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The role of glutathione-*S*-transferase polymorphisms on clinical outcome of ALI/ARDS patient treated with *N*-acetylcysteine

Mandana Moradi [a], Mojtaba Mojtahedzadeh [a], Ali Mandegari [b,c], Mohammad Sadegh Soltan-Sharifi [b], Atabak Najafi [d], Mohammad Reza Khajavi [d], Molook Hajibabayee [a], Mohammad Hossein Ghahremani [b,e,*]

[a] *Department of Clinical Pharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[b] *Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[c] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran*
[d] *School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[e] *Department of Molecular Medicine, School of Advanced Technology in Medicine, Tehran University of Medical Sciences, Tehran, Iran*

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Summary
Oxidative stress has a proven role in pathophysiology of acute respiratory distress syndrome. The antioxidant drugs, especially *N*-acetylcysteine (NAC) have been used for years to overcome oxidative stress effects in patients. In the present study we have investigated the effects of NAC treatment (IV NAC in 150 mg/kg at the first day followed by 50 mg/kg/day for three days) on 27 ICU patients with ALI/ARDS considering the glutathione-*S*-transferase genetic variations, as an important enzyme contributing in oxidative stress pathways. The results indicated that NAC improved oxygenation (increase in PaO_2/FiO_2) and decreased mortality rate in treated patients compared to control group ($p < 0.05$). Evaluation of three isoforms of glutathione-*S*-transferase (GST M1, P1 and T1), in these patients have showed an association between GST M1 null, and GST M1 and T1 double null polymorphisms with increased mortality in control group, suggesting antioxidant therapy critical for this group of patients.

* Corresponding author. Department of Toxicology and Pharmacology, Faculty of Pharmacy, New Bldg, Room J-309, Tehran University of Medical Sciences, 14155 Tehran, Iran. Tel./fax: +98 21 6695 9102.
E-mail address: mhghahremani@tums.ac.ir (M.H. Ghahremani).

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Introduction

Acute respiratory distress syndrome (ARDS)/acute lung injury (ALI) is a serious clinical problem, which is a contributor to the morbidity and mortality of patients in intensive care units all over the world, impairing tremendous human and financial costs. Despite advances in supportive care, the mortality rate in patients with the ALI/ARDS is still high and varies from 10% to as high as 90%[1] resulting in approximately 80,000 deaths yearly in the United States.[2] Since the original description of ARDS/ALI, investigators have endeavored to elucidate the mechanisms regulating the pathways leading to lung injury in ALI/ARDS, with the ultimate goal of developing therapeutic tools to ameliorate or prevent lung injury in patients at risk. One important fact in the pathogenesis of ALI/ARDS, and one that is the focus of our study, is oxidative injury to the lung mediated by Reactive Oxygen Species (ROS).[3–5] This leads to cell injury by various mechanisms including direct DNA damage, lipid peroxidation, oxidation of proteins,[6–8] releasing of proteases and inactivation of antioxidant and antiprotease enzymes,[9] and alteration of transcription factors such as activator protein-1 and nuclear factor (NF)-κB, leading to enhanced expression of proinflammatory genes.[10,11]

Glutathione system (GSH/GSSG) is an important and the most abundant antioxidant in the lung that decreases in lung inflammatory conditions such as ARDS/ALI.[12] Thus one strategy to limit oxidative lung injury is to restore the oxidant/antioxidant balance by augmenting the intracellular pool of glutathione using its precursors, such as *N*-acetylcysteine (NAC).[13–15] NAC can stimulate GSH synthesis, enhance glutathione-*S*-transferase (GST) activity, promote detoxification, and act directly on reactive oxidant radicals. Moreover, NAC reduces the formation of proinflammatory cytokines, such as interleukin-8 and tumor necrosis factor-[alpha].[16] Protective effects of NAC have been described in many experimental and clinical models of ARDS. In mechanically ventilated patients with acute lung injury, NAC has increased cellular glutathione content, decreased the blood levels of conjugated dienes, variables of oxidative stress,[17] improved systemic oxygenation and reduced ventilatory requirements,[18] improved chest radiograph edema and vascular resistance resulting better survival rate.

Reports on administration of NAC in ARDS are still conflicting and some trials have not demonstrated its efficacy.[19] Therefore, we hypothesized that genetic differences in study populations, especially in genes involved in oxidative stress, may have contributed to these diverse results. Glutathione-*S*-transferases (GSTs) have considerable impact upon oxidative stress in critically ill patients. GSTs are a complex multigene family of enzymes that are widely distributed in the animal kingdom and catalyze the conjugation of glutathione to a wide range of compounds and possess many biological functions, such as detoxification of xenobiotics, removal of reactive oxygen species and regeneration of *S*-thiol proteins.[20] These enzymes have important role in oxidative stress pathways, and data support the view that allelism in different GST genes mediates susceptibility and outcome in different diseases,[21–26] especially in the cases mediated by oxidative stress. The major GST enzyme in the human lung is GST P1, with the common polymorphism leading to replacement of the amino acid Val with Ile at codon 105. This substitution results in three possible genotypes: 105 Ile/Ile, 105 Ile/Val, or 105 Val/Val, and also changes the enzyme active site and it's substrate-specific catalytic activity.[21,23] These polymorphisms have been studied widely as a potential contributor in asthma, COPD and lung cancers, but related available data are still conflicting.[25–27] Since *GST M1, GST P1,* and *GSTT1* are the most commonly examined genes in GST family, with documented role in oxidative stress pathways,[25–28] in this study we have investigated the role of polymorphisms of these three classes of enzyme in clinical response of ARDS patients treated with NAC.

Subjects and methods

Recruitment of subjects

Following ethics committee approval (by the Institutional Review Board of Medical Ethics, Pharmaceutical Sciences Research Center/Tehran University of Medical Sciences), 30 consecutive mechanically ventilated patients meeting criteria for ALI/ARDS, were recruited between July 2005 and April 2006 from the General ICU of Sina hospital, one of the principal teaching hospitals of Tehran University of Medical Sciences. ALI/ARDS was defined according to the criteria established by the American–European Consensus Conference on ARDS[29] (acute onset, $PaO_2/FiO_2 < 300$ mmHg, bilateral infiltrates seen on frontal chest radiograph, and pulmonary artery occlusion pressure below 18 mmHg). The patients must also have SIRS concomitantly (Systemic Inflammatory Response Syndrome: 2 or more of the following conditions, temperature $> 38\ ^\circ C$ or $< 36\ ^\circ C$, heart rate > 90 - beats/min, respiratory rate > 20/minor $PaCO_2 < 32$ mmHg, WBC $> 12{,}000$ or < 4000 cells/mm^3 or 10% bands).

The patients with $PaO_2/FiO_2 > 300$, age < 18 years, hepatic or renal failure not due to septic shock and pregnancy were excluded. The design was a prospective randomized single blinded, placebo controlled clinical trial.

Clinical protocol

Following randomization (simple randomization) patients received either NAC, or the equal volume of placebo (5% dextrose) as soon as the clinical diagnoses of ALI/ARDS were established (within few hours of admission). The dose of NAC in this study was 150 mg/kg diluted in 5% dextrose that was infused in a period of 20 min at first day and then 50 mg/kg/day diluted in 5% dextrose for 3 additional doses in 3 consecutive days. All patients received routine supportive therapy for ALI/ARDS including mechanical ventilation, fluid managements and treatments for their underlying pathology. None of these patients received any investigational drug or surfactant.

At the beginning of the study and initiating NAC, the past medical history and physical, laboratory and homodynamic variables as well as ventilatory support measures were recorded and the presence of comorbid conditions (see the

subsequent discussion) was noted. The APACHE II score was calculated within 6 h of initiation of NAC.[30] Physiologic and laboratory characteristics prior to initiation of NAC were measured to determine the development of non-pulmonary organ-system dysfunction (ODS) between the time of ICU admission and initiation of our intervention or after that. The characteristics of the patient's population are summarized in Table 1. In all patients we recorded daily oxygenation indices (PaO_2, FiO_2 and PaO_2/FiO_2) during the course of NAC administration, length of intubations, and hospital mortality as our clinical end points.

Definitions

On the basis of previously published data, comorbid conditions most likely to affect mortality are as follows.[31]

Malignancy (active, untreated or undergoing current treatment), cirrhosis (biopsy proven or with evidence of portal hypertension), HIV infection (serologic evidence of infection with or without AIDS), organ transplantation (history of bone morrow, liver or kidney transplantation), chronic obstructive pulmonary disease (COPD), alcohol abuse, intravenous drug use (IVDU), chronic steroid use (current use of corticosteroid for >2 weeks and dose > 20 mg/day), and diabetes mellitus.

Non-pulmonary organ dysfunction was defined as such a dysfunction developing after admission to the hospital and persisting through the time of initiation of mechanical ventilation or during mechanical ventilation in a specific organ system, as follows.

Hepatic dysfunction (total serum bilirubin > 2.0 mg/dl, along with a prothrombin time > 3 s longer than the control value), renal dysfunction (serum creatinine > 2 mg/dl), hematologic dysfunction (platelet count of $<75{,}000/mm^3$, WBC $< 1000/mm^3$ or evidence of disseminated intravascular coagulation (DIC)), neurological dysfunction (obtundation, seizure, hemorrhage or acute cerebrovascular accident), and gastrointestinal dysfunction (ileus lasting > 24 h or hemorrhage requiring transfusion).

Blood sampling

Blood samples were taken (approximately 7 ml) from indwelling arterial cannula in a tube containing 0.1 mmol/l EDTA as an anticoagulant, for genotype analysis peripheral blood samples were centrifuged (3000 rpm, and 10 min) to separate buffy coat layer, containing white blood cells. Then stored at −80 °C for DNA isolation and genotype analyses.

Table 1 Baseline demographic characteristics of patients.

	NAC group ($n = 14$)	Control group ($n = 13$)	*P* value
Age (years)	48.4 ± 5.5	49.2 ± 4.5	0.912
Gender (M/F)	9/5	8/5	0.598
APACHE II	18.3 ± 1.0	21.1 ± 2.0	0.198
PaO_2/FiO_2 (mmHg)	194.5 ± 40.5	139.1 ± 15.3	0.225
Organ dysfunction	14	13	0.756
Systemic comorbidities	6	6	0.863

Data are presented as median ± SE. For statistical analysis Fisher's Exact Test (for gender), *t*-test (for age, PaO_2/FiO_2), Chi-square tests (for systemic comorbidity) and Mann–Whitney test (for organ dysfunction) were used.

Genotype analysis

DNA was isolated using salting out method. The DNA yield was estimated by measuring the optical density at 260 nm in a spectrophotometer prior to PCR reaction. A multiplex PCR was performed to detect the presence or absence of the *GST M1* and *GST T1* genes, according to the protocol described previously by Abdel-Rahman et al.[32] This technique conclusively identified GST M1 null and GST T1 null genotypes, corresponding to the deletion of both alleles and β-globin as an internal positive control. Primers used to amplify the *GST M1* genotypes were 5′-GAA CTC CCT GAA AAG CTA AAG C-3′ and 5′-GTT GGG CTC AAA TAT ACG GTG G-3′ as forward and reverse primers, respectively, resulting in a 219 bp band, and for the *GST T1* were 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′ and 5′-TCA CCG GAT CAT GGC CAG CA-3′ as forward and reverse primers, respectively, resulting in a 450 bp band. As an internal control, the β-globin gene was amplified using 5′-GAA GAG CCA AGG ACA GGT AC-3′and 5′ CAA CTT CAT CCA CGT TCA CC 3′ as forward and reverse primers, respectively, giving a 267 bp product. PCR reactions were resolved in an ethidium bromide-stained 2% agarose gel electrophoresis. The absence of the GST M1- or GST T1-specific fragments indicated the corresponding null genotype (*0/*0), whereas the β-globin-specific fragment confirmed the presence of amplifiable DNA in the reaction mixture.

Genotyping for the *GST P1* codon 105 (Ile 105 Val) was determined by PCR-RFLP method in a separate reaction. The subclasses were amplified using 5′-TCA TCC TTC CAC GCA CAT CC-3′ as forward and 5′-GCA GGT TGT GTC TTG TCC CAG-3′ as reverse primers, then the 280 bp PCR product was digested with the restriction enzyme Alw 261(BsmAL) 10 U/50 μl at 37 °C for 6 h and analyzed by separation in a 3% agarose gel and visualized with ethidium bromide. The homozygous Ile genotype was identified by a 280 bp band, the homozygous Val genotype was identified by the presence of 150 and 124 bp bands. The heterozygous type exhibited all three bands.

PCR reaction mixture contained 2 mM of $MgCl_2$, 0.2 mM of dNTP, 1× PCR buffer, 1.2 U of Taq DNA polymerase, 100 ng of DNA template and 5 pmol/μl of both forward and reverse primers in deionized sterile water in total volume of 50 μl.

Amplification was carried out with touch down method as follows: 94 °C for 30 s (denaturation), 60 °C (−0.5 °C/cycle, 10 cycle) for 30 s (annealing) and 72 °C for 30 s (extension), then 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s for 35 cycle and 72 °C for 10 min as final extension.

Materials

All PCR reagents and ALW26I were purchased from Fermentas, Ukraine. Electrophoreses reagents were from Invitrogen, USA, other chemicals were obtained from Merck, Germany.

Statistical analyses

Data were analyzed using Chi-square (2×2), three-dimensional contingency table (Chi-square $2 \times 2 \times 2$), one-way and two-way ANOVAs with Tukey post-test and independent sample *t*-test accordingly. The *p* value less than 0.05 was set as significant. The statistical tests were performed using SPSS11.5 (SPSS Inc.) and Excel 12.0 (Microsoft Corp.) softwares.

Results

Over the study time period 30 patients were recruited (15 in each group) but one from the treatment and two from the control group were withdrawn (died before completion of study period). Fourteen of the 27 studied patients received NAC, and 13 were given placebo. The demographic data are summarized in Table 1. The groups were well matched in terms of age, male/female distribution, baseline APACHE II and PaO_2/FiO_2, organ dysfunction during ICU stay and systemic comorbidities.

The genotype frequencies of the GST M1, T1 and P1 polymorphism in both (NAC and control) groups are shown in Table 2. In control group, the GST M1 gene was deleted in 11 cases (84.6%), and deletion of the GST T1 gene was identified in 5 cases (38.5%). Deletions of both genes in a genotype were identified in 5 cases (38.5%) in control group. In this group of patients, 9 cases (69.2%) were homozygous for the GST P1 (Ile/Ile), 3 cases (23.1%) were homozygous for (Val/Val) allele and 1 patient was (7.7%) heterozygous for the (Ile/Val) alleles.

In the NAC group, deletion of the GST M1 gene was found in 7 cases (50%), and the GST T1 gene deletion in 7 (50%) cases. Deletion of both genes was identified in 3 (21%) cases. In this group of patients, 8 (57.1%) were homozygous for the GST P1 (Ile/Ile) allele, 5 cases (35.7%) were homozygous for the GST P1 (Val/Val) allele and 1 (7.1%) heterozygous for the (Ile/Val) alleles.

The differences in the GST M1, P1 and T1 genotype distribution between the NAC and the control group were not statistically significant ($p > 0.05$). Also insignificant difference between groups ($p > 0.05$) in simultaneous deletions of both genes in a genotype (double null genotype) was observed.

Table 2 Genotype frequencies of the GST M1, T1 and P1 polymorphism in both (NAC and control) groups.

	NAC group ($n = 14$) *n* (%)	Control group ($n = 13$) *n* (%)	*P* value
GST M1			
Null (−)	7 (50)	11(84.6)	0.066
Present (+)	7 (50)	2 (15.4)	
GST T1			
Null (−)	7 (50)	5 (38.5)	0.547
Present (+)	7 (50)	8 (61.5)	
GST P1			
Ile homozygote	8 (57.1)	9 (69.2)	0.77
Val homozygote	5 (35.7)	3 (23.1)	
Heterozygote	1 (7.1)	1 (7.7)	
GST M1, T1			
Null (−)	3 (21)	5 (38.5)	0.33

P value was calculated using Chi-square tests. The Ile105Val variant of *GST P1* was genotyped in all subjects.

Clinical outcome

Clinical outcomes are listed in Table 3. The overall hospital mortality rate was less in NAC group (35.7%; $p < 0.05$) but no statistically significant difference between groups, in duration of mechanical ventilation or length of ICU stay was observed ($p > 0.05$). Baseline oxygenation index (PaO_2/FIO_2), was similar in both groups, but improved significantly ($p < 0.05$) from day 0 to day 4 in the NAC-treated group. Correlation test did not demonstrate any association between intubation period and oxygenation changes in our study.

In order to assess the potential correlation between systemic comorbidities or simultaneous organ dysfunction and hospital mortality, the only major clinical end point that shows statistically significant difference between NAC and control group, we used Chi-square tests for intra group assessment. Systemic comorbidities had no effect on mortality either in NAC or in control group ($p > 0.05$), however, simultaneous organ dysfunction showed a correlation with increased mortality in control group ($p < 0.05$). *t*-test analyses showed no statistically significant difference, in oxygenation indices between dead and live patients in different days of our study ($p > 0.05$). In addition, to address whether GST M1, T1 and P1 variant allele is associated with increased mortality, or poor oxygenation, we performed three-dimensional Chi-square ($2 \times 2 \times 2$) and two-way ANOVA with Tukey post-test, respectively. Our results indicated that the oxygenation had been improved in NAC-treated group compared to control patients; however, there was no significant association between GST polymorphism and oxygenation (Table 4). On the other hand, a significant association was observed between increased mortality with GST M1 null and double deletion of both genes (M1, T1), in control group ($p < 0.05$).

Table 3 Clinical outcomes in NAC and control groups.

	NAC group ($n = 14$) *n* (%)	Control group (n = 13) *n* (%)	*P* value
Hospital mortality	5 (35.7)	10 (76.9%)	0.031
Duration of mechanical ventilation	24.8 ± 8.5	32.9 ± 9.8	0.539
Length of ICU stay	32.9 ± 7.6	42.1 ± 10.3	0.475
PaO_2/FiO_2 2nd day	227.3 ± 23.9	155.0 ± 15.5	0.020
PaO_2/FiO_2 3rd day	344.0 ± 38.3	166.5 ± 119.0	<0.001
PaO_2/FiO_2 4th day	440.9 ± 47.5	151.2 ± 24.6	<0.001

P values were calculated using Chi-square tests (for hospital mortality), *t*-test (for ventilation and ICU stay) and one-way ANOVA with Tukey post-test (for PaO_2/FiO_2).

Table 4 GST polymorphisms and oxygenation.

	Oxygenation in genotype					
	NAC			Control		
	Day 2	Day 3	Day4	Day 2	Day 3	Day4
GST M1						
Null (−)	214 ± 18.4	266 ± 28.7	380 ± 60.1***	147 ± 17.4	161 ± 22.2	152 ± 29.2
Present (+)	240 ± 45.6	421 ± 59.4*	501 ± 70.3***	196 ± 6.0	192 ± 19.7	144 ± 20.0
GST T1						
Null (−)	239 ± 45.0	319 ± 56.7	399 ± 66.0**	155 ± 31.4	160 ± 29.4	171 ± 51.2
Present (+)	215 ± 20.2	368 ± 45.2**	482 ± 69.6***	154 ± 17.8	170 ± 26.4	138 ± 26.4
GST P1						
Ile/Ile	198 ± 17.2	327 ± 41.2*	449 ± 65.4***	173 ± 18.4	174 ± 24.9	171 ± 33.7
Val/Val	222 ± 26.8	313 ± 63.8	382 ± 71.5**	104 ± 19.5	153 ± 39.2	112 ± 6.2
Ile/Val	485	633	660	144	130	310
GST M1, T1						
Null (−)	203 ± 38.9	245 ± 53.4	346 ± 128.8	155 ± 31.3	160 ± 29.3	171 ± 51.2

P value was calculated using two-way ANOVA with Tukey post-test. The Ile105Val variant of *GST P1* was genotyped in all subjects.
* $p < 0.05$ compared to their corresponding control group.
** $p < 0.01$ compared to their corresponding control group.
*** $p < 0.001$ compared to their corresponding control group.

Furthermore, NAC treatment reduced mortality in GST M1, T1 and double deletion (M1, T1) patients significantly. The $2 \times 2 \times 2$ Chi-square analysis indicated a correlation between GST M1 null polymorphism and increase in mortality ($\chi^2 = 15.97$, df = 4, $p < 0.01$). Moreover, there was a trend toward involvement of GST T1 polymorphism ($\chi^2 = 7.18$, df = 4, $p > 0.10$) in mortality rate of ARD patients. These results indicated the importance of NAC treatment in ARD patients with GST M1 null and GST M1, T1 double null polymorphisms. These data are summarized in Table 5.

Discussion

Recent research has proven the inflammatory mediators as key players in the pathogenesis of ALI/ARDS.[33] Oxidative injury compromising endothelial barrier integrity, have documented role in the pathogenesis of ALI/ARDS[34] and the oxidative burden has been investigated in these patients. Ortoloni et al. have found that expired ethane, malondialdehyde (MDA), oxidized and reduced glutathione in the epithelial lining fluid of ARDS patients correlate with oxidative changes in lungs.[35] In oxidative stress situation, reduced glutathione (GSH) is one of the main intracellular low molecular weight thiol that acts as a nucleophilic scavenger and as an enzyme-catalyzed antioxidant in the event of electrophilic/oxidative tissue injury.[36] As a matter of fact, it has been suggested that repletion of glutathione may safely be accomplished with NAC, an extremely safe agent with a wide toxic therapeutic window in ALI/ARDS patients, and such treatment may shorten the duration of lung injury.[37] Our earlier study have showed that treatment with NAC can increase extracellular total antioxidant power and total thiol molecules and improve intracellular glutathione and the outcomes of patients with ALI/ARDS.[38]

In the present study, we have demonstrated that intravenous NAC can improve oxygenation in ALI/ARDS patients, as evidenced by the significant increase in PaO_2/FiO_2 from day 1 to day 4 compared to control group ($p < 0.05$). This increase is so much that the median of PaO_2/FiO_2 was over the threshold for definition of ALI/ARDS in the third and fourth day of our study in NAC group. These observations are in agreement with the findings of an earlier study by Suter et al. which showed that intravenous NAC could

Table 5 GST polymorphisms and mortality.

	Death in a genotype		χ^2	*P* value
	NAC	Control		
GST M1				
Null (−)	3/7*	10/11	15.97	<0.01
Present (+)	2/7	0/2		
GST T1				
Null (−)	2/7	5/5	7.18	>0.10
Present (+)	3/7	5/8		
GST P1				
Ile/Ile	3/8	6/9	6.90	>0.10
Val/Val	1/5	3/3		
Ile/Val	1/1	1/1		
GST M1, T1				
Null (−)	1/3*	5/5	ND	ND

P value was calculated using three-dimensional Chi-square ($2 \times 2 \times 2$) for GST polymorphism and mortality. The Ile105Val variant of *GST P1* was genotyped in all subjects.
* $p < 0.05$ mortality rate compared to control group calculated by *t*-test. ND, not determined.

improve systemic oxygenation and reduce the need for ventilatory support in patients presenting with mild to moderate ARDS.[18] Another study by Bernard indicated that NAC has favorable effects in pulmonary vascular resistance, static compliance, oxygen delivery and oxygen consumption.[39]

However, we observed that compared to control group, NAC treatment did not significantly decrease the period of ICU stay and ventilatory support requirements. The correlation analyses indicate no relationship between oxygenation and intubation period in NAC group. This perhaps is due to other complications, for example decreased level of consciousness has made the extubation of some of these patients impossible in spite of acceptable oxygenation indices.

The results of the present study showed that NAC not only ameliorated the ALI/ARDS by improving oxygenation but also decreased mortality rate in ALI/ARDS patients ($P < 0.05$), as the most important clinical outcome in our study. A correlation between simultaneous organ dysfunction and increased mortality in control group was observed. Since both groups had comparable organ dysfunction at baseline, we can conclude that NAC has ameliorated the negative effects of this parameter on mortality in treatment group. The better survival in NAC group is expected to be related to better oxygenation in these patients; however, the statistical analyses do not show such an association. Thus, one can conclude that these are two separate conditions, and pathologic pathways interfering with oxygenation may be different from those contributing to mortality although further study is needed to make firm conclusion. In this regard, Domenighetti and Suter showed that in a group of patients with established ARDS, NAC neither improved systemic oxygenation nor reduced the need for ventilatory support.[19] These controversies may be due to different study designs in dosing, time of starting NAC and study populations.

In the last few years there has been enormous interest in determining the possible role of different polymorphisms in association with different pathologic conditions, as with ARDS. There are some known polymorphisms with proved diagnostic or prognostic role in these patients. For example the angiotensin-converting enzyme polymorphisms (insertion/deletion) have been reported as significant prognostic factor for the outcome of patients with ARDS[40] or the variations of the vascular endothelial growth factor gene may contribute to the prognosis in patients with ARDS via VEGF production.[41] There are various studies indicating the impact of genetic variability in glutathione-S-transferase (GST) isoforms M1, T1 and P1, mainly cancer prognosis, metabolism of xenobiotic and neurological disorders,[42–45] which contribute to individual susceptibility differences to oxidative stress. Furthermore, recently it has been reported that GST polymorphism contributes to progression from bronchial hyperresponsiveness to asthma in adults.[46] Based on these observations, we investigated the role of GST polymorphism, in our study population, to evaluate individual response to antioxidant drug NAC, as one of the possible differences in various studies about NAC in ALI/ARDS. Our results indicate that there is a significant correlation between GST M1 null polymorphism and increased mortality in control group. Moreover, we also observed a similar association with mortality and double deletion of GST M1 and GST T1 in control group of patients. Furthermore, our results indicated that in ARD patients with GST M1 null and M1, T1 double deletion, NAC treatment significantly lowers the mortality rate. Although there is a trend toward involvement of GST T1 polymorphism in mortality rate of ARD patients, no statistically significant association between the single GST T1 null/present or GST P1 polymorphisms and mortality was observed. It is noteworthy to mention that there are no significant differences in baseline distribution of three classes of GST enzymes (GST M1, GST T1 and GST P1) between NAC and control group. These results suggest that patients with absence of GST M1 gene or deletion of both GST M1 and GST T1, are more vulnerable to oxidative stress contributing to ALI/ARDS and are in immediate need of antioxidant therapy. It should be mentioned that in patients with GST T1 null and GST P1 Val/Val homozygote polymorphism, an increase in mortality rate in control group has been observed although it is not statistically significant which is perhaps because of the small number of patient in these groups. On the other hand, GST polymorphisms showed no association with the fluctuation in oxygen indices in both NAC and control groups, although there was better oxygenation in some polymorphisms. As the GST polymorphisms, one of the oxidative stress related enzymes, was more prominent in altering mortality compare to oxygenation, we can assume that confronting with oxidative stress is an important mechanism contributing to mortality. Perhaps other mechanisms in addition to antioxidant effects of NAC, are contributing to improve oxygenation in patients. Furthermore, the role of GST polymorphisms in pathogenesis and prognosis of different lung diseases have been investigated.[23–26] The GST P1 is the major isoform in the human lung, with the common polymorphism leading to replacement of the amino acid Val with Ile at codon 105. This substitution results in three possible genotypes: 105 Ile/Ile, 105 Ile/Val, or 105 Val/Val, and also changes the enzyme's active site and its substrate-specific catalytic activity.[21,23] These polymorphisms have been studied widely as a potential contributor in asthma, COPD and lung cancers, however, the data are still conflicting.[25–27] Our result indicated a significant increase in oxygenation in NAC-treated patients compared to control; however, no significant association between GST polymorphism and oxygenation was observed.

Based on these observations, one can assume that discrepancies in clinical studies can be explained by genetic variations in GST isoforms of studied population. As a result, our study indicates the importance of GST genotyping in predicting patient's clinical response to NAC treatment.

In conclusion our results indicate that treatment with NAC has a beneficial effect in ALI/ARDS patients by improving oxygenation and decreasing mortality rate. Furthermore, the genotyping of GST M1, P1 and T1 classes will have further impact on outcome of treatment, since NAC is required in patients with GST M1 null or GST M1 and T1 double null genotypes. Although this study proved major benefit of NAC treatment in ALI/ARDS and requirement of GST genotyping, further studies with larger patient sample size will provide better treatment protocols.

Conflict of interest statement

The authors declare that none of them have any financial or any other sort of conflict of interest in relation to this study that may influence their interpretation of the results.

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