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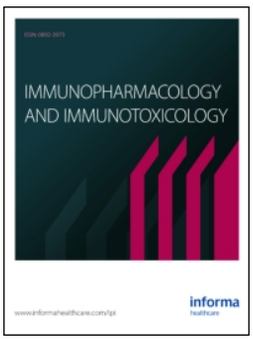

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ORIGINAL ARTICLE

Sulfur mustard triggers oxidative stress through glutathione depletion and altered expression of glutathione-related enzymes in human airways

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ABSTRACT

Context: Sulfur mustard (SM) is a lipophilic and reactive chemical compound that targets human airway system.

Objective: Glutathione (GSH) depletion, oxidative stress (OS) status, and changes in expression of GSH-dependent antioxidant enzymes were considered in human mustard lungs.

Materials and methods: Lung biopsies and bronchoalveolar lavage (BAL) were collected from non-exposed ($n = 10$) individuals and SM-exposed patients ($n = 12$). Alterations in expression of GSH-dependent enzymes were studied using RT^2 Profiler^{TM} PCR array. OS was evaluated by determining BAL fluid levels of total antioxidant capacity (TAC), malondialdehyde (MDA), and GSH.

Results: Mean TAC (0.142 ± 0.027 μmol/l) and GSH (4.98 ± 1.02 nmol/l) in BAL fluids of control group was significantly higher ($p < .05$) than those in SM-exposed patients (TAC $= 0.095 \pm 0.018$ μmol/l and GSH$= 3.09 \pm 1.02$ nmol/l), while MDA level in BAL fluids of these patients (0.71 ± 0.06 nmol/l) was significantly ($p = .001$) higher than that in controls (0.49 ± 0.048 nmol/l). Glutathione peroxidases (GPXs), glutathione-s-transferases (GSTs), and glutathione synthetase (GSS) enzymes were overexpressed in mustard lung biopsies, while glutathione reductase (GSR) was significantly downregulated (14.95-fold).

Conclusions: GSH depletion induced by *GSR* downregulation may be a major mechanism of SM toxicity on human lung. Despite overexpression of *GSTs* and *GPXs* genes, GSH depletion may decline the productivity of these enzymes and total antioxidants capacity, which is associated with OS.

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KEYWORDS
Sulfur mustard; lung; antioxidants; glutathione; oxidative stress

Introduction

Sulfur mustard is a chemical and cytotoxic agent that has been developed and used during World War I [1,2]. The highest unconventional use of SM occurred in Iran–Iraq war (1980–1988) [3], where nearly one-third of patients are still suffering from the late effects [4,5]. Pathological and harmful effects of SM on various organs, especially on lung tissue, have been frequently reported in previous studies [6,7]. It has been reported to be associated with deficiency in host defense system, immunological deficiency, dermatologic, hematologic, psychological, neurological, ocular, gastrointestinal and immunological complications, as well as reproductive and sleep disorders [8]. Numerous studies indicated that SM can cause various complications in respiratory system, including asthma, chronic bronchitis, bronchiectasis, pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), and bronchiolitis obliterans [9,10].

Although several cellular and molecular mechanisms are proposed for toxicological effects of SM on pulmonary function, the actual mechanism is not-well understood. For this reason, there is no appropriate treatment to suppress pulmonary injuries long-term after exposure to SM. Furthermore, current medications only improve symptoms but generally do not decelerate progression of the disease. Therefore, further studies are required to understand molecular mechanisms of SM activity on lung function long-term after exposure.

Oxidative stress (OS) induced by massive production of reactive oxidative species (ROS) and depletion of intracellular glutathione (GSH) can be considered as one of the main mechanisms of SM toxicity long-term after exposure. Overproduction of ROS and molecular damage of GSH-related antioxidants, particularly glutathione peroxidases (GPXs), glutathione reductase (GSR), glutathione synthase (GSS), and glutathione-s-transferases (GSTs), are the hypothesized events which may contribute in lung injury after the late effects of SM toxicity [2,11]. These enzymes, especially GPXs and GSTs, use GSH to protect cells against free radicals (Figure 1). Therefore, reduced levels of intracellular GSH may affect activity or productivity of these enzymes and increase ROS production and OS. Accordingly, expression analysis of GSH-dependent enzymes in mustard lungs can provide an

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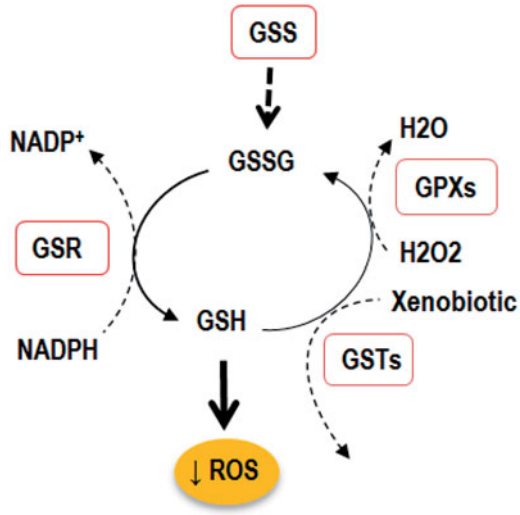

Figure 1. Correlation between GSH production and GSH-dependent enzymes. GSSG is produced from GSS enzyme activity and then converted to its reduced form (GSH) under GSR activity. GSH is a co-factor for the activity of several enzymes such as GPXs and GSTs, which are involved in ROS scavenging. GSR: glutathione reductase; GSS: glutathione synthase; GPX: glutathione peroxidase; GST: glutathione-s-transferase; ROS: reactive oxygen species; OS: oxidative stress; GSH: reduced glutathione; GSSG: oxidized glutathione.

appropriate tool to investigate these molecular mechanisms. The activity of these enzymatic antioxidants and some non-enzymatic antioxidants such as vitamin C, E, zinc, copper, and GSH is referred to total antioxidant capacity (TAC). In this study, we aim to consider alterations of OS markers such as TAC, GSH, and malondialdehyde (MDA) in bronchoalveolar lavage (BAL) fluid of SM-exposed patients. Furthermore, changes in the expression of GSH-dependent enzymes caused by SM will be considered in the lung of injured patients.

Materials and methods

Subjects

Lung biopsy specimens were collected from patients who had a documented exposure to SM during the Iran–Iraq war and non-exposed subjects. Our patients had chronic lung disease caused by SM, which was confirmed and diagnosed by pulmonologists in hospital. Furthermore, they are under evaluation by our research team for several years. They did not have any other chronic respiratory problems such asthma, allergy, and so on. The mean time after SM exposure among selected patients was 27.33 ± 0.57 years. The Ethics Review Board of the Baqiyatallah University of Medical Sciences approved this study and all patients signed an informed consent form. Pulmonary function tests (PFT) such as FEV1 (forced expiratory volume in 1s), FVC (forced volume vital capacity), and FEV1/FVC were determined by spirometry before samples collection. Patients were excluded if they had other chronic lung diseases, COPD, cardiovascular (CVD) disease, autoimmune disease, lung cancer, diabetes mellitus, drug addictions, >65 years, smoking habitat, and history of organ transplant recipients. Patients with history of exposure to other toxic agents were excluded. All selected patients were clinically stable and those who had used anti-inflammatory or antioxidant supplements such as N-acetyl cysteine and curcumin for at least 2 weeks prior to study were also excluded. Furthermore, patients who are using bronchodilator were excluded from the study. Eventually, 12 SM-exposed patients with mild and moderate SM-lung injury ($50\% < FEV1 < 80\%$) and 10 non-exposed persons were entered into the study.

Preparation of BAL fluids and lung biopsy samples

The process of sample collection including BAL fluid and biopsy specimen is described by our colleagues in previous works [11–13]. Briefly, all biopsies were taken at the same area of lung and there was no injury at the site of biopsies under bronchoscopy examinations. Chest X-ray was also provided for each patient to consider any possible injury sites. Biopsies were then immediately immersed in RNA Later reagent (R0901 SIGMA, St. Louis, MO), stored at $-80\,^{\circ}C$ until RNA extraction. BAL fluids were collected under tracheal wash by endotracheal tube. A wedged bronchoscope was applied for BAL fluids collection. BAL was performed in the right middle lobe with a total volume of 200 ml of sterile isotonic saline solution ($37\,^{\circ}C$). Aspirated BAL fluids were collected in a 50-ml conical tube. About 20 ml of BAL fluid recovered from each subject were centrifuged for 10 min at $300 \times g$ to separate the BAL cells from the fluids.

About 20 ml of fluid was centrifuged for 10 min at $300 \times g$ to separate the BAL cells from the fluids and then the BAL fluid was stored at $-20\,^{\circ}C$ until further assessment of TAC, MDA, and GSH values.

TAC measurement

TAC in BAL fluid samples was measured using FRAP (ferric reducing of antioxidant power) method that is previously described by Benize [14]. Briefly, 5 ml of fluids were centrifuged at $14{,}000 \times g$, $4\,^{\circ}C$ for 7 min. Supernatants were removed for the assessment of TAC. About 1.5 ml of FRAP reagent (including acetate buffer 300 mM, pH 3.6, TPTZ 10 mM, and ferric chloride 20 mM) was added to each tube and kept in water bath at 37 C for 5 min. Then, 50 µl of supernatants was added to each tube, and again kept in water bath at 37 C for 10 min. After 10 min, the absorbance was evaluated at 593 nm [12].

MDA measurement

MDA level in BAL fluid was measured using the thiobarbituric acid (TBA) method [15–17]. Briefly, 5 ml of BAL fluids were centrifuged for 7 min at $2000 \times g$ and then 100 µl of supernatants was added in 900 µl of distilled water into a glass tube. About 500 µl of TBA reagent (including 0.67 g of 2-TBA dissolved in 100 ml of distilled water along with 0.5 g NaOH and 100-ml glacial acetic acid) was added to each tube and then incubated in a boiling water bath for at least 1 h. After cooling in room temperature, each tube was centrifuged for 10 min at $4000 \times g$ and the absorbance of supernatants was recorded at 534 nm.

GSH measurement

GSH level of BAL fluids was determined according to the Tietz method [18]. Briefly, 800 µl of 3 mM NaHPO along with 100 µl of 0.04% 5,5-dithiobis 2-nitrobenzoic acid (DTNB) in 0.1% sodium citrate were added to BAL fluids. The absorbance of the solution was monitored at 412 nm using a UV spectrophotometer.

RNA extraction, cDNA synthesis, and RT-PCR

Total RNA of lung biopsy specimens was extracted using the RNX-Plus (SinaClon; RN7713C, Karaj, Iran) kit and then further purified by RNeasy Mini Kit (Qiagen; Cat No-74104, Hilden, Germany) according to the manufacturer's instructions.

Changes in expression of GSH-dependent genes in the lung biopsies of all samples were measured using RT^2 Profiler^{TM} PCR Array kit (Qiagen; PAHS-065ZA-6) that has been described in a previous work [11]. An equal amount of RNA (1 µg) was applied for cDNA synthesis using a RT^2 First strand kit (Qiagen; Cat No-330401). An equal amount of cDNA was mixed with RT^2 SYBR green/ROX qPCR Mastermix (QIAGEN Company, Cat No: 330522), and distributed to each PCR array well containing portions of specific genes. For all study genes, PCR was performed in 96-well plates, according to manufacturer's guidelines. Reaction volume per each well was 25 µl and holding stage was 95.0 °C 10:00 min. Cycle stages were also as the following: 40 cycles; 95.0 °C 15s; 60.0 °C 01:00 min. Beta-2-micro globulin (B2M) was used as a reference gene for normalization of the gene expression.

Statistical analysis

Data are presented as mean ± SD. An independent *t*-test was considered to compare the mean of parametric data, such as age, weight, BMI, PFT results between two groups. Data were analyzed using SPSS, version 19 and a probability of less than .05 was considered as significant. Gene expression data were evaluated using $2^{-\Delta Ct}$ method. Each sample was assessed in triplicate for gene expression data. Delta Ct (ΔCT) was calculated using the following formula: [ΔCT = CT (target) − CT]. Gene expression level was determined by $2^{-\Delta Ct}$ method.

Results

Clinical complications among SM-exposed patients are described in Table 1. Cough (91.66%), shortness of breath (83.33%), and sleep disorders (83.33%) were the most common complications among the patients. Mean of PFTs values and demographic information of cases and controls are presented in Table 2. There was no significant difference in the baseline characteristics between two groups; however, mean of weight among SM-exposed patients (78.0 ± 4.36 kg) was significantly ($p < .01$) greater than controls (66.53 ± 2.47 kg). As expected, PFTs values in SM-exposed patients were lower than that in the control group. PFTs results had demonstrated both obstructive and restrictive spirometric patterns among SM-exposed patients. The mean values of FVC, FEV1, and FEV1/FVC (FEV1%) in SM-exposed patients were significantly lower than those in control group ($p < .05$). Total lung capacity (TLC) and residual volume (RV) in SM-exposed patients (72.29 ± 5.6 and 75.06 ± 3.57, respectively) were higher ($p < .01$) than in the control group (56.43 ± 3.51 and 63.29 ± 5.71, respectively).

Table 1. Demographic characteristics and pulmonary function tests in patient and control groups.

Parameters	Control group ($n = 10$)	Patients group ($n = 12$)	p value
Age (year)	48.33 ± 24.58	52.0 ± 10.81*	.5
Weight (kg)	66.53 ± 2.47	78.0 ± 4.36	<.01
BMI (kg/m^2)	25.61 ± 1.55	25.13 ± 1.17	.44
FEV1 (% predicted)	95.33 ± 12.09	73.64 ± 4.16	.031
FVC (% predicted)	95.72 ± 9.5	76.68 ± 3.29	.034
FEV1/FVC	104.42 ± 11.78	71.55 ± 8.1*	.018
TLC	75.06 ± 3.57	72.29 ± 5.6*	<.01
RV	63.29 ± 5.71	56.43 ± 3.51	<.01

Control group: non-chemical subjects; patients group: chemical injured subjects; BMI: body mass index; FEV1: forced expiratory volume in 1s; FVC: forced volume vital capacity; TLC: total lung capacity; RV: residual volume.
*$p < .05$ is considered as significant difference.

Table 2. The most common clinical complications among SM-exposed patients.

Parameters	Frequency
Complications (%)	
Cough	11 (91.66%)
Shortness of breath	10 (83.33%)
Sleep disorders	10 (83.33%)
Wheezing	9 (75%)
Decreased lung sounds	9 (75%)
High sputum	8 (66.66%)
Chest tightness	8 (66.66%)
Chest pain	7 (58.33%)

Mean concentrations of FRAP, GSH, and MDA in BAL fluids of the controls and SM-exposed patients are seen in Figure 2. Significant differences were found in mean concentrations of FRAP, GSH, and MDA between the two groups. Control group demonstrated significantly higher FRAP value in their BAL fluids than SM-exposed patients group (0.142 ± 0.027 µmol/l vs. 0.095 ± 0.018 µmol/l; $p = .01$). Moreover, control group had significantly higher level of GSH in their BAL fluid compared to with SM-exposed patients (4.98 ± 1.02 nmol/l vs. 3.09 ± 1.02 nmol/l; $p = .013$). The mean of MDA concentration in SM-exposed patients was significantly higher than the controls ($p = .001$). The levels of MDA in controls and patients were 0.49 ± 0.048 nmol/l and 0.71 ± 0.06 nmol/l, respectively. The ratio of BAL fluid MDA from SM-exposed patients to controls was 1.44.

We found that most of the examined genes were significantly differentially expressed in lung of the SM-exposed patients ($p < .05$), with greater than a two-fold change in expression. A scatter plot depicts upregulation and downregulation fold changes of studied genes between SM-exposed patients and controls (Figure 3(A)).

Upon comparing *GPXs* genes expression profiles in mustard lungs, it was found that the expression of three *GPXs* genes including *GPX1*, *GPX2,* and *GPX7* was significantly higher in SM-exposed patients than control group. No remarkable alterations were detected for expression of other *GPXs* genes (*GPX3*, *GPX4, GPX5,* and *GPX6*) between two groups. Statistically significant upregulation of all three studied *GSTs* genes including *GSTP1*, *GSTZ1,* and *MGST3* expression was also observed in lung samples of the SM-exposed patients. Additionally, a significant rise in

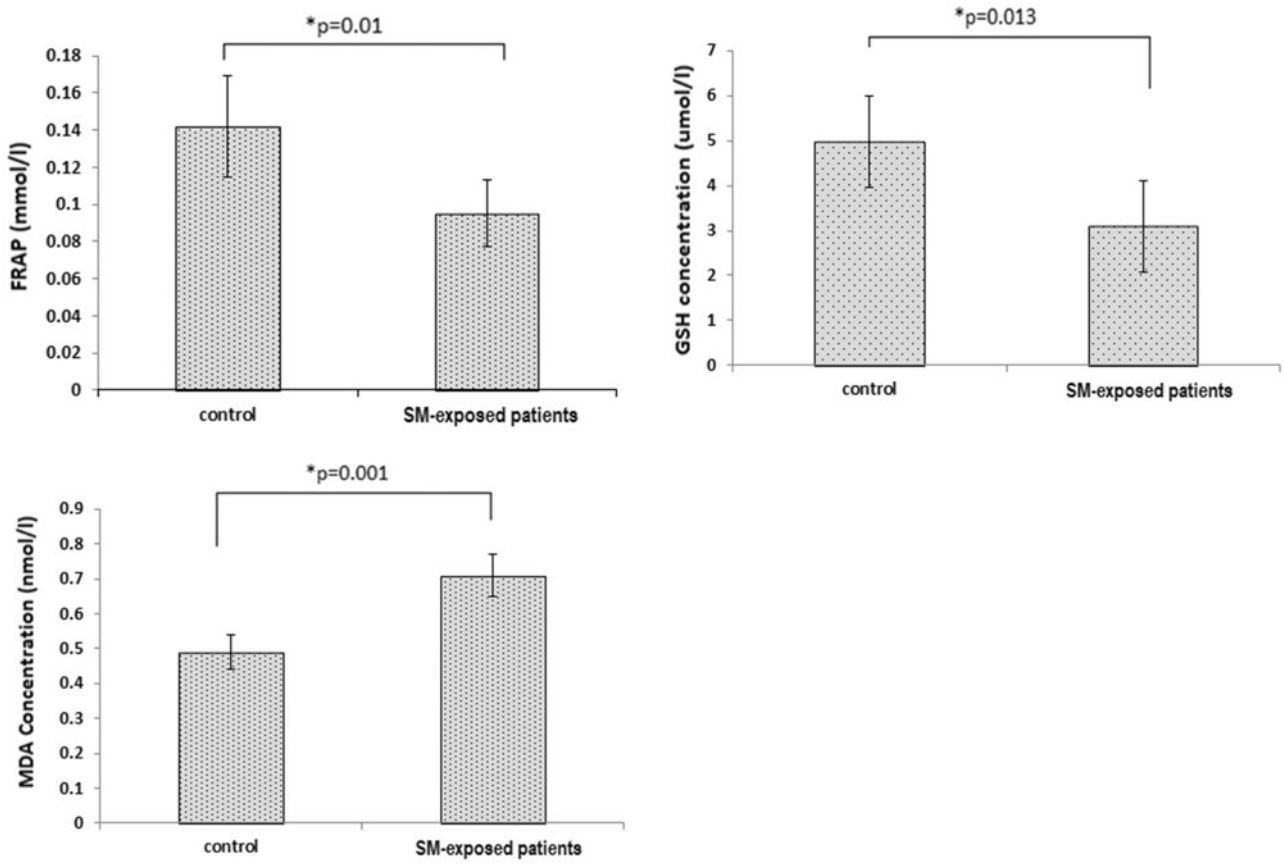

Figure 2. Comparison of FRAP, GSH, and MDA levels in BAL fluid of the SM-exposed patients and controls. Concentration of FRAP and GSH in BAL fluid of controls was significantly ($p = .01$ and $p = .013$, respectively) higher than in SM-exposed patients, while MDA level in BAL fluid of SM-exposed patients was significantly higher than in controls ($p = .001$).

expression was found for *GSS*, which encodes GSS enzyme. In contrast, a significant reduced expression was seen for *GSR*, encoding GSR enzyme. Figure 3(B) depicts fold changes ratio of the studied genes expression in SM-exposed patients compared to controls. Expression of all examined genes was in the order *GPX2* > *GSTZ1* > *GSS* > *MGST3* > *GSTP1* > *GPX1* > *GPX7* > *GPX4* > *GPX5* > *GPX3* > *GSR*. Among the most upregulated genes was the *GPX2*, which was overexpressed by +13.88-fold ($p < .0001$), while the *GSR* was the most downregulated gene (−14.96-fold; $p = .008$). SM-exposed individuals also showed overexpression of *GSS* +6.47 ($p = .0001$), *GPX1* +3.93 ($p < .0001$), and *GPX7* +2.2-folds ($p = .00004$) compared to the controls. No significant changes were detected for expression of other *GPXs* including *GPX3, GPX4, GPX5,* and *GPX6* in mustard lungs. All lung biopsy samples of SM-injured patients were found to overexpress *GSTs* genes at the highest level. SM-exposed individuals demonstrated expression of *GSTZ1* +7.36 ($p = .0002$), *MGST3* +6.04 ($p = .00007$), and *GSTP1* +4.5 ($p = .0004$), higher than those of controls that reveal. No significant changes were detected for expression of *PRDX4* and *PRDX5* genes; however, a trend for overexpression of *PRDX5* (1.72-fold) was observed.

Discussion

Results of the current study demonstrated different expression of GSH-dependent genes in the lung tissue specimens of SM-injured patients. Additionally, a reduction for the mean of GSH and TAC levels along with an increase in mean of MDA content were found in BAL fluids of the patients compared with controls. These data indicate an increased OS status and GSH depletion in BAL fluids of the SM-exposed patients. GSR downregulation may be the main reason for GSH depletion in lungs of the patients because we found a significant reduction in expression of *GSR* in mustard lungs. GSR is a central enzyme of cellular antioxidant defense. It reduces oxidized glutathione disulfide (GSSG) to the sulfhydryl form (GSH), which is an important cellular antioxidant [19,20]. Therefore, GSR downregulation can be considered as an actual mechanism of SM toxicity in patients lungs because it is associated with GSH depletion (Figure 4). GSH is also considered as the key survival antioxidant and oxyradical scavenger and its depletion enhances the cytotoxic effects of ROS and enhancement toxicity in lung endothelial cells [20]. Previous studies reported that late effects of SM exposure is associated with reduced level of GSH in serum and BAL fluids [21–23]. Several clinical trial studies revealed that treatment with GSH precursors, especially with N-acetylcysteine (NAC), compensates GSH depletion and consequently decrease markers of OS and toxicity induced by SM [24,25]. For example, NAC use has been shown to improve acute outcome after SM inhalation in pigs [26]. These findings point out the important function of cellular GSH pools, as protective mechanism against OS and lung injuries.

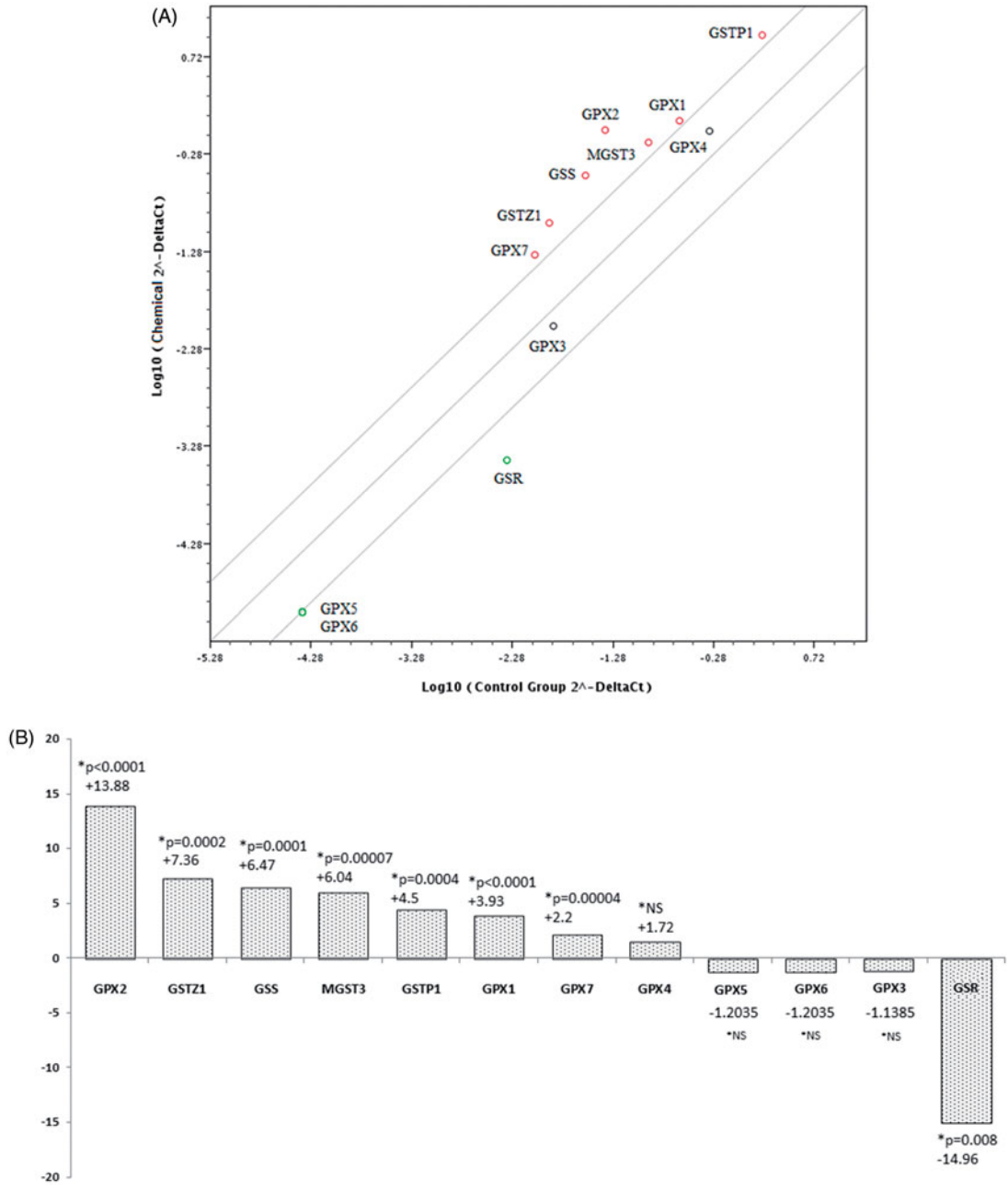

Figure 3. (A) Scatter plot of upregulation and downregulation of all examined genes in the lung tissue of SM-exposed patients compared to controls. Each point represents a gene, and colors correspond to the ranges of the negative $\log_{10}$ P and $\log_2$ fold change values. The black line indicates normal expression. Black circles: no statistically significant expressed genes (GPX4 and GPX3). Green circles: downregulated genes (GSR, GPX5, and GPX6). Red circles: upregulated genes (GSTP1, GPX1, GPX2, MGST3, GSS, GSTZ1, and GPX7). (B) Fold changes ratio of all examined genes expression in SM-exposed patients to controls. Among the upregulated genes in lung of the patients was the *GPX2* (+13.88-fold; $p < .0001$), while the *GSR* was the most downregulated gene (−14.96-fold; $p = .008$). SM-exposed individuals also showed overexpression of *GSTZ1* +7.36 ($p = .0002$), *GSS* +6.47 ($p = .0001$), *MGST3* +6.04 ($p = .00007$), *GSTP1* +4.5 ($p = .0004$), *GPX1* +3.93 ($p < .0001$), and *GPX7* +2.2-folds ($p = .00004$) higher than those of controls that reveal. We did not find a significant alteration in expression of *GPX3–6*. *$p < .05$ is considered as significant difference; *NS: non-significant.

Our study has also revealed an altered expression of *GSTs* genes in mustard lungs. A major function of both cytosolic and microsomal GST enzymes is conjugation of GSH to oxidized cellular macromolecules in order to facilitate their elimination and limit tissue injury [27,28]. GSTs are one of the major antioxidants in human airways, because they facilitate detoxification of various electrophilic molecules, including carcinogens, mutagens, and several therapeutic drugs, by conjugation with GSH [29,30]. Therefore, higher expression of *GSTs* is closely linked with enhancement of drug detoxification [31]. In this research, upregulation of *GSTs* genes, including *MGST3*, *GSTP1,* and *GSTZ1* was seen in the lung tissues of the patients, which indicates the toxicity effects of SM in their lungs. GSTs are one of the major antioxidants in human airways, which facilitate detoxification of various electrophilic molecules, including carcinogens, mutagens, and several therapeutic drugs, by conjugation with GSH [30]. Our findings are consistent with previous works that reported overexpression of *GSTs*, especially *GSTP1*, *GSTA1,* and *GSTM1* in airway wall and BAL fluid of SM patients [22,28].

Higher expression of *GPXs* family (*GPX1*, *GPX2,* and *GPX7*) was also observed in our study. The enzyme GPXs is a

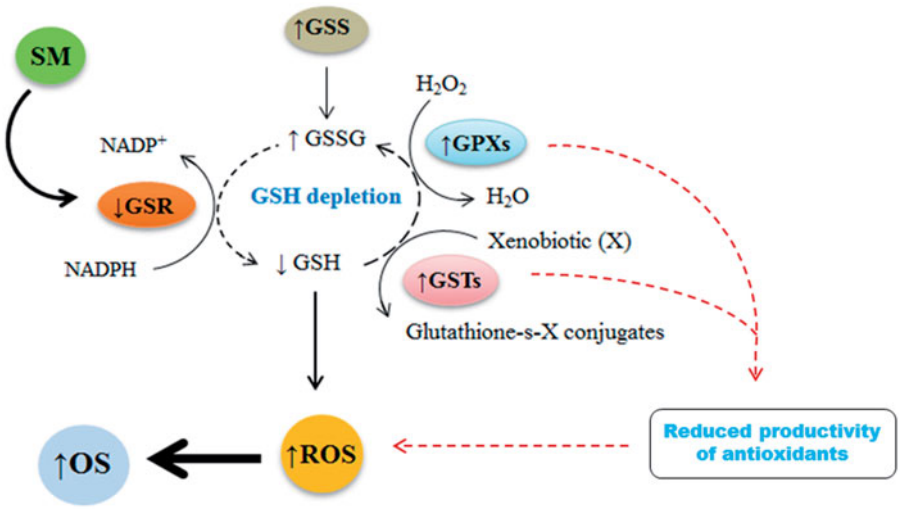

Figure 4. SM induces intracellular GSH depletion in mustard lungs via downregulation of GSR. There is a tight link between SM, GSH depletion, ROS production, and oxidative stress in mustard lungs. GSH depletion induced by SM and GSR deficiency can empty cellular GSH pool for GSTs and GPXs activity and as the result increase oxidative stress and toxicity effects. SM: sulfur mustard; GSR: glutathione reductase; GSS: glutathione synthase; GPX: glutathione peroxidase; GST: glutathione-s-transferase; ROS: reactive oxygen species; OS: oxidative stress; GSH: reduced glutathione; GSSG: oxidized glutathione.

selenocysteine-containing protein, which serves as an important factor for cellular defense against oxidant stress by utilizing GSH to H_2O_2 [32,33]. It scavenges hydrogen and lipid peroxides, thereby protects the body against OS. In the absence of this enzyme, reduction in antioxidant defense occurs, which leads to increased ROS accumulation, and can also elicit numerous pathophysiological consequences [34]. Moreover, it might provide a barrier against hydroperoxides. However, several lines of studies have demonstrated that SM declines the activity of GPXs and GSTs enzymes in the human lung tissue [22,35], which may be related to depletion of GSH. Sawale et al. found that exposure to 2-chloroethyl ethyl sulfide (CEES) depletes intracellular GSH level and activity of GSR, GPX, and GST enzymes, which play a major role in preventing ROS production and OS [36]. Therefore, there is a tight link between GSH contents and activity of these antioxidants in oxidatively damaged cells.

Although increased expression of GSTs and GPXs enzymes illustrates a mechanism to protect mustard lung against ROS-induced damages and further OS, this is apparently not sufficient to overwhelm ROS production because decreasing GSH levels mediated by SM may decline the productivity of these enzymes in lung cells. It can be supported with our results that revealed reduced levels of TAC in BAL fluids of the patients. Furthermore, GSH depletion induced by GSR downregulation can empty cellular GSH pool for GSTs and GPXs activity and as the result increase OS and toxicity effects of SM (Figure 4). Since GSH is produced from GSSG by the action of GSR, downregulation of GSR can be associated with decreased level of GSH as we observed in our study. We speculated that decreased levels of GSH may induce positive regulation or feedback for GS expression to compensate GSH depletion. But downregulation of GSR maintains GSH depletion, while GSSG may be increased. Nevertheless, it would be well if we could also measure GSSG level or GSH contents in biopsies, but due to limitations of tacking tissue samples we could not provide this assessment. However, we could not check GSH level in biopsy samples, as it was impossible for us to get larger samples size for additional works. Therefore, it should be evaluated in further studies.

We could not consider expression of these enzymes at the protein level. Although GST gene was overexpressed, it may not be translated to protein due to abnormalities resulted by SM. Although GSH was significantly declined, we speculate that increased OS may lead to induction of expression of some enzymatic antioxidants genes such as GSTs, GPXs, and GSS to scavenge ROS. Since intracellular GSH is depleted in SM-exposed patients, the productivity and activity of GSH-dependent enzymes may be severely declined. Previous studies also reported decreased activity of these GSH-dependent enzymes that support our suggestion. Nevertheless, it can be considered as a limitation of our study and it was better if we considered this different expression at protein levels, too. Data being analyzed were gene expression results and do not reflect protein expression because upregulated genes do not necessarily translate into higher protein expression levels. We would like to bind our genomics findings with another study at proteomics levels, but due to the following reasons it was relatively impossible for us or to any other researchers. The process of taking lung tissue samples in these patients is very difficult because lungs of the SM-exposed patients are very sensitive to invasive sampling method and there is a high risk of bleeding, heart arrest, and bronchial spasm among them. Additionally, larger tissue samples could be provided by surgery to study expression patterns at the protein levels. Unfortunately, surgery is more invasive than the bronchoscopy method for these patients, and we did not admit to obtain tissue samples with this method. Therefore, we could provide only three to four small biopsy samples using the bronchoscopy method, which are only enough to consider gene expression but not protein expression patterns. Due to these limitations, previous studies considered protein expression pattern in BAL fluids rather than tissue specimens among these patients.

In conclusion, we have shown that mustard lungs are associated with downregulation of GSR that is associated with

GSH depletion. Although expression of *GPXs* and *GSTs* genes was increased, reduced level of GSH may decline the productivity of these enzymes, and consequently leads to ROS accumulation and OS. Therefore, GSH depletion induced by GSR downregulation can be considered as one of the main reasons for cytotoxic effects of SM in lung cells. Antioxidant therapy is recommended as a potential treatment to minimize the SM-induced OS among intoxicated patients.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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