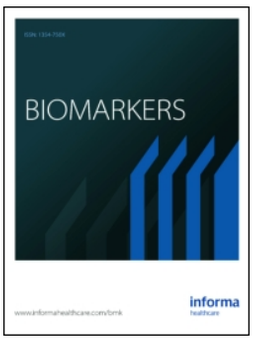

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RESEARCH ARTICLE

NMR spectroscopy-based metabolomic study of serum in sulfur mustard exposed patients with lung disease

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ABSTRACT

Sulfur mustard (SM) is a vesication chemical warfare agent for which there is currently no antidote. Despite years of research, there is no common consensus about the pathophysiological basis of chronic pulmonary disease caused by this chemical warfare agent. In this study, we combined chemometric techniques with nuclear magnetic resonance (NMR) spectroscopy to explore the metabolic profile of sera from SM-exposed patients. A total of 29 serum samples obtained from 17 SM-injured patients, and 12 healthy controls were analyzed by Random Forest. Increased concentrations of seven amino acids, glycerol, dimethylamine, ketone bodies, lactate, acetate, citrulline and creatine together with the decreased very low-density lipoproteins (VLDL) levels were observed in patients compared with control subjects. Our study reveals the metabolic profile of sera from SM-injured patients and indicates that NMR-based methods can distinguish these patients from healthy controls.

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Human serum; metabolomics; mustard gas; nuclear magnetic resonance

Introduction

Thousands of Iranians were exposed to sulfur mustard (2,2′-dichlorodiethyl sulfide, SM) during Iran–Iraq war (1983–1988). It has been estimated that hundreds of thousands Iranians were exposed to SM during the Iran-Iraq war, and lots of SM-affected individuals are suffering from current respiratory and eye drawbacks (Emad & Rezaian, 1997). Chronic bronchitis, bronchiectasis and lung fibrosis have been announced as late problems of SM exposure (Emad & Rezaian, 1997). According to newly published reports Bronchiolitis Obliterans (BO) should be considered as a major long-term sequel following SM exposure (Dompeling et al., 2004; Ghanei et al., 2004; Thomason et al., 2003).

SM-intoxicated patients show signs similar to coughing, wheezing and dyspnea. these patients usually suffer from several diseases of the respiratory tract, and it is therefore, not always facile to achieve the proper diagnosis. Until now, the criteria for the detection and management of the people who were exposed to SM have been based on clinical observations, lung function tests, high-resolution computed tomography (HRCT) (not accurate) and bronchoscopy (an invasive method) (Bagheri et al., 2003; Bijani & Moghadamnia, 2002; Freitag et al., 1991; Ghanei et al., 2008).

Metabolomics is the comprehensive assessment of endogenous metabolites (<1000 Da) from biofluids, tissues, exhaled breath condensate (EBC), and so on, being influenced by genetic modifications, pathological stimuli, or the environmental factors (Nicholson et al., 1999). Metabolomics has been proven to be an acceptable and reproducible platform technology, capable of capturing key molecular signatures and characteristics of diseases at different stages and progression (Dunn et al., 2005; Ng et al., 2012).

To date, there has not been a study using metabolomics approach to analyze veterans exposed to SM. In this study, we employed proton nuclear magnetic resonance (^{1}H-NMR) metabolomics method to analyze serum from SM-exposed patients more than 20 years after exposure, in order to recognize the patients, and improve understanding of disease mechanisms.

Materials and methods

Study population

A total of 29 people in the age range of 40–55 years were included in the study. All of the samples from male volunteer were selected. The patients were recruited from the outpatient clinic of the department of pulmonology of the Sasan Hospital (Tehran, Iran) from July 2012 to October 2013. The patients diagnosis was made on their existing documents in Iran–Iraq war (1983–1988). Exclusion criteria were pneumonia,

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acute bronchitis, asthma, chronic obstructive pulmonary disease (COPD) (due to smoking) and the other pulmonary diseases, cigarette smoking, diabetic and heart disease.

The extent of the tissue injury depends on the duration and intensity of the exposure (Renshaw, 1946). The degree of airflow obstruction of patients exposed to mustard gas was staged according to GOLD guidelines on the basis of FEV_1 and FEV_1/FVC (FEV_1: Forced expiratory volume in one second, FVC: Forced vital capacity) into five groups: normal, mild, moderate, severe, or very severe if FEV_1 was [80<, 80≤, 50–80, 30–49%, or <30%] and FEV_1/FVC [normal >70, the other groups <70%] of the predicted value, respectively (Rabe et al., 2007).

Seventeen very severe patients were recruited according to the percentage of FEV_1 and FEV_1/FVC. Selected patients ($n = 17$ males; mean age, 47.88 ± 7.79 years) and control group ($n = 12$ males; mean age, 47.84 ± 7.67 years) were matched for age and gender. Patients with very severe status at lung lesion had received inhaled corticosteroid and could not stop treatment, because of the severity of their disease.

Informed consent was obtained from all patients. The study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (SBMU).

Specimen of blood was drawn from every patient; the collected blood was immediately centrifuged. The serum samples were shipped on dry ice, stored in aliquots at −80 °C until use.

NMR data acquisition

All ^{1}H NMR spectra were recorded on a Bruker DRX500 MHz spectrometer (Karlsruhe, Germany) operating at 500.13 MHz, equipped with 5 mm high-quality NMR tubes (Sigma Aldrich, Johannesburg, RSA), with the temperature set at 298 K. The NMR lock signal was obtained by adding 10% D_2O (deuterium oxide, 99.9% D, Aldrich Chemicals Company, Döttingen, Switzerland). For serum samples, we used the Carr-Purcell-Meiboom-Gill (CPMG) spin-echo pulse sequence, $\pi/2\text{-}t_D\text{-}\pi\text{-}t_D$. The CPMG sequence reduces broad resonances from high molecular weight compounds and facilitates the observation of low molecular weight metabolites (Brown et al., 1977).

The acquisition parameters included spectral width 8389.26 Hz, time domain points 32 K, relaxation delay 2 s, number of scans 154, and acquisition time: 1.95 s, spectrum size 32 K and line broadening 0.3 Hz.

Data processing

All spectra were manually corrected for phase and baseline using the XWINNMR (version3.5, Bruker Spectrospin Ltd, Billerica, MA). All serum spectra were referenced to the methyl doublet of lactate (1.33 ppm) within XWINNMR (Fardet et al., 2007; Tang et al., 2004). Each NMR spectrum was segmented into 408 regions of equal width (typically 0.02 ppm) using the ProMetab software (version prometab_ v3_3) (Viant, 2003) in MATLAB (version 6.5.1, The MathWorks, Cambridge, UK). The region from δ4.2 to δ5.5, which contained the residual water signal, was excluded.

Before pattern recognition analysis, each integral region is normalized. The data obtained was mean centered, and then processed with Random Forest (RF).

Statistical analysis

Random Forest is a supervised learning algorithm suitable for high-dimensional data analysis. It fits many classification trees to a data set, and class prediction is made by majority vote of trees. To determine the correct prediction of the Performance of the RF algorithm, we performs a type of cross-validation by using of out-of-bag (OOB) samples. On average, each tree is grown using about second-third of the training data, leaving about one-third of the instances as OOB (OOB is as test sample). Because the OOB observations were not used in the fitting of the trees, the OOB estimates are cross-validated accuracy estimates, and represent an unbiased estimation of the classification error (Breiman, 2001). Variable importance is evaluated by measuring the increase of the OOB error when it is permuted.

In all cases, a forest of 500 trees was grown. RF analysis was performed using the RF package in MATLAB for statistical computing, similar to our previous studies (Fathi et al., 2013, 2014; Mehrpour et al., 2013). This package is a readily accessible implementation of the RF algorithm and can be downloaded from the website (http://code.google.com/p/randomforest-matlab/downloads/list).

Metabolite set enrichment analysis

To identify the most significantly affected metabolic pathways, a set of significantly altered metabolites was used as the input for the Metabolite Set Enrichment Analysis (MSEA). The MSEA was performed with a freely accessible web-based program (www.metaboanalyst.ca) (Xia et al., 2009, 2012). After normalization, Over Representation Analysis (ORA) was used for comprehensive screening of affected pathways. *p* Values and False Discovery Rates (FDR) are reported.

Results

Patient characteristics

The characteristics of the study subjects are shown in Table 1. Forced expiratory volume in 1 s (FEV_1) and FVC were reduced in patients compared with healthy subjects.

Serum spectral discrimination between healthy subjects and patients

The 0.7–9 ppm region of serum samples was used to investigate the metabolites characterizing serum. The region from 0.9 to 4.2 ppm contained distinguished metabolites between patients and healthy subjects. Figure 1 depicts representative spectra of SM-exposed patients. Supervised RF analysis correctly identified 15 (88%) of 17 patients exposed to SM, and 10 (83%) of 12 controls. The diagnostic accuracy for the training and test sets were 91% and 80%, respectively.

The metabolite resonances were identified according to assignments published in the literature (Nicholson et al., 1995) and on-line databases (http://hmdb.ca). It was found that metabolites related to amino acid metabolism (L-glutamine, L-glutamate, L-asparagine, L-lysine, L-glycine, L-proline, and 4-hydroxyproline), lipid metabolism (glycerol), keton bodies (3-hydroxybutyrate and acetoacetate), two organic acids (lactate and acetate), urea pathway (citrulline), dimethylamine, and creatine pathway (creatine) were increased in patients whereas, decreased levels of low-density lipoproteins (VLDL) were shown in patients as compared to healthy control (Table 2).

Receiver operating characteristic (ROC) curve is the most common metric used to explain the performance of medical diagnostic tests for discriminant problems (Obuchowski et al., 2004; Pepe et al., 2001; Zweig & Campbell, 1993) and is recommended for metabolomic biomarker discovery studies (Xia et al., 2013). Based on the predicted response values, ROC curve was plotted, and its corresponding area under the curve (AUC) value was calculated for the training and test sets (Figure 2 (a,b)). AUC value was 0.9 for both of them, suggesting that the NMR-based fingerprints have the potential to be used to distinguish SM-exposed patients and healthy controls.

A confusion matrix is designed to include the real and predicted classifications performed by a classification model. The confusion matrix of the RF classification model for the training and test sets and other classification parameters are shown in Tables 3 and 4.

Table 1. Demographic and pulmonary function test of very severe statues at lung lesion (because of SM) and control participants.

Characteristics	Control group	Very severe group
Number	12	17
Age (year)	47.84 ± 7.67	47.88 ± 7.79
Height (cm)	174.26 ± 4.8	171.35 ± 6.8
Weight (kg)	78.84 ± 9.29	74.23 ± 15.86
BMI (kg/m^2)	25.96 ± 2.9	25.29 ± 5.42
FEV_1 (% predicted)	95.84 ± 8.3	28.26 ± 10.2[a]
FVC (%)	89.26 ± 8.1	40.46 ± 15.26[a]
FEV_1/FVC (%)	85.68 ± 3.44	59.73 ± 16.17[a]

Values are presented as mean ± SD. BMI: body mass index; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity. Compared with the control group: [a]$p < 0.001$.

Metabolite set enrichment analysis

MSEA was used to test metabolic pathways enrichment in each group. MSEA indicated that protein biosynthesis and ammonia recycling, as well as arginine and proline metabolism, urea cycle, glutathione metabolism, ketone body metabolism, aspartate metabolism, glutamate metabolism, and pyruvate metabolism pathways are significantly associated with SM-intoxicated patients (Figure 3).

Discussion

Non-targeted metabolomics is well suited for biomarker discovery, allowing investigation of profile all detectable metabolites in a single experiment. The potential discovery of biomarker panels is facilitated by the investigation of multiple markers; also it can describe systemic effects of disease. We have applied metabolomics to the analysis of serum samples from SM-exposed patients to assist in the drive to

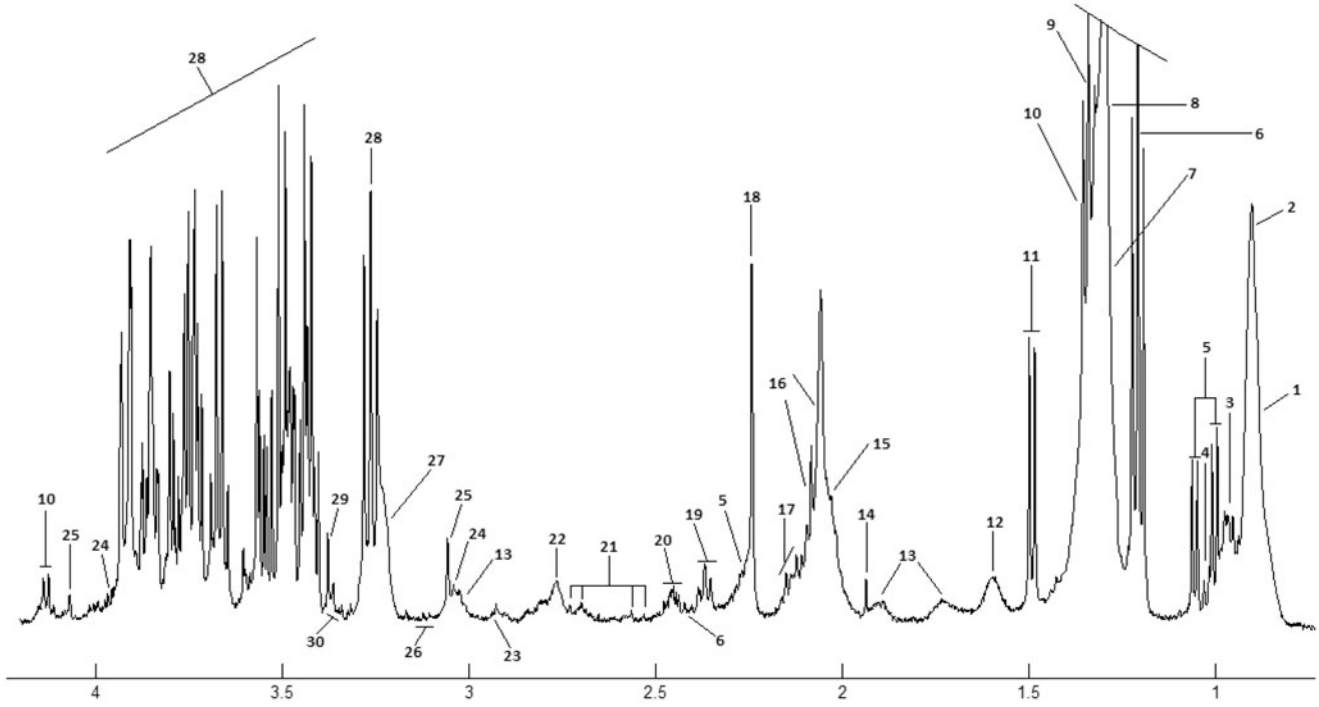

Figure 1. Representative 500 MHz one-dimensional Carr–Purcell–Meiboom–Gill (1D-CPMG) 1H-nuclear magnetic resonance (NMR) spectrum of SM-exposed patients, serum samples measured at 298 K. The following metabolites are identified: 1, Lipid: LDL CH3–(CH_2)n; 2, Lipid: VLDLCH3–(CH_2)n; 3, Leucine; 4, Isoleucine; 5, Valine; 6, 3-Hydroxybutyrate; 7, Lipid: LDL CH3–(CH_2)n; 8, Lipid: VLDL (CH_2)n-CO; 9, Threonine; 10, Lactate; 11, Alanine; 12, Lipid: VLDL CH_2–CH_2–CO; 13, Lysine; 14, Acetate; 15, Lipid: CH_2–CH=CH; 16, N-acetylated glycoproteins; 17, Glutamate + Glutamine; 18, Acetoacetate; 19, Glutamate; 20, Glutamine; 21, Citrate; 22, Lipid: C=CCH_2C=C; 23, Asparagine; 24, Creatine; 25, Creatinine; 26, Citrulline; 27, Choline; 28, α & β-Glucose; 29, Hydroxyproline; 30, Proline.

Table 2. List of serum metabolites with larger contribution for the discrimination between patients and control, identified by analysis of random forest.

Number	Metabolite	δ ^{1}H (p.p.m.)[a]	Assignment	Multiplicity[b]	*p* Value[c]	Direction of variation[d]
1	3-Hydroxybutyrate	1.2, 2.40	γ-CH_3, half α-CH_2	d, m	<0.001	↑
2	VLDL	1.30	$CH_2CH_2CH_2CO$	m	0.001>	↓
3	Lactate	1.33, 4.11	CH_3, CH	d, q	<0.05	↑
4	Lysine	1.91	β-CH_2	m	<0.001	↑
5	Acetate	1.94	CH_3	s	<0.05	↑
6	Acetoacetate	2.23	CH_3	s	<0.05	↑
7	Glutamate	2.35	half γ-CH_2	m	<0.05	↑
8	Glutamine	2.42	half γ-CH_2	m	<0.05	↑
9	Dimethylamine	2.71	CH_3	s	<0.05	↑
10	Asparagine	2.94	half β-CH_2	dd	<0.05	↑
11	Creatine	3.04, 3.93	CH_3, CH_2	s, s	0.001	↑
12	Citrulline	3.13	γ-CH_2	t	<0.05	↑
13	Proline	3.34	half δ-CH_2	m	<0.05	↑
14	4-Hydroxyproline	3.37	γ-CH	ddd	0.001	↑
15	Glycine	3.54	CH_2	s	<0.001	↑
16	Glycerol	3.56, 3.63	half CH_2, half CH_2	dd, dd	<0.001	↑

[a]Chemical shift of signal used for quantification.
[b]Multiplicity: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; VLDL, very low-density lipoproteins.
[c]*p* Value calculated by independent *t*-test.
[d]Increased or decreased in patients compared to control serum.

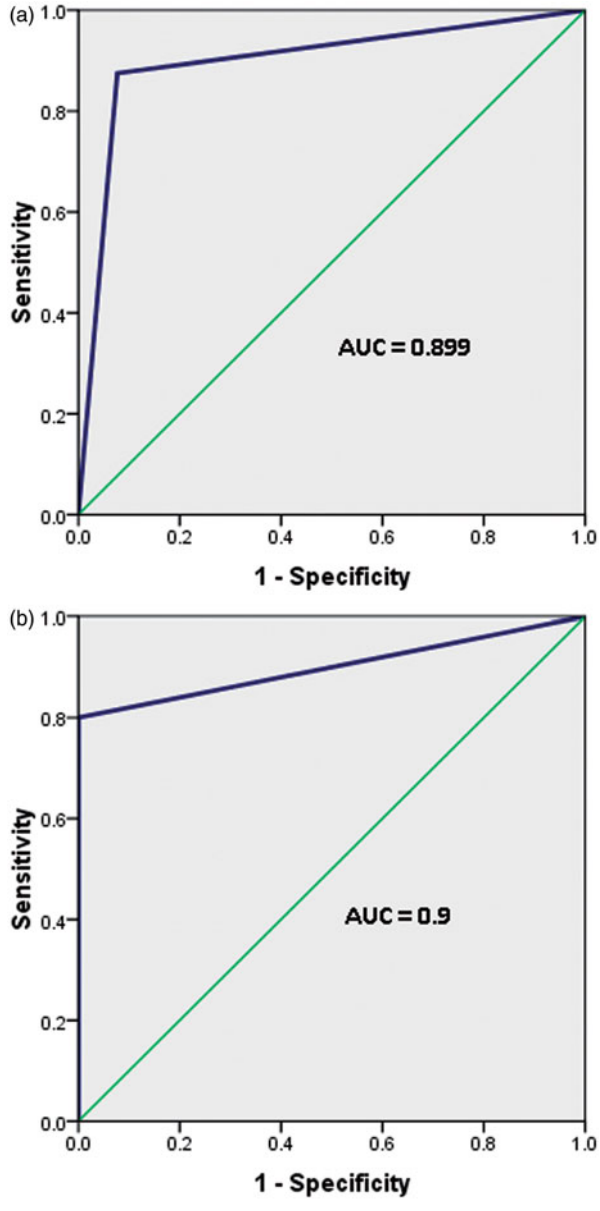

Figure 2. Receiver operating characteristic (ROC) curve for (a) train and (b) test. Areas under the ROC curve are 0.899 and 0.9 for train and test, respectively.

Table 3. Confusion matrix for training and test set.

	Observed	Very severe class	Predicted Control class
Training set	Very severe class	12	1
	Control class	1	7
Test set	Very severe class	3	1
	Control class	1	3

Table 4. The calculated error and non-error rates of the classification index and the classification performances of training and test sets.

	Error rate	Non-error rate	Specificity	Sensitivity	Accuracy
Training set	0.09	0.91	0.87	0.92	0.91
Test set	0.2	0.8	0.75	0.75	0.8

discover biomarkers that may reflect diagnosis of patients more predictively than HRCT and less invasive than bronchoscopy, which are currently used.

To the best of our knowledge, this study is the first to demonstrate differences between SM-injured patients and healthy subjects using NMR-based metabolomic fingerprints of human serum.

In this study, 16 metabolites were altered in SM-exposed patients, as compared with those in healthy controls (Table 2). The major group of altered endogenous metabolites in the serum of SM-intoxicated patients contained products of glycolysis, amino acids, ketone bodies and molecules related to lipid catabolism. As shown in Figure 3, the metabolic processes found to be most significantly altered between SM-injured patients and healthy controls were protein biosynthesis, followed by ammonia recycling, arginine and proline metabolism, urea cycle, glutathione metabolism, ketone body metabolism, aspartate metabolism, glutamate metabolism and pyruvate metabolism.

Lactate is the end product of anaerobic glycolysis. Levels of lactate were elevated in the sera of SM-injured patients, consistent with increased anaerobic glycolysis. Anaerobic glycolysis increases as a result of tissue hypoxia, lung injury, and ischemia (De Backer, 2003). Moreover, more pyruvate was converted into lactate rather than entering into the TCA cycle pathway, presumably due to lack of sufficient oxygen supply.

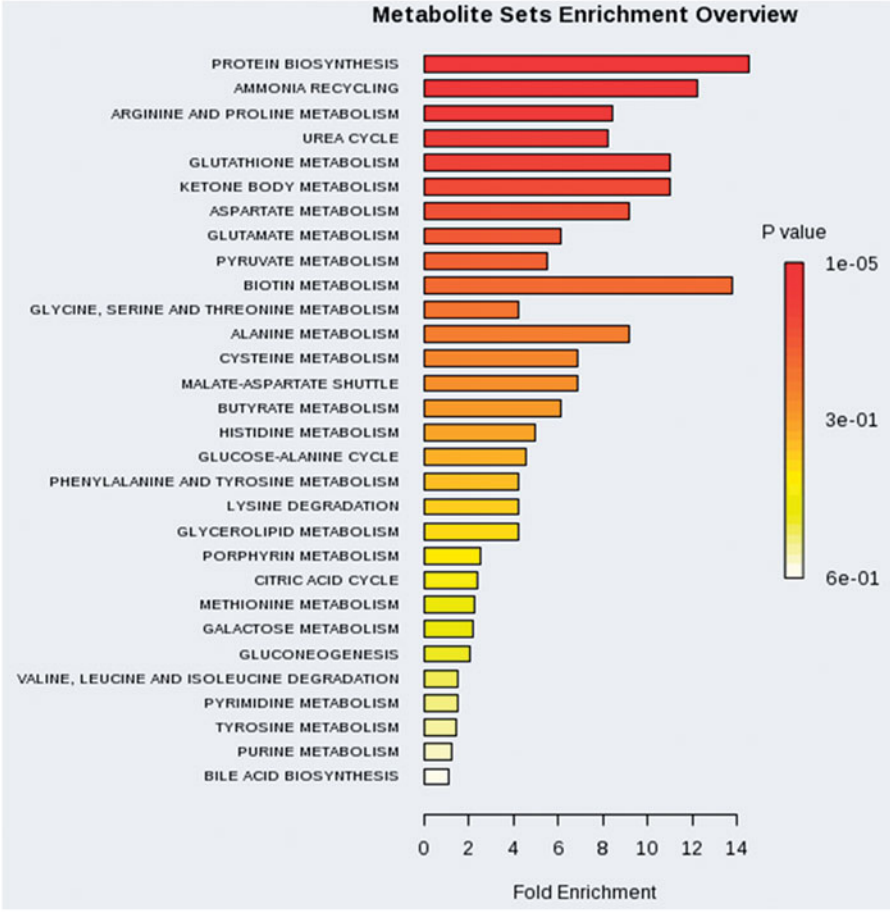

Figure 3. Results of the metabolite set enrichment analysis (MSEA) for SM-exposed patients. Differences in metabolic pathways noted between SM-intoxicated patients and healthy controls are shown in this map. The horizontal bar graph summarizes the most significant metabolite sets identified during the analysis. The most significant differences include protein biosynthesis (p value = 9.61E-6, FDR = 7.69E-4), ammonia recycling (p value = 1.87E-4, FDR = 0.00749), and arginine and proline metabolism (p value = 8.39E-4, FDR = 0.0224). FDR: false discovery rate.

It has been reported that exposure to hypoxia increases serum concentration of interleukin-6 (IL-6) and also elevates IL-1 and tumor necrosis factor-α (TNF-α) production by human blood mononuclear cells (Ghezzi et al., 1991; Klausen et al., 1997). Furthermore, existing studies on the short-term effects of SM showed increased levels of IL-1, IL-6, and TNF-α in human skin cells exposed to SM (Arroyo et al., 1999, 2001) and studies on the long-term effects of this chemical warfare agent demonstrated elevated serum levels of IL-6 and highly sensitive C-reactive protein (hsCRP) in veterans exposed to SM (nearly 20 years ago) compared to control group (Attaran et al., 2010). Beside, it is shown that the inflammation is associated with the induction of oxidative stress. For instance, some pro-inflammation cytokines such as IL-6 and TNF-α induce oxidative stress (OS) and reactive oxygen species (ROS) production (Dong et al., 2007; Wassmann et al., 2004). These findings are in agreement with our previous work that demonstrated exposure to SM may increase OS (Alamdari et al., 2015; Nobakht et al., 2015).

Our data showed an increase in seven amino acids, including glutamate, glutamine, lysine, asparagine, proline, 4-hydroxyproline and glycine in sera from SM-exposed patients relative to those of healthy controls, suggesting alterations in protein metabolism in very severe patients after more than 20 years SM exposure.

The levels of glycine, lysine, glutamate, proline and 4-hydroxyproline were significantly increased in the serum of SM- exposed patients compared to control group. Since glycine, lysine, glutamate, proline and 4-hydroxyproline are collagen metabolites (Keshari et al., 2005; Schiller et al., 2001), increased levels of these amino acids are associated with collagen breakdown. This result is consistent with previous studies that reported exposure to SM clearly increases the rate of osteopenia and osteoporosis and also causes the corneal collagen degradation (Agin et al., 2004; Naderi et al., 2010). It is well known that loss of bone collagen occurs in osteoporosis (Shuster, 2005). Agin and et al. evaluated the rate of osteoporosis in asthmatic patients compared to SM-induced patients who were receiving corticosteroids as the treatment for chronic pulmonary complications and observed that SM clearly increases the rate of osteoporosis (Agin et al., 2004). Therefore, osteoporosis and collagen degradation in SM-injury patients aren't because of corticosteroids usage. Note of, collagens of the various types constitute about 15% of the dry weight of human lung tissue and are the major protein group. Types I and III are the most abundant of the lung collagens, co-distributing in airways and blood vessels as well as in the interstitium (Laurent, 1986). It suggests that lung collagens change in parallel pattern with other tissues and it can be one of the causes of lung lesion in these patients.

However, this hypothesis is inconsistent with Ghanei results that reported increases in submucosal collagen in lung tissue from 15 SM-injury patients, severe (6 cases) and mild (9 cases) exposed to SM (Ghanei et al., 2008). Therefore, more comprehensive studies need to clarify lung collagens.

The levels of VLDL were detected to be decreased in serum of patients compared to healthy controls. It might be related to increased lipid metabolism in patients. Furtheremore, we found that acetoacetate and 3-hydroxybutyrate were increased in SM-injury patient sera. 3-Hydroxybutyric acid and acetoacetate constitute ketone bodies, which are important byproducts of fatty acid oxidation in the liver and serve as a major source of energy in the heart and brain (Wolfe et al., 1982). In this study the metabolic profile of increased ketone bodies and decreased VLDL is consistent with enhanced lipid degradation in SM-exposed patients relative to healthy controls. Also in current study the significant difference for SM-intoxicated patients was related to glycerol, suggesting lipid degradation or/and cell membrane rearrangement.

SM-exposed patients and patients with COPD have similar pulmonary indications. Interestingly, in our study, hallmarks of patients exposed to SM, collagen metabolites (increased 4-hydroxyproline, proline, glycine, glutamine and glutamate) and ketone body (increased levels of acetoacetate and 3-hydroxybutyrate), were significantly altered in SM-exposed patients. In contrast, in COPD patients (due to smoking) increased levels of methylhistidine (a muscle protein) and reduced levels of branched-chain amino acids (BCAAs) as well as 4-hydroxyproline and proline (COPD patients GOLD IV) in serum were shown (Nobakht et al., 2014; Ubhi et al., 2012). It suggests that metabolomic-based diagnostics not only can distinguish patients with SM compared with healthy controls, but also can recognize these patients in comparison with other pulmonary diseases with similar clinical symptoms such as COPD.

Conclusion

The current study represents the first investigation of serum metabolic changes in SM-induced patients using NMR. As a result, 16 significantly changed metabolites were identified in patients compared to controls. It was found that metabolites related to lipid metabolism and collagen metabolism were significantly increased. Furthermore, the levels of lactate elevated in patients compared to control group. Lactate is indicative of tissue hypoxia that can lead to inflammation and OS. In brief, our results demonstrated that a combination of NMR spectroscopy and chemometric techniques offers a useful tool for the identification of markers of SM-injury patients and discriminates these patients from other pulmonary diseases with similar symptoms.

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Disclosure statement

The authors report no declarations of interest. The authors alone are responsible for the content and writing of this article.

References

Agin K, Rajaee A, Mehrabi M, et al. (2004). Osteoporosis among asthmatic patients exposed to mustard gas compared with non-exposed asthmatics. Tanaffos 3:7–11.

Alamdari DH, Gh BFNM, Oskouie AA, et al. (2015). Prooxidant-antioxidant balance in mustard airway disease with different severity. Eur Respir J 46:PA3915.

Arroyo C, Schafer R, Kurt E, et al. (1999). Response of normal human keratinocytes to sulfur mustard (HD): cytokine release using a non-enzymatic detachment procedure. Hum Exp Toxicol 18:1–11.

Arroyo CM, Broomfield CA, Hackley BE. (2001). The role of interleukin-6 (IL-6) in human sulfur mustard (HD) toxicology. Int J Toxicol 20:281–96.

Attaran D, Lari SM, Towhidi M, et al. (2010). Interleukin-6 and airflow limitation in chemical warfare patients with chronic obstructive pulmonary disease. Int J Chronic Obstruct Pulmonary Dis 5:335–40.

Bagheri M, Hosseini S, Mostafavi S, Alavi S. (2003). High-resolution CT in chronic pulmonary changes after mustard gas exposure. Acta Radiologica 44:241–5.

Bijani K, Moghadamnia A. (2002). Long-term effects of chemical weapons on respiratory tract in Iraq–Iran war victims living in Babol (North of Iran). Ecotoxicol Environ Safety 53:422–4.

Breiman L. (2001). Random forests. Machine Learn 45:5–32.

Brown FF, Campbell ID, Kuchel PW, Rabenstein DC. (1977). Human erythrocyte metabolism studies by 1H spin echo NMR. FEBS Lett 82:12–16.

DE Backer D. (2003). Lactic acidosis. Intensive Care Med 29:699–702.

Dompeling E, JöBSIS Q, Vandevijver N, et al. (2004). Chronic bronchiolitis in a 5-yr-old child after exposure to sulphur mustard gas. Eur Respir J 23:343–6.

Dong R, Wang Q, He X, et al. (2007). Role of nuclear factor kappa B and reactive oxygen species in the tumor necrosis factor-a-induced epithelial-mesenchymal transition of MCF-7 cells. Brazil J Med Biol Res 40:1071–8.

Dunn WB, Bailey NJ, Johnson HE. (2005). Measuring the metabolome: current analytical technologies. Analyst 130:606–25.

Emad A, Rezaian GR. (1997). The diversity of the effects of sulfur mustard gas inhalation on respiratory system 10 years after a single, heavy exposure analysis of 197 cases. Chest J 112:734–8.

Fardet A, Canlet C, Gottardi G, et al. (2007). Whole-grain and refined wheat flours show distinct metabolic profiles in rats as assessed by a 1H NMR-based metabonomic approach. J Nutrit 137:923–9.

Fathi F, Kyani A, Darvizeh F, et al. (2013). Relationship between serum level of selenium and metabolites using 1HNMR-based metabonomics in Parkinson's disease. Appl Mag Reson 44:721–34.

Fathi F, Majari-Kasmaee L, Mani-Varnosfaderani A, et al. (2014). 1H NMR based metabolic profiling in Crohn's disease by random forest methodology. Magn Reson Chem 52:370–6.

Freitag L, Firusian N, Stamatis G, Greschuchna D. (1991). The role of bronchoscopy in pulmonary complications due to mustard gas inhalation. Chest J 100:1436–41.

Ghanei M, Mokhtari M, Mohammad MM, Aslani J. (2004). Bronchiolitis obliterans following exposure to sulfur mustard: chest high resolution computed tomography. Eur J Radiol 52:164–9.

Ghanei M, Tazelaar HD, Chilosi M, et al. (2008). An international collaborative pathologic study of surgical lung biopsies from mustard gas-exposed patients. Respirat Med 102:825–30.

Ghezzi P, Dinarello CA, Bianchi M, et al. (1991). Hypoxia increases production of interleukin-1 and tumor necrosis factor by human mononuclear cells. Cytokine 3:189–94.

Keshari KR, Zektzer AS, Swanson MG, et al. (2005). Characterization of intervertebral disc degeneration by high-resolution magic angle spinning (HR-MAS) spectroscopy. Magn Reson Med 53:519–27.

Klausen T, Olsen NV, Poulsen TD, et al. (1997). Hypoxemia increases serum interleukin-6 in humans. Eur J Appl Physiol Occup Physiol 76:480–2.

Laurent G. (1986). Lung collagen: more than scaffolding. Thorax 41:418–28.

Mehrpour M, Kyani A, Tafazzoli M, et al. (2013). A metabonomics investigation of multiple sclerosis by nuclear magnetic resonance. Magn Reson Chem 51:102–9.

Naderi M, Jadidi K, Falahati F, Alavi SA. (2010). The effect of sulfur mustard and nitrogen mustard on corneal collagen degradation induced by the enzyme collagenase. Cutan Ocular Toxicol 29:234–40.

Ng D, Salim A, Liu Y, et al. (2012). A metabolomic study of low estimated GFR in non-proteinuric type 2 diabetes mellitus. Diabetologia 55:499–508.

Nicholson JK, Foxall PJ, Spraul M, et al. (1995). 750 MHz 1H and 1H-13C NMR spectroscopy of human blood plasma. Anal Chem 67:793–811.

Nicholson JK, Lindon JC, Holmes E. (1999). 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 29:1181–9.

Nobakht M, Gh BF, Aliannejad R, et al. (2014). The metabolomics of airway diseases, including COPD, asthma and cystic fibrosis. Biomarkers 20:5–16.

Nobakht M, Gh BF, Oskouie AA, et al. (2015). Pro-oxidant–antioxidant balance in Iranian veterans with sulfur mustard toxicity and different levels of pulmonary disorders. Drug Chem Toxicol. [Epub ahead of print]. DOI: 10.3109/01480545.2015.1122033.

Obuchowski NA, Lieber ML, Wians FH. (2004). ROC curves in clinical chemistry: uses, misuses, and possible solutions. Clin Chem 50:1118–25.

Pepe MS, Etzioni R, Feng Z, et al. (2001). Phases of biomarker development for early detection of cancer. J Natl Cancer Inst 93:1054–61.

Rabe KF, Hurd S, Anzueto A, et al. (2007). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Critic Care Med 176:532–55.

Renshaw B. (1946). Mechanisms in production of cutaneous injuries by sulfur and nitrogen mustards. Chem Warfare Agents Related Chem Probl. 1:479–520.

Schiller J, Naji L, Huster D, et al. (2001). 1H and13C HR-MAS NMR investigations on native and enzymatically digested bovine nasal cartilage. Magn Reson Mater Phys Biol Med 13:19–27.

Shuster S. (2005). Osteoporosis, a unitary hypothesis of collagen loss in skin and bone. Med Hypothes 65:426–32.

Tang H, Wang Y, Nicholson JK, Lindon JC. (2004). Use of relaxation-edited one-dimensional and two dimensional nuclear magnetic resonance spectroscopy to improve detection of small metabolites in blood plasma. Anal Biochem 325:260–72.

Thomason JW, Rice TW, Milstone AP. (2003). Bronchiolitis obliterans in a survivor of a chemical weapons attack. JAMA 290:598–9.

Ubhi BK, Cheng KK, Dong J, et al. (2012). Targeted metabolomics identifies perturbations in amino acid metabolism that sub-classify patients with COPD. Mol BioSyst 8:3125–33.

Viant MR. (2003). Improved methods for the acquisition and interpretation of NMR metabolomic data. Biochem Biophys Res Commun 310:943–8.

Wassmann S, Stumpf M, Strehlow K, et al. (2004). Interleukin-6 induces oxidative stress and endothelial dysfunction by overexpression of the angiotensin II type 1 receptor. Circulation Res 94:534–41.

Wolfe R, Shaw J, Durkot M. (1982). Energy metabolism in trauma and sepsis: the role of fat. Progr Clin Biol Res 111:89–109.

Xia J, Broadhurst DI, Wilson M, Wishart DS. (2013). Translational biomarker discovery in clinical metabolomics: an introductory tutorial. Metabolomics 9:280–99.

Xia J, Mandal R, Sinelnikov IV, et al. (2012). MetaboAnalyst 2.0-a comprehensive server for metabolomic data analysis. Nucleic Acids Res 40:W127–33.

Xia J, Psychogios N, Young N, Wishart DS. (2009). MetaboAnalyst: a web server for metabolomic data analysis and interpretation. Nucleic Acids Res 37:W652–60.

Zweig MH, Campbell G. (1993). Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 39:561–77.