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# Four sulfur mustard exposure cases: Overall analysis of four types of biomarkers in clinical samples provides positive implication for early diagnosis and treatment monitoring

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ABSTRACT

In one event, Chinese male individuals accidentally exposed to unknown chemicals and emerged erythema or blisters on contacted organism derma, then hospitalized. To identify the causative agents, blood, urine and exudate samples were collected from the patients during the therapeutic course. Five established liquid chromatography–mass spectrometry (LC–MS) and gas chromatography (GC)–MS methods were employed to analyze the samples. Here, an overall analysis of four types of sulfur mustard biomarkers, including the hydrolysis/oxidation products, β-lyase metabolites, DNA adducts and hemoglobin adducts, was conducted toward the samples from exposed individuals. The results of all the four types of biomarkers in different biomedical matrices showed high relevance, and verified that this exposure is indeed originated from sulfur mustard. The concentrations of the biomarkers in specimens revealed a good correlation with the severity of the patient's symptom. The concentration-time profile demonstrated that most of the biomarkers quickly achieved maximum at the beginning of the course, and then decreased and kept a detectable level until the 7th day after exposure. The DNA adducts in urine samples still appeared on the 30th day, and the N-terminal valine adducts in hemoglobin could be monitored for over 90 days, which was meaningful for the concurrent study of clinical samples. To the best of our knowledge, this work provides the total analysis and profile of four categories of biomarkers in human specimens for the first time, and the good accordance between concentration and level of burns, between time course and biomarkers will be of great importance for early diagnosis and medical treatment monitoring of sulfur mustard exposure.


## 1. Introduction

Sulfur mustard (SM, U.S. Code HD, chemical name 2,2′-dichloroethyl sulfide) is a powerful vesicant and a biological alkylating agent, which can function *via* inhalational, cutaneous and ocular routes of exposure [1,2]. It has

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been employed as a chemical warfare agent since World War I, and has a continued strike in more recent Middle Eastern conflicts [3]. SM was also a predominant agent found in the chemical weapons abandoned in China by the Japanese imperialist army after World War II. The old shells or containers of SM are still a large threat to civilian health. To date, accidental human injuries by SM occurred from a variety of situations have been reported [4–8]. Because of its ease of preparation, SM also poses a potential threat to public safety if abused by terrorists.

As a bifunctional alkylating agent, SM reacts rapidly with numerous nucleophiles, *i.e.*, water, glutathione, DNA and proteins under physiological conditions *via* the intermediate episulfonium ion (Fig. 1). The corresponding four major metabolic routes have been identified in animal models (*e.g.*, rat and mouse) [9–13]. The first pathway involves the direct oxidation product of SM, bis-β-chloroethyl sulfoxide (SMO), the directly hydrolyzed metabolite thiodiglycol (TDG), and its oxidation product thiodiglycol sulfoxide (TDGO). The second pathway involves a reaction with abundant glutathione and then undergoing oxidation to the sulfone followed by β-lyase cleavage, leading to the formation of 1,1′-sulfonylbis[2-S-(N-acetylcysteinyl) ethane] (SBSNAE), 1,1′-sulfonylbis[2-(methylthio) ethane] (SBMTE), 1-methylsulfinyl-2-[2-(methylthio)ethylsulfonyl]ethane (MSMTESE) and 1,1′-sulfonylbis [2-(methylsulfinyl) ethane] (SBMSE). The third way is the reaction on the critical nucleophilic sites in DNA to produce SM-DNA adducts. The major sites of DNA alkylation by SM include $N^7$, $O^6$ positions of guanine, $N^3$ position of adenine, and interstrand or intrastrand crosslinks at the $N^7$ position of guanine, and adducts of $N^7$-[2-[(2-hydroxyethyl)thio]ethyl]-guanine ($N^7$-HETEG), $O^6$-[2-[(2-hydroxyethyl)thio]ethyl]-guanine ($O^6$-HETEG), $N^3$-[2-[(2-hydroxyethyl)thio]ethyl]-adenine ($N^3$-HETEA), and bis[2-(guanin-7-yl)ethyl] sulfide (Bis-G) are correspondingly formed. The last pathway involves the reaction with various amino acid residues present in proteins, among which the HETE-valine (HETE-Val) adduct of hemoglobin and HETE-cysteine adduct of albumin are of the most attention now.

The biological fate of SM has been fully investigated in rats, while a complete understanding of SM in humans is lacking. This is due that, biological samples of alleged victims available for analysis are rare or difficult to be achieved, and new trace analytical methods to detect SM attached to long-lived proteins, DNAs, and other biomarkers are only vast improved in the last decade by virtue of booming chromatography–mass spectrometry technique. Basically, now researchers have the feasible tools to access the archived or freshly collected specimens, typical blood or urine samples, and find evidence after SM attack or accident.

For two subjects between 2 and 3 days after an accidentally cutaneous exposure to SM, free metabolites have been studied in the urine samples, the glutathione derived β-lyase metabolites SBMSE, MSMTESE, and hydrolysis products TDG, TDGO were identified in both individuals [14]. The β-lyase metabolites of SM in the urine of casualties are proposed as unequivocally diagnostic indicators of SM poisoning in human [15]. With regard to archived blood samples of four hospitalized Iranian casualties 5–10 days after exposure and one individual 2 days after an accidental exposure to SM, SM-alkylated hemoglobin adducts including HETE-histidine and HETE-Val adducts have been found for SM exposure [16]. In another case, archived blood samples of two hospitalized Iranians 22 and 26 days post a suspected SM exposure were tested positive for both the HETE-Val adduct and $N^7$-HETEG [17]. For nine Iranian individuals exposed to SM, the archived blood specimens were tested positive for SM-alkylated albumin [18] and HETE-Val adduct of SM-hemoglobin [19]. In a most recent report, for two individuals 2–42 days after exposure, positive results of biomedical samples were obtained for SM poisoning. Notably, only in this case, two different biological matrices from an individual were used to mutually verify the SM exposure, in which the free metabolites of SM in urine and SM-albumin adduct in blood were tested positive [15,20].

All above-mentioned reports indicated some similarity in metabolism of SM in human and rat. Generally, hydrolyzed/oxidation metabolites and β-lyase cleavage products formed in relatively high concentrations in early samples and persist only for several days due to the relatively rapid elimination, while DNA- and protein adducts existed for a relatively long time and thus offer valuable evidence for verification of SM exposure even weeks to months later. However, these researches only investigated one or up to two types of biomarkers in the clinic specimens, which cannot directly and comprehensively demonstrate the biological fate of SM in humans.

To address this issue, this paper provides a total analysis of four types of SM biomarkers in three different biomedical matrices collected from individuals who exposed to a vesicating agent in one accident. To identify the causative agent, blood, urine and blister exudate fluid samples collected from four representative cases with different extent of exposure symptoms were detected. A series of liquid chromatography–mass spectrometry (LC–MS) and gas chromatography (GC)–MS methods previously established and validated in our laboratory [13,21–27] were employed to measure and profile the hydrolysis/oxidation products, β-lyase metabolites, DNA adducts and hemoglobin adducts in the samples. The correlation between concentration of biomarkers and level of burns were investigated. The detection windows of the four types of biomarkers were suggested, and thus a choice of suitable biomarker in certain biomedical samples were promoted. To the best of our knowledge, this is the first and detailed paper of four kinds of SM biomarkers in a typical exposure event in worldwide, it will be of great importance for early clinical diagnosis and medical treatment monitoring of SM exposure.

## 2. Materials and methods

### 2.1. Caution

SM is a highly reactive alkylation vesicant and cytotoxic agent. Handling of this agent should be carried out in the well-ventilated fume hood. The use of gloves and stringent protective measures must be adopted.