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# Supersulfide formation in the sinus mucosa of chronic rhinosinusitis

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## Abstract

**Objectives:** Disruption of the oxidative stress defense system is involved in developing various diseases. Sulfur compounds such as glutathione (GSH) and cysteine (CysSH) are representative antioxidants in the body. Recently, supersulfides, including reactive persulfide and polysulfide species, have gained attention as potent antioxidants regulating oxidative stress and redox signaling. However, their involvement in the pathogenesis of chronic rhinosinusitis (CRS) remains unclear.

**Methods:** To clarify the changes in sulfur compounds within the sinus mucosa of each CRS subtype, we measured sulfur compound levels in the sinus mucosa of control individuals ($n = 9$), patients with eosinophilic CRS (ECRS) ($n = 13$), and those with non-ECRS (nECRS) ($n = 11$) who underwent sinus surgery using mass spectrometry.

**Results:** GSH and CysSH levels were significantly reduced, and the glutathione disulfide (GSSG)/GSH ratio, an oxidative stress indicator, was increased in patients with ECRS. Despite the absence of notable variations in supersulfides, patients with ECRS and nECRS exhibited a significant reduction in glutathione trisulfide (GSSSG), which serves as the precursor for supersulfides.

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Jun Suzuki and Tomotaka Hemmi contributed equally to this study.

**Conclusions:** This study is the first quantitative assessment of supersulfides in normal and inflamed sinus mucosa, suggesting that sulfur compounds contribute to the pathogenesis of CRS.

**Level of Evidence:** N/A.

**KEYWORDS**

antioxidants, chronic rhinosinusitis, sinus mucosa, sulfur compounds, supersulfides

# 1 | INTRODUCTION

Chronic rhinosinusitis (CRS) is a heterogeneous disease caused by various etiologies, resulting in nasal discharge, nasal obstruction, olfactory disturbances, and other distressing symptoms. According to the phenotypes, CRS is classified into two types depending on the presence or absence of nasal polyps: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP).[1] Recently, the classification of CRS has shifted to be based on the presumed underlying inflammatory subtypes, that is, endotypes of CRS: type 2 inflammation or non-type 2 inflammation.[2–4] Type 2 CRS is characterized by type 2 inflammation orchestrated by T helper 2 (Th2) cells that involves interleukins (IL)-4, IL-5, IL-13, and local immunoglobulin E (IgE). This inflammation results in tissue eosinophilia and can contribute to polyp formation. CRS is also subdivided into eosinophilic CRS (ECRS) and non-eosinophilic CRS (nECRS) based on the clinical criteria developed by the Japanese Epidemiological Survey of Refractory Eosinophilic Rhinosinusitis (JESREC).[5] ECRS is typically based on type 2 inflammation accompanied by nasal polyps with eosinophil-dominant inflammatory cell infiltration. Considering the refractory and recurrent nature of ECRS, elucidation of the pathogenesis of ECRS needs a different approach besides the immunological approach. Recently, the oxidative stress defense system has attracted attention as a factor affecting the pathogenesis of CRS.[6]

Oxidative stress is caused by generating reactive oxygen species (ROS) induced by environmental stimuli, aging, and genetic factors. ROS induce protein aggregation and organelle damage, resulting in cellular and tissue injury and the initiation and progression of diseases.[7] The respiratory system directly contacts the outside world and is vulnerable to oxidative stress because of its complex conductive airways.[6] Studies of airway diseases and oxidative stress have focused on the development of lower respiratory tract diseases. Oxidative stress is involved in the pathogenesis of bronchial asthma through the amplification of airway inflammation and hyperreactivity[8] Exogenous oxidative stress from cigarettes, endogenous oxidative stress from activated inflammatory cells, and reduced antioxidants induce high levels of oxidative stress in the lungs, driving the pathophysiology of chronic obstructive pulmonary disease (COPD).[9] However, few studies have examined the effects of oxidative stress on CRS pathogenesis. Possible causes of oxidative stress include air pollution, smoking, and other exogenous oxidants; an excess of endogenous oxidants such as hydrogen peroxide; and a decrease in reduced antioxidants such as thioredoxin, glutathione (GSH), and nuclear factor erythroid

2–related factor 2 (Nrf2).[6,7] Recently, reactive sulfur species, especially supersulfides, defined as hydropersulfides and polymeric sulfur species with sulfur catenation, have attracted attention as new redox-regulated molecules.[10–16]

Sulfur is abundant in the body and more readily available for redox reactions due to its ability to release and accept electrons more readily than oxygen. Sulfur compounds with thiol groups (RSH) have antioxidant potential and are representative antioxidants in the body, such as cysteine (CysSH) and GSH.[17] Supersulfides, that is, reactive persulfide and polysulfide species such as cysteine persulfide (CysSSH) and glutathione persulfide (GSSH), are highly reactive and powerful antioxidants that regulate oxidative stress and redox signaling.[10,18] The production of supersulfides in lung-resident cells and epithelial lining fluid (ELF) decreases in patients with COPD.[19] However, no studies exist on the changes in the production of supersulfides in patients with CRS. Moreover, GSH has been reported to decrease in the sinus mucosa of patients with CRS[20]; however, the differences between the endotypes of CRS, e.g., between ECRS and nECRS, have not been clarified. Therefore, in this study, we quantified the amount of sulfur compounds, including supersulfides, in human sinus mucosa obtained from surgical specimens to clarify the changes in supersulfides in each CRS subtype.

# 2 | MATERIALS AND METHODS

## 2.1 | Patients

Patients with nasal and sinus diseases who underwent sinus surgeries at Tohoku University Hospital or Tohoku Kosai Hospital between December 2020 and May 2022 participated in the study with informed consent. Age, sex, comorbidities (branchial asthma, atopic dermatitis, and allergic rhinitis), preoperative oral steroid use, laboratory data, pathological findings, including eosinophil infiltration, and paranasal sinus computed tomography (CT) images were obtained from medical records. Paranasal sinus mucosa from the uncinate process was collected from all patients during surgeries and promptly frozen in liquid nitrogen and stored in a deep freezer at $-80°$. The analysis was carried out each time a certain number of samples were collected.

This study was approved by the Ethics Committee of Tohoku University Graduate School of Medicine (2020-1-211). Written informed consent was obtained from all the participants. The control group

**TABLE 1** Diagnostic criteria of eosinophilic chronic rhinosinusitis.

| Diagnostic criterion of eosinophilic chronic rhinosinusitis | |
| --- | --- |
| **Factor** | **Score** |
| Disease side: both side | 3 |
| Nasal polyp | 2 |
| CT shadow: ethmoid ≥ maxillary | 2 |
| Eosinophils of peripheral blood | |
| 2 < ≤5% | 4 |
| 5 < ≤10% | 8 |
| 10% < | 10 |
| **Diagnosis** | **JESREC score** |
| ECRS | ≥11 |
| nECRS | ≤10 |

*Note*: Definite diagnosis of ECRS was made by histological analysis: the mean eosinophil count in 3 high-power fields must be 70 or more. Abbreviations: CT, computed tomography; ECRS, eosinophilic chronic rhinosinusitis; JESREC, Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis; nECRS, non-ECRS.

consisted of patients with cysts or benign sinonasal tumors who did not have anterior ethmoid sinusitis. We collected the normal mucosa of the uncinate process of the affected side. ECRS and nECRS were diagnosed according to JESREC (Table 1).[5] CT scan scores were evaluated using the Lund-Mackey score (LM score).[21] Briefly, each paranasal sinus (maxillary, anterior ethmoid, posterior ethmoid, sphenoid, and frontal) and ostiomeatal complex were assigned a score of zero for absent opacification, one for partial opacification, and two for complete opacification. A maximum LM score of 24 was obtained for both sides.

## 2.2 | Measurement of supersulfides using HPE-IAM labeling

Liquid chromatography-electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) combined with β-(4-hydroxyphenyl)ethyl iodoacetamide (HPE-IAM) trapping was used to measure the sulfur compounds according to our previous reports.[10,22] Metabolites were extracted using a solution containing 5 mM HPE-IAM in 70% methanol and homogenized. The lysates were harvested, incubated at 37°C for 20 min, and centrifuged at $14,000 \times g$. After centrifugation, aliquots of the lysate supernatants were diluted 20–50 times with 0.1% formic acid containing known amounts of an isotope-labeled internal standard (50–200 nM). The sulfide metabolites in each sample were quantified using a Nexera UHPLC system (Shimadzu, Kyoto, Japan) and LCMS-8060 (Shimadzu, Kyoto, Japan) LC-ESI-MS/MS. Samples were injected and separated using the YMC-Triart C18 column (50 mm × 2.0 mm inner diameter), eluted with a methanol mobile phase through a linear gradient (0%–90%) for 15 min in the presence of 0.1% formic acid at a flow rate of 0.2 mL/min at 40°C. CysSH, CysSSH, GSH, GSSH, glutathione trisulfide (GSSSH), oxidized

glutathione disulfide (GSSG), oxidized glutathione trisulfide (GSSSG), cystine, hydrogen sulfide (HSH), hydrogen disulfide (HSSH), and hydrogen trisulfide (HSSSH) were identified and quantified by multiple reaction monitoring (MRM) based on their specific parameter, as previously performed.[10,18,22] The concentration of metabolites in the sample was calculated by dividing the peak area of target metabolite in the measured sample by the peak area of added isotope-labeled internal standard and multiplying the result by the internal standard concentration. The mechanisms for the formation and maintenance of these sulfur compounds are shown in Figure 1.

## 2.3 | Statistical methods

Statistical analyses were conducted using Prism 9 (GraphPad Software, San Diego, CA, USA) for the Kruskal-Wallis test with Dunn's multiple comparison tests for clinical data and one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests for mass spectrometry data. Data were presented as median and interquartile ranges (IQRs) or mean ± standard deviation. Statistical significance was set at $p < .05$.

## 3 | RESULTS

### 3.1 | Characteristics of the patients

A total of 33 patients were included in this study: the control group ($n = 9$), the nECRS group ($n = 13$), and the ECRS group ($n = 11$) (Table 2). The ECRS group had a mean number of mucosal eosinophils count ≥70/high-power field in all patients. The median age of each group was 68.0 (range 53.5–72.5) years in the control, 57.0 (range 49.0–63.5) years in nECRS, and 49.0 (range 36.0–67.0) years in ECRS groups. No significant difference was found among these groups (Table 2 and Figure 2A). The ECRS group had the following characteristics: bilateral lesions (100%), bronchial asthma complications (63.6%), higher blood eosinophil counts, LM scores, and JESREC scores (Table 2 and Figure 2B–D).

### 3.2 | Quantification of cysteine and glutathione and their polysulfide species

We first quantified the amounts of sulfur compounds, including CysSH, GSH, and their supersulfides, in the uncinate process mucosa using LC-ESI-MS/MS (Figure 3). The amount of CysSH in the ECRS group (1355 ± 189.4 pmol/mg protein) was significantly decreased compared with that in the control group (1770 ± 383.2 pmol/mg protein, $p = .0043$) and the nECRS group (1698 ± 219.5 pmol/mg protein, $p = .0098$) (Figure 3A). Similarly, the GSH amount in the ECRS group (3382 ± 362.9 pmol/mg protein) significantly decreased compared to the control group (4305 ± 638.9 pmol/mg protein, $p = .0044$) and patients with nECRS (4047 ± 700.8 pmol/mg protein,
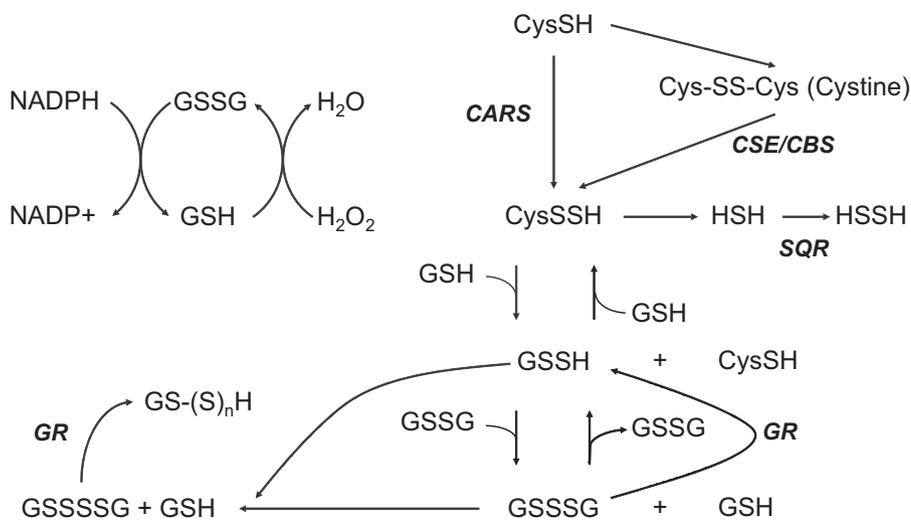
**FIGURE 1** Formation and maintenance of supersulfides. CSE, cystathionine γ-lyase; CBS, cystathionine β-synthase; CARS, cysteinyl tRNA synthetase; SQR, sulfide-quinone oxidoreductase; GR, glutathione reductase.

**TABLE 2** Characteristics of participants.

| | Control ($n = 9$) | | nECRS ($n = 13$) | | ECRS ($n = 11$) | |
|---|---|---|---|---|---|---|
| Age | 68.0 [53.5–72.5] | | 57.0 [49.0–63.5] | | 49.0 [36.0–67.0] | |
| Sex (male) | 3 | 33.3% | 9 | 69.2% | 5 | 45.5% |
| Bronchial asthma | 0 | 0% | 0 | 0% | 7 | 63.6% |
| Allergic rhinitis | 1 | 11.1% | 10 | 76.9% | 8 | 72.7% |
| Atopic dermatitis | 0 | 0% | 1 | 7.7% | 1 | 9.1% |
| Smoking | 5 | 55.6% | 6 | 46.2% | 8 | 72.7% |
| Polyps | 0 | 0% | 0 | 0% | 11 | 100% |
| Bilateral lesion | 0 | 0% | 3 | 23.1% | 11 | 100% |
| Preoperative oral steroid | 0 | 0% | 0 | 0% | 2 | 18.2% |
| Blood eosinophil (%) | 1.1 [0.5–3.4] | | 2.9 [1.4–4.7] | | 8.9 [6.4–15.3] | |
| LM score | 2 [1–2] | | 8 [6–9] | | 18[16–22] | |
| JESREC score | 0 [0–4] | | 4 [2–9] | | 15 [15–17] | |

*Note*: Data are presented as median (interquartile ranges).
Abbreviations: ECRS, eosinophilic chronic rhinosinusitis; JESREC, Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis; LM score, Lund-Mackey score; nECRS, non-eosinophilic chronic rhinosinusitis.

$p = .0266$) (Figure 3C). No significant differences were observed in the quantities of persulfide and polysulfide species (CysSSH, GSSH, and GSSSH) (Figure 3B, D, E).

### 3.3 | Quantification of hydrogen sulfide and their persulfide and polysulfide species

Next, we quantified the amounts of other reduced inorganic sulfur species, including hydrogen sulfide and their persulfide and polysulfide species (Figure 4). The amount of HSH in the nECRS group (49.8 ± 10.4 pmol/mg protein) was significantly increased compared with that in the control group (37.8 ± 8.7 pmol/mg protein, $p = .0219$) and the ECRS group (36.5 ± 9.9 pmol/mg protein, $p = .0067$) (Figure 4A). The amount of HSSH in nECRS group (6.6 ± 2.2 pmol/mg protein) was significantly increased compared with that in the ECRS group

(4.7 ± 1.5 pmol/mg protein, $p = .0454$) (Figure 4B). No significant differences were observed in the amount of HSSSH (Figure 4C).

### 3.4 | Quantification of oxidized sulfur metabolites and evaluation of oxidative stress level

We also quantified the amount of oxidized sulfur species (Figure 5). Although no significant differences were observed in the amounts of cystine and GSSG (Figure 5A,B), the amount of GSSSG in the control group (3.4 ± 0.9 pmol/mg protein) was significantly higher than that in the nECRS group (2.0 ± 0.4 pmol/mg protein, $p < .0001$) and the ECRS group (2.4 ± 0.4 pmol/mg protein, $p = .0014$) (Figure 5C). Finally, we calculated the ratio of GSSG/GSH, an index for oxidative stress. As shown in Figure 5D, the GSSG/GSH ratio of the ECRS group (0.301 ± 0.151) was significantly higher than that of the control
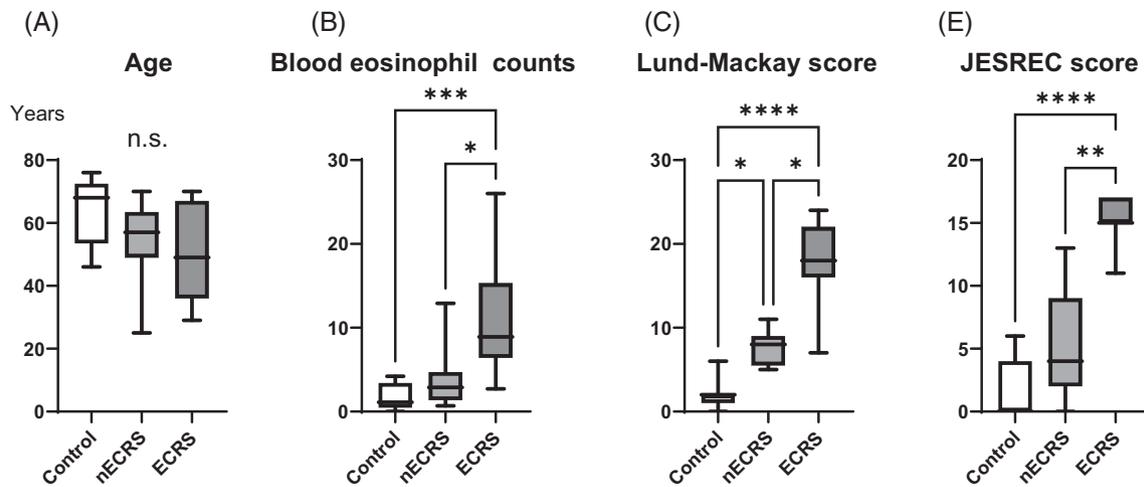
**FIGURE 2** Statistical analysis of clinical data. (A) Age, (B) blood eosinophil counts, (C) Lund-Mackay score, and (D) Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis (JESREC) score. Data information: midlines represent the median, boxes the interquartile range (25th–75th percentile), and whiskers the range of data. Statistical significance was determined using the Kruskal-Wallis test and Dunn's multiple comparisons test. *$P < .05$; **$P < .01$; ***$P < .005$; ****$P < .0001$. ECRS, eosinophilic chronic rhinosinusitis; nECRS, non-eosinophilic chronic rhinosinusitis; n.s., not significant.

**FIGURE 3** Determination of reduced sulfur compounds including supersulfides in the sinus mucosa. Cysteine (CysSH) (A), cysteine persulfide (CysSSH) (B), glutathione (GSH) (C), glutathione persulfide (GSSH) (D), glutathione trisulfide (GSSSH) (E) levels in the sinus mucosa of the control ($n = 9$), nECRS ($n = 13$), and ECRS ($n = 11$) groups were quantified. Supersulfides are marked with "†". Data are presented as mean ± standard deviation. Data were analyzed using the one-way analysis of variance with Tukey's multiple comparison tests. *$P < .05$; **$P < .01$.
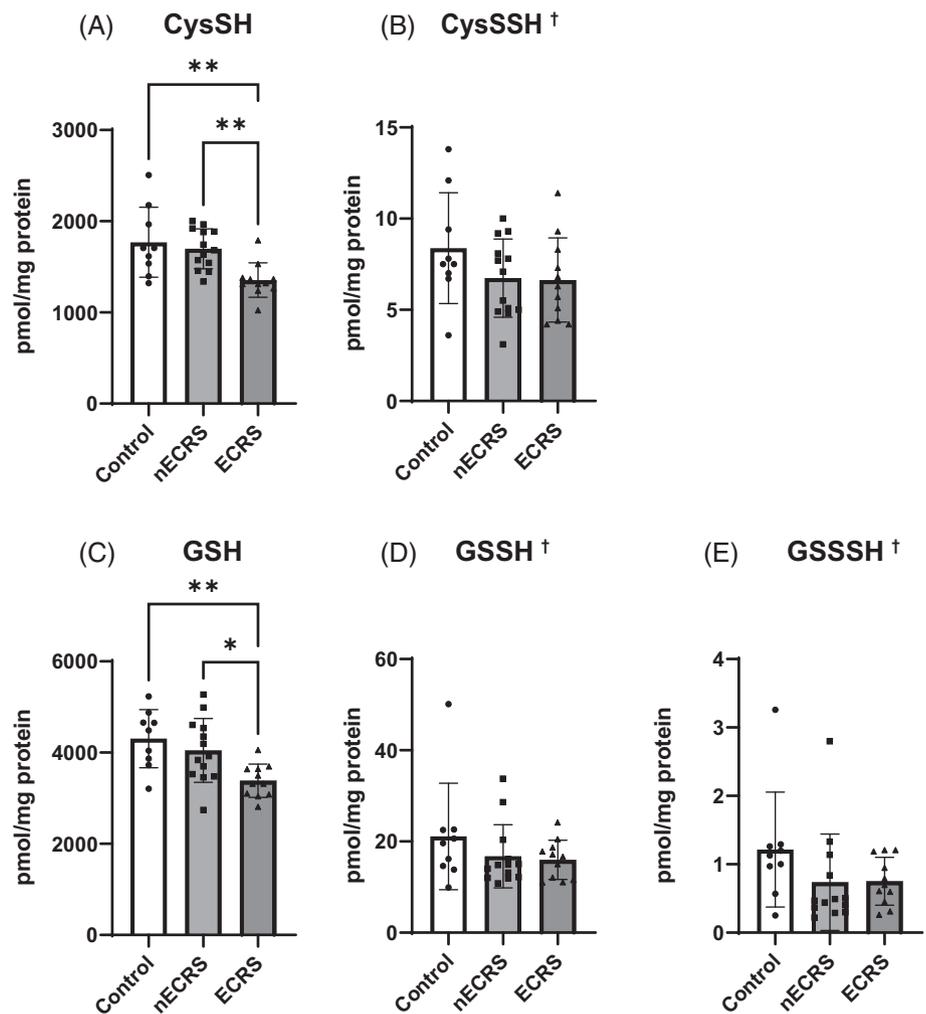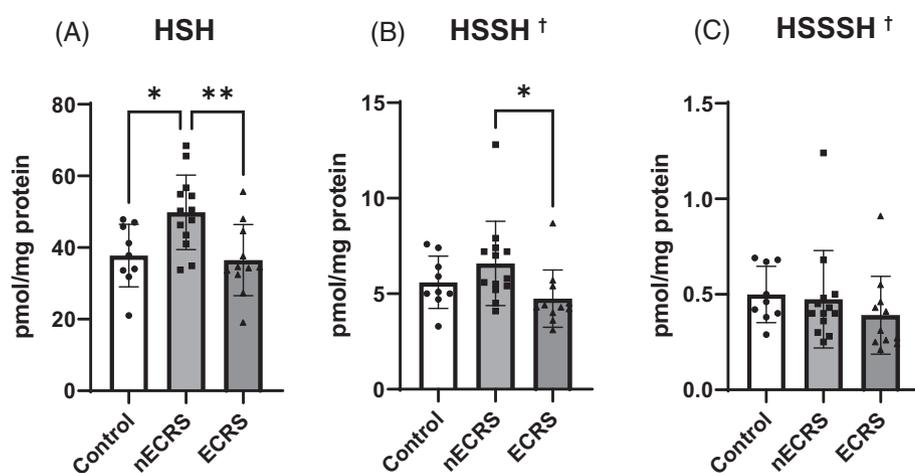
**FIGURE 4** Determination of the other reduced inorganic sulfur species in the sinus mucosa. Hydrogen sulfide (HSH) (A), hydrogen disulfide (HSSH) (B), and hydrogen trisulfide (HSSSH) (C) levels in the sinus mucosa of the control ($n = 9$), nECRS ($n = 13$), and ECRS ($n = 11$) groups were quantified. Supersulfides are marked with "†". Data are presented as mean ± standard deviation. Data were analyzed using the one-way analysis of variance with Tukey's multiple comparison tests. *$P < .05$; **$P < .01$.
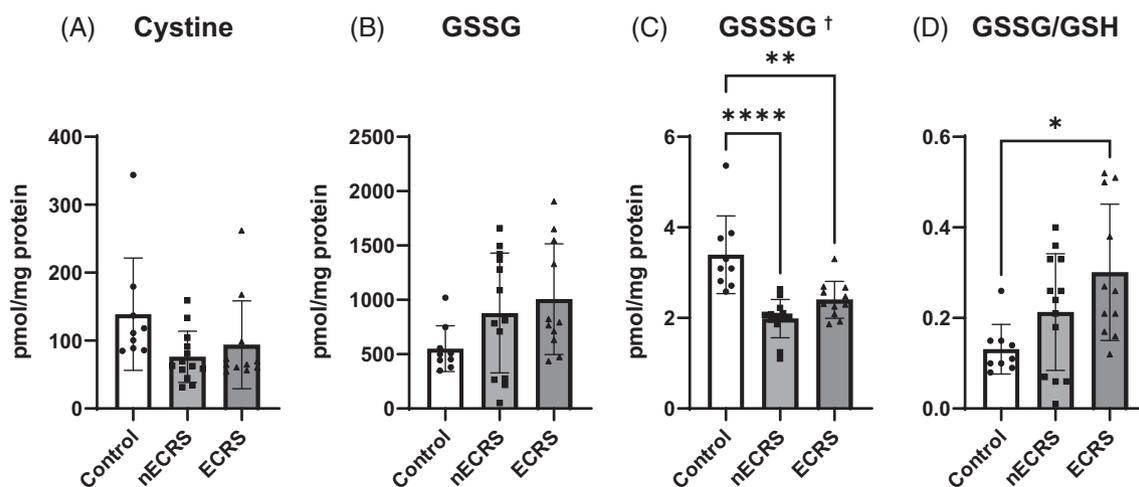


**FIGURE 5** Determination of oxidized sulfur species in the sinus mucosa. Cysteine (A), oxidized glutathione disulfide (GSSG) (B), and oxidized glutathione trisulfide (GSSSG) (C) levels in the sinus mucosa of the control ($n = 9$), nECRS ($n = 13$), and ECRS ($n = 11$) groups were quantified. Supersulfides are marked with "†". The ratio of GSSG/glutathione (GSH), an index for oxidative stress, is shown in (E). Data are presented as mean ± standard deviation. Data were analyzed using the one-way analysis of variance with Tukey's multiple comparison tests. *$P < .05$; **$P < .01$; ****$P < .0001$.

group ($0.131 \pm 0.055$, $p = .0117$), suggesting that the sinus mucosa of patients with ECRS have higher oxidative stress levels.

## 4 | DISCUSSION

In this study, we revealed that GSH, a representative intracellular antioxidant, and CysSH, a component element of GSH, were significantly decreased and that the GSSG/GSH ratio, an indicator of oxidative stress levels, was increased in the sinus mucosa of patients with ECRS. Furthermore, we found that GSSSG, the source of supersulfides, was significantly decreased in both patients with ECRS and nECRS, with no notable differences observed in the supersulfides. Only a few studies have examined the relationship between sulfur compounds and sinusitis pathogenesis.[20,23,24] To the best of our knowledge, this is the first study to quantitatively evaluate supersulfides in normal and

inflamed sinus mucosa, suggesting that sulfur compounds contribute to the pathogenesis of CRS.

GSH is a tripeptide composed of three amino acids: glutamic acid, CysSH, and glycine. GSH is abundant in cells in its reduced form and is converted to its oxidized form (GSSG) when exposed to oxidative stress. Therefore, the intracellular GSSG/GSH ratio indicates cytotoxicity.[25] Previous studies have revealed that GSH decreases in the sinus mucosa of patients with CRS[20] and that the severity of CRS and tissue GSH levels have a significant negative correlation.[23] In this study, GSH and its substrate CysSH were significantly decreased in patients with ECRS, supporting a previous finding that GSH decreases in severe CRS cases. Recently, an increase in GSSG levels in polyps with refractory CRS has been reported by metabolomic analysis using liquid chromatography coupled with a mass spectrometer.[24] Moreover, a reduction in the expression of glutathione peroxidase 3 has been reported in polyps from patients with CRSwNP compared to the

sinus mucosa from normal individuals.[26] In this study, GSSG did not increase in patients with nECRS or ECRS, whereas the GSSG/GSH ratio significantly increased in patients with ECRS. Our results suggest that increased oxidative stress and decreased GSH levels are involved in the pathogenesis of refractory CRS.

HSH is a representative gaseous mediator along with nitric oxide and carbon monoxide. Endogenous HSH participates in the pathogenesis of several diseases and functions as an inflammatory mediator in various tissues.[27] In the respiratory system, endogenous HSH has been shown to alleviate inflammatory responses, oxidative stress, and pulmonary fibrosis and to have broad antiviral activity.[27,28] Past studies revealed that HSH and its synthases, cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), increase in the human nasal and sinus mucosa of patients with allergic rhinitis and CRS.[29,30] In the paranasal sinuses, CBS and CSE are localized to the superficial part of the mucosa and submucosal glands; however, CSE is also found in the vascular endothelium.[29] Therefore, involvement of HSH in various pathological conditions and symptoms of CRS has been suggested. In a previous report from Korea, the amount of HSH in the sinus mucosa was not significantly different between patients with CRSwNP and CRSsNP.[29] Nevertheless, in this study, the amount of HSH in the sinus mucosa was significantly higher in the patients with nECRS than in those with ECRS alone. The ECRS group in this study was presumed type-2 inflammation cases with high eosinophilic infiltration and high rates of bronchial asthma complications. In contrast, when considering the "regiotype" of the CRS, particularly the higher proportion of non-type 2 inflammation in CRSwNP in Asia,[31] the CRSwNP group in the previous report from Korea may have included numerous non-type 2 inflammation cases. Notably, no bronchial asthma complications were reported in patients with CRSwNP or CRSsNP in that study.[29] Our results can also be interpreted as follows: patients with ECRS consumed more HSH than those with nECRS, potentially due to the elevated oxidative stress observed in ECRS. However, a possibility exists that HSH production is decreased in patients with ECRS. Therefore, future studies on sulfur compounds and their synthases focusing on endotypes rather than CRS phenotypes are warranted.

Supersulfides are present in vivo at various levels, such as persulfides and polysulfides of GSH and homocysteine, through sulfur transfer reactions from CysSSH.[18] Due to their excess sulfur atoms, supersulfides exhibit greater nucleophilicity and antioxidant capacity than simple thiol compounds, suggesting their role as a significant defense system against oxidative stress in a cytoprotective manner.[10,12,18] Furthermore, supersulfides regulate the quality control and energy metabolism of mitochondria.[10] Supersulfides in bronchial epithelial cells and ELF of patients with COPD are decreased compared to patients without COPD.[19] Protective functions of supersulphides in viral airway infections, including influenza and COVID-19, have recently been reported in patients with aged lungs and chronic lung diseases, such as COPD.[12] These studies indicate that a decrease in supersulphides may lead to an increase in oxidative stress. This stress may be involved in the development of various airway diseases, and supersulphide supplementation is a potential treatment. In contrast, some supersulfides with protective effects against oxidative stress are

upregulated in the aqueous and vitreous humors of eyes with diabetes mellitus.[32] In this study, we did not find significant changes in the amount of low-molecular-weight polysulfides, such as CysSSH and GSSH, in the sinus mucosa of patients with CRS. However, GSSSG, which has been noted as a donor of supersulfides such as glutathione polysulfides, was significantly decreased in both ECRS and nECRS groups. Considering that supersulfides are present in the ELF covering the surface of the lower airway,[19] they are expected to be present in the ELF covering the surface of the upper airway. The detection of supersulfides in exhaled breath has also been reported.[12] Therefore, the amount of supersulfides in the nasal discharge and nasal exhaled breath of patients with CRS may differ among the control, patients with ECRS, and patients with nECRS, which should be a topic for future research.

Several previous studies have evaluated whether antioxidants can serve as a treatment for CRS.[6] Previous experiments that evaluated the representative antioxidants flavones[33] and resveratrol[34] reported promising effects on inflammation; however, they were performed on cultured cells and mice, respectively. Evaluation of antioxidants on human CRSwNP has been limited to the promising report on oral administration of erdosteine, which has both anti-inflammatory and antioxidant effects.[35] Therefore, no clear clinical conclusions have been reached. Regarding supersulfides, GSSSG has been reported to prevent retinal inflammation,[36] and synthesized N-acetyl-L-cysteine polysulfides have been reported to inhibit lipopolysaccharide-induced pro-inflammatory responses and protect mice from lethal endotoxin shock.[37] These results indicate the possibility of using GSSSG as a candidate drug for CRS if the drug can be efficiently administered to the localized area of inflammation. Further experiments are required to confirm this hypothesis.

Our study had several limitations. First, this was an exploratory study that examined whether sulfur compounds, including supersulfides, could be detected in the sinus mucosa. Therefore, we did not examine extracellularly secreted sulfur compounds. The quantification of extracellular sulfur compounds is the next issue to be addressed. Second, the enzymes that produce supersulfides still need to be evaluated. The protein expression of CBS and CSE, which are HSH-producing enzymes, has been confirmed in the sinus mucosa.[29] However, the localization of mitochondrial cysteinyl-tRNA synthetase (CARS2), which is attracting attention as a principal cysteine persulfide synthase,[10] has yet to be established. Analysis of the localization of CARS2 in the sinus mucosa is an issue that should be addressed in future studies. Finally, CRS was classified into two groups, ECRS and nECRS, based on the CRS endotypes. Inflammation is classified into three types depending on the cytokines produced: type 1, type 2, and type 3.[38] CRS is broadly classified into two types: type 2 or non-type 2 (types 1 and 3); however, mixed and unclassifiable types also exist.[4] To perform a precise classification of CRS regarding endotypes, the usefulness of detailed machine learning techniques such as cluster analysis and principal component analysis has been reported.[39] However, we believe that our categorization of CRS into ECRS ($\approx$ type 2 inflammation) and nECRS ($\approx$ non-type 2 inflammation) in this study is reasonable at present because it is supposed to capture the

differences in the pathogenesis of CRS more accurately than the phenotypic classification based on the presence or absence of polyps.

# 5 | CONCLUSIONS

In summary, we found decreased amounts of CysSH, GSH, and GSSSG and an increased ratio of GSSG/GSH in the sinus mucosa of patients with ECRS. Moreover, oxidative stress mediated by sulfur compounds may be involved in the pathogenesis of ECRS.

## CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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