH/(−-SH+-S-S-)) and increase of disulfide to native thiol ratios and disulfide to total thiol (-S-S-/-SH)) and (-S-S-/ (-SH+-S-S-).

These results are in line with the finding that CRS is affected by the thiol/disulfide homeostasis. Besides, the patients with CRS without NP showed higher SH levels than the control group did. According to this study, the CRS without the NP group had a low SH level and there was no statistically significant difference between CRS without the NP group and the control group. The CRS without the NP group showed 442.91 mol/L while the control group showed 475.78 mol/L.

In summary, it is shown that an increase in disulfide and a decrease in thiol in the patients with chronic sinusitis were caused by the balance between the thiol and disulfide while the control group didn't show it. It was found in the current study that the CRS with the NP group showed significantly lower SH levels and significantly higher TT levels ($p<0.05$) than the CRS without the NP group showed. The CRS with NP group showed significantly lower SH and TT levels than the control group showed.

The SS levels in CRS with NP was high but not significant, as compared to the control group.

This study has some limitations such as the need for more extensive studies for assessment of the relationship between the balance of thiol and disulfide and eosinophilic and non-eosinophilic nasal polyp.

CONCLUSION

It is concluded that dynamic thiol and disulfide homeostasis changes into the formation of disulfide due to oxidation of thiol among the patients with CRS. The reason is that the antioxidant system is not sufficient for NPs patients. More longitudinal studies should be conducted on the tissue samples to specify whether CRS with NP is affected by the thiol/disulfide homeostasis as a predictive risk factor. The antioxidants should be studied more to study NPs prevention.

Conflict of interest : The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: The study was approved by the Yildirim Beyazit University Faculty of Medicine (Ethics Committee Decision no: 03/12/2014-224).

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