Proteomics analysis of chronic skin injuries caused by mustard gas

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Abstract

Sulfur mustard (SM) is an alkylation and forming chemical that was widely used by Iraqi forces during the Iran–Iraq wars. One of the target organs of SM is the skin. Understanding the mechanisms involved in the pathogenesis of SM may help better identify complications and find appropriate treatments. The current study collected ten SM-exposed patients with long-term skin complications and ten healthy individuals. Proteomics experiments were performed using the high-efficiency TMT10X method to evaluate the skin protein profile, and statistical bioinformatics methods were used to identify the differentially expressed proteins. One hundred twenty-nine proteins had different expressions between the two groups. Of these 129 proteins, 94 proteins had increased expression in veterans’ skins, while the remaining 35 had decreased expression. The hub genes included RPS15, ACTN1, FLNA, HP, SDHC, and RPL29, and three modules were extracted from the PPI network analysis. Skin SM exposure can lead to oxidative stress, inflammation, apoptosis, and cell proliferation.

Keywords: Proteomics analysis, Mustard gas, Chemical injuries, Systems biology, Chemical agents

Introduction

Sulfur mustard (SM) could be a chemical operations agent that causes blisters on the skin and mucous membranes [1]. Quite 90% of patients exposed to dichloroethyl sulfide showed various skin lesions within the damaged areas. Skin may absorb about 20% of this blistering agent during exposure. About 70% of chemicals can accumulate within the epidermis and the rest within the basement membrane and dermis [2]. SM appears to wreck the skin by disrupting cell proliferation [3]. The Skin symptoms of SM exposure include itching, burning, painless erythema or sunburn, hypopigmentation, hyperpigmentation, and melanoma in exposed and unexposed areas [4, 5]. The mechanism of SM skin symptoms is not fully understood. Acute skin symptoms caused by SM can emerge as itching, erythema, blistering and burning sensation reckoning on the dose and duration of exposure [5, 6]. 2 to 24 h after exposure to SM, only a tiny low number of basal cells show nuclear changes like chromatin loss. Hydropic degeneration of the cytoplasm, and liquefactive necrosis of the epithelium has been reported in acute exposure [7]. The blisters usually appear as small vesicles after about 17 h of exposure to SM, but usually heal within three weeks [8]. In mild cases, the skin lesions could also be limited to erythema that darkens in about 15 days, while the superficial layers of the epidermis become scaly without causing an actual skin defect [9]. Histological studies of skin exposed to SM show vaso-dilation and neutrophil leakage, indicating the assembly of vasoactive mediators and chemical adsorbsents within the damaged areas [10]. Interleukins IL-1α/β, IL-6, IL-8, and TNF-α are released shortly after exposure to SM [11]. The NF-κB pathway and mitogen-activated protein kinases are significantly involved in the regulation of genes encoding inflammatory cytokines after SM injury.
Increased formation of reactive oxygen species and decreased glutathione production occur after exposure to SM [13]. Various metabolites are formed following the reaction of SM with glutathione, which may be detected within the urine [14]. NO signaling pathways also are important in modulating inflammation and necrobiosis in sulfur mustard-exposed skin cells [15]. Although vast amounts of knowledge are obtained in previous research to know the pathophysiology of SM toxicity, many questions remain and more research is needed to answer them [16]. Our main goal during this study was to gauge the expression of different skin proteins in chemical veterans exposed to SM and to search out a possible link between common skin problems including pruritus, eczema, atopic eczema, hyperpigmentation and hypopigmentation and melanoma with changes in the expression of skin proteins among these patients.

Materials and methods

Statement

The research ethics committee of Baqiyatallah University of Medical Sciences has approved the project (Approval ID: IR.BMSU.REC.1398.114). Informed consent was obtained from all subjects, and all methods were carried out in accordance with the relevant guidelines and regulations of REC. All experiments and relevant protocols were performed in accordance with the relevant guidelines and regulations of Baqiyatallah University of Medical Sciences.

Patients and controls

After filling in the questionnaire and informed consent, ten patients (SM, moderate to severe) and ten controls who were matched in sex and age were included in the study. Sample inclusion and exclusion criteria are stated in Table 1. The patients had medical records and their mustard injuries were confirmed by expert Dermatologists. Skin biopsy was done the same dermatologist from the inner part of the arm near the axillary fossa. The size of sampling was about 2 * 1 cm² and the depth of it was hypodermic fat. After cleaning of the sample from blood, it was inserted it in normal saline and then prepared for laboratory examination.

Sample preparation

Skin samples were resuspended in 800 μl of 1% sodium deoxycholate in 100 mM NH₄HCO₃ was added to the samples and vortexed vigorously; the samples were centrifuged at 5000 RPM. The protein solution was lyophilized and covered with parafilm. According to the manufacturer’s instructions, protein concentrations were determined using the BCA assay (CAT NO:23235 Thermo Scientific™). The disulfide bond of cysteine in the protein was reduced with 10 mM dithiothreitol (DTT) at 37 °C for 1 h, followed by alklylation with 20 mM iodoacetamide (IAA) for 45 min in a dark place with room temperature. The remaining IAA in the sample was quenched with 10 mM DTT for 15 min in a dark with room temperature. Proteolysis of samples using Lys-C protease (protein to enzyme ratio 100:1) was performed overnight at 28 °C, followed by trypsin digestion (protein to enzyme ratio 100:1) for 6 h at 37 °C. The pH of the sample was adjusted to approximately 3 using a final concentration of approximately 1% trifluoroacetic acid (TFA) and then desalted using a solid phase extraction disc containing styrene–divinylbenzene, and stopped tips (Empore SDBRPS 47 mm Extraction Disc, SUPLCO). Stage tips were self-packed into pipette tips, peptides were bound to the stage tips, washed with 0.2 percent TFA, and lastly eluted with 80 percent acetonitrile: 5% ammonium hydroxide. The peptides were vacuum centrifuged and then reconstituted in 200 mM HEPES pH 8.8 before being quantified using the Pierce quantitative colorimetric peptide assay (CAT NO:23235 Thermo Scientific™).

Sample labeling

Peptides were labeled with tandem mass tag (TMT) reagent according to the manufacturer’s instructions (CAT NO: 90110 Thermo Scientific™). Briefly, anhydrous acetonitrile was added to each vial labeled with TMT, followed by shaking for 5 min and short centrifugation. Aliquots of individual peptide samples were labeled with one of the individual TMT tags (10 tags total). Labeling was performed at room temperature for 1 h with occasional shaking.

To quench excess TMT label in the samples, 5% hydroxylamine was added to each sample, shaken, and incubated at room temperature for 15 min. A label check experiment was performed by mixing 1.5 μl of each individually labeled vacuum-dried TMT sample to ensure

Table 1: Inclusion and exclusion criteria of patients and controls

| Inclusion criteria       | Exclusion criteria                                      |
|--------------------------|---------------------------------------------------------|
| Adult male               | Taken corticosteroids or other specific drugs for 2 weeks |
| Non-smoker               | Diabetic, Hypertension, Gout, Asthma and Hyperlipidemia  |
| Age between 45 and 60 years | Surgery in the last 3 months                             |
| A well-documented exposure with SM | Seasonal allergies in the last 6 months                   |
|                          | Treated with antibiotics for 2 weeks                      |
that the same amount of total peptide was pooled in all samples prior to sample pooling. Samples were reconstituted in 2 μN, 0.1 μl water and analyzed by LC connected to a mass spectrometer (QExactive, Thermo Fisher, USA). Normalization factors were obtained from label validation experiments, and samples of TMT-labeled peptides were pooled in a 1:1 ratio across all samples and dried under vacuum. Samples were purified by C18 solid phase extraction desalting (SPE, SepPak, Waters) and centrifuged under a vacuum to dryness. The peptide mixture was fractionated into 96 fractions by high pH reverse phase HPLC (Hph), which were then pooled into 17 fractions before LCMS/MS analysis. Hph HPLC fractions of each TMT kit were reconstituted with sample loading buffer (2% acetonitrile, 97.9% water, 0.1% formic acid) and subjected to LC–MS/MS analysis.

**Preparation of the samples for MS analysis**
The Hph HPLC fractions of each TMT set were reconstituted with sample loading buffer (2% acetonitrile, 97.9% water, 0.1% formic acid) and subjected to LC–MS/MS analysis.

**LC–MS/MS analysis**
**1D data dependent acquisition (DDA) of peptides on QExactive Quadrupole-Orbitrap (QE-classic)**
TMT-labeled peptide samples were injected into an in-house packed trap column and desalted with loading buffer. The peptide was eluted from the trap into an in-house packed analytical column with linear gradients of mobile phases A and B (mobile phase B (30%) over 110 min at a flow rate of 300 nL/min across the gradient). The eluent from the trap was separated on the analysis column. The column eluate was fed to the ion source of the mass spectrometer. An electrospray voltage of 2.6 kV was applied through the fluid connection upstream of the column. Peptide precursors from 350 to 1850 m/z were scanned at 70 k resolution using a 1 × 106 target AGC. The 10 strongest ions in the previous survey scan were fragmented by high energy collision dissociation (HCD) using a collision energy of 35 normalized with a separation width of 0.7 m/z. For MS/MS analysis, only precursors with charge levels of +2 to +4. In the MS procedure, the minimum signal required for the MS2 trigger was 2.5 × 104, the AGC target value for MS2 was 2 × 105, and the maximum injection time for MS2 was 250 ms. The MS/MS scan resolution was set to 70 k. The dynamic exclusion was set to 90 s.

**Protein identification and quantification**
The mass spectrometric data files for each sample set were searched using Proteome Discoverer (version 2.1, Thermo Scientific). Uniprot database (181005_UniPr_HUMAN_Revi+Unrevi.fasta, Ref: http://www.uniprot.org) containing 95,106 human proteins including isoforms and unreviewed (Homo sapiens) was used for searching the data. The quantitative ratios were generated using the quantitative values found in channel 126 (control) as the denominator.

**Data processing**
TMT quantitative proteomic analysis of human samples resulted in the identification of 3586 high confident (Protein, Peptide and PSM FDR < 1%) proteins in skin samples. Density plot and Box Plot analysis of normalized data (Fig. 1) show similar protein ratio distribution (ratio with reference to channel 126, a control sample) across all samples within each respective data set, therefore, a

![Fig. 1](image_url) Density plot (left panel) and Box plots (right panel) of post-normalized data showing variability in the TMT-MS data skin
relative abundance comparison was performed. Normalization is accomplished by dividing abundance values by the total intensity of the provided array (i.e., the sum of all abundance values).

Statistical analysis
The differentially abundant proteins were analyzed using the Limma Packages [17], written in R. The Bioconductor software was used to analyze the data, which fits a linear model to each protein and then uses the Bayes technique to the predicted variances to increase power. The results were validated by the ANOVA test separately. Comparison of normalized protein areas between control and patient samples showed a total of 129 differentially abundant proteins (Fold change ≥ 1.2 and Fold change ≤ 0.8, statistically significant differences between patient and control were accepted as p-value < 0.05) in skin samples. The reason for choosing this Fold change was due to the chronic nature of the disease. Selecting a higher threshold makes it difficult to find the altered proteins for patients with chronic disease. Moreover, regarding the limited number of samples, the adaptation of Adjusted p-value for a low number of samples limits the validation of significant proteins.

Gene ontology and pathway enrichment
Gene Ontology (GO) was used to define gene functions in biological process (BP), molecular function (MF) and cellular component (CC) aspects. Enrichr is an online enrichment analysis tools that provides a functional annotation set to understand the biological meaning behind a large list of genes or proteins [18]. The functional enrichment analysis of the statistically significant proteins, including GO and KEGG pathway enrichment analysis [19], was conducted using Enrichr.

PPI network and network analysis
The list of proteins was analyzed using the online website STRING (https://string-db.org/, version 11.5) for protein–protein interaction analysis [20]. Then, the software Cytoscape was used to establish a PPI network. The Network Analyzer in Cytoscape was utilized to calculate node degree [21]. Also, the MCODE plugin was used to perform modular analysis [22], with the parameters set as follows: a Degree Cutoff = 2, Node Score Cutoff = 0.2, K-Core = 2, and Max. Depth = 100.

Results
Identification of significant abundance proteins
This study evaluated the protein profiles of 10 healthy individuals and 10 chemical warfare veterans with a history of SM exposure were evaluated. A total of 129 proteins were identified with significant increasing and decreasing regulatory changes, including 35 proteins with down-regulation and 94 proteins with up-regulation (Additional file 1). Table 2 shows the top 10 down-regulated skin proteins and Table 3 show the top 10 up-regulated skin proteins.

Among the 129 proteins in this study that were associated with changes in statistically significant expression, their role and importance in the occurrence of some skin problems such as pruritus, inflammation, eczema, and melanoma observed in chemical warfare exposed to sulfur SM are different.

GO and pathway enrichment analyses
The list of GO analysis and the KEGG pathway are shown in Additional file 2. Figure 2 shows the top 10 enrichment analysis for all significant abundance proteins. The top 10 KEGG pathway of significant abundance proteins are found in Fig. 2a. In terms of BP, the top 10 significant abundance proteins are found in Fig. 2b. As far as MF is found in Fig. 2c. CC is found in Fig. 2d.

PPI network and identification of hub genes
A PPI network of the proteins was constructed using the STRING and Cytoscape tool. The PPI network contained 129 nodes, including 94 upregulated genes and 35 downregulated genes, and 121 edges; (Fig. 3a). The term “degree” in the PPI network means a number of interactions between two genes (nodes). The hub genes of the PPI network were filtered with a cut-off value of degree > 6. As a result, six hub genes were identified. The hub genes included RPS15, ACTN1, FLNA, HP, SDHC, and RPL29. Three modules were extracted from the PPI network, including module 3 with five nodes (i.e., NDUF6, SDHC, DUFA12, COX6C, and UQCR10) (Fig. 3b), module 2 with five nodes included HBG1, HBB, HBD, HBA1, and HP (Fig. 3c), and module 1 with six nodes of TPM4, MYH11, ACTN1, TPM2, FLNA, and TPM1 (Fig. 3d).

Discussion
Proteomic analysis of skin biopsy samples could aid in the discovery of potential disease biomarkers, as well as provide a better knowledge of disease pathophysiology. In the current study, TMT-based proteomics technologies on the skin of SM-exposed patients were used to show a different pattern compared to healthy controls. This is the first study to evaluate the skin’s global proteome profiles in these patients in comparison with the control group.

Our findings showed six hub proteins (RPS15, ACTN1, FLNA, HP, SDHC, and RPL29) that three modules were extracted from the PPI network. Module 1 with five nodes includes NDUF6, SDHC, DUFA12, COX6C, and UQCR10 (KEGG Pathway Enrichment: Oxidative
Table 2  Top 10 skin proteins with significant down-regulation in chemical warfare victims

| Accession ID | P-value | Fold change | Gene name          | Protein name                   | Pathogenesis pathway                                                                                                                                                                                                 |
|--------------|---------|-------------|--------------------|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| P20718       | 2.5E−03 | 0.31        | GZMH               | Granzyme HOS = Homo sapiens OX = 9606 GN = GZMH PE = 1 SV = 1 | Due to the fact that granzyme H is primarily expressed in CD3−CD56+ NK (natural killer) cells of the immune system, decreased expression of this protein is associated with impaired induction of target cell death in pathogen-infected cells |
| P69891       | 9.3E−03 | 0.54        | HBG1               | Hemoglobin subunit gamma-1 OS = Homo sapiens OX = 9606 GN = HBG1 PE = 1 SV = 2 | Dysfunction of blood coagulation, Impairment of cellular oxidant detoxification, Disruption of hydrogen peroxide catabolic process, Impairment of antigen delivery to T cells and their activation, inability of T cells to proliferate and secrete cytokines which are normally involved in the immune response, Disruption of other parts of the immune response cascade, including B cells |
| A0A182DWH4   | 1.8E−02 | 0.56        | HLA-DRB3           | HLA class II histocompatibility antigen, DR beta 3 chain OS = Homo sapiens OX = 9606 GN = HLA-DRB3 PE = 1 SV = 1 | Impairment of antigen delivery to T cells and their activation, inability of T cells to proliferate and secrete cytokines which are normally involved in the immune response, Disruption of other parts of the immune response cascade, including B cells |
| P16455       | 2.6E−06 | 0.66        | MGMT               | Methylated-DNA–protein-cysteine methyltransferase OS = Homo sapiens OX = 9606 GN = MGMT PE = 1 SV = 1 | Inability to replicate DNA                                                                                                                                                                                                 |
| O95864       | 4.6E−02 | 0.67        | FADS2              | Fatty acid desaturase 2 OS = Homo sapiens OX = 9606 GN = FADS2 PE = 1 SV = 1 | Dysfunction of the skin barrier and changes in the structure of the fat structure in the stratum corneum                                                                                                                                 |
| P69905       | 2.5E−02 | 0.68        | HBA1               | Hemoglobin subunit alpha OS = Homo sapiens OX = 9606 GN = HBA1 PE = 1 SV = 2 | Disruption of the catabolic processes of hydrogen peroxide, oxidation-reduction, oxygen delivery to various tissues, disruption of the cell death, and small molecule metabolic process |
| P68871       | 4.3E−02 | 0.70        | HBB                | Hemoglobin subunit beta OS = Homo sapiens OX = 9606 GN = HBB PE = 1 SV = 2 | Disruption of the catabolic processes of hydrogen peroxide, oxidation-reduction, oxygen delivery to various tissues, disruption of the cell death, Disorder of small molecular metabolic process small molecule metabolic process, Dysregulation of nitric oxide biosynthetic process |
| Q9ULE0       | 8.0E−06 | 0.72        | WWC3               | Protein WWC3 OS = Homo sapiens OX = 9606 GN = WWC3 PE = 1 SV = 3 | Increased cell proliferation and metastasis                                                                                                                                                                                                 |
| O95969       | 3.2E−02 | 0.72        | SGCB1D2            | Secretoglobin family 1D member 2 OS = Homo sapiens OX = 9606 GN = SGCB1D2 PE = 2 SV = 1 | Impaired inflammation modulation, tissue repair disorders and tumorigenesis, Disorder of cellular physiological process                                                                                                                                 |
| O14556       | 1.6E−02 | 0.73        | GAPDH5             | Glyceraldehyde-3-phosphate dehydrogenase, testis-specific OS = Homo sapiens OX = 9606 GN = GAPDH5 PE = 1 SV = 2 | Increased glycolytic metabolism, tumor progression and metastatic proliferation of tumor cells, Disruption of the various membrane, cytoplasmic and nuclear transmissions |
**Table 3** Top 10 skin proteins with significant up-regulation in chemical warfare victims

| Accession ID | P-value | Fold change | Gene name | Protein name | Pathogenesis pathway |
|--------------|---------|-------------|-----------|--------------|----------------------|
| P82932       | 1.8E-04 | 1.79        | MRPS6     | 28S ribosomal protein S6, mitochondrial | Disorder of oxidative phosphorylation process and disruption of mitochondrial protein synthesis, Decreased cell survival, impaired glycolic metabolism, and many biological activities |
| P05161       | 1.0E-02 | 1.62        | ISG15     | Ubiquitin-like protein ISG15 | Dysfunction of intracellular proteins and immune responses |
| Q9UDW1       | 1.9E-06 | 1.60        | UQCR10    | Cytochrome b-c1 complex subunit 9 | Cell death and apoptosis, Production of reactive oxygen species (ROS) |
| Q96988       | 2.5E-02 | 1.58        | TSR2      | Pre-rRNA-processing protein TSR2 | Inhibition of NF-κB transcriptional activity and disruption of the apoptotic process |
| K7ELC2       | 7.4E-03 | 1.54        | RPS15     | 40S ribosomal protein S15 | Loss of cell nucleus integrity and irregularities in cell growth and proliferation |
| P09669       | 1.8E-02 | 1.50        | COX6C     | Cytochrome c oxidase subunit 6C | Irregularities in the process of apoptosis and oxidative phosphorylation of tissue cells |
| O95715       | 4.3E-03 | 1.49        | CXCL14    | C-X-C motif chemokine 14 | Disruption of cell migration, impaired regulation of immune activity, especially anti-bacterial immunity, impaired use of CD14+ DC precursors and inflammation |
| P16403       | 3.5E-02 | 1.47        | H1-2      | Histone H1.2 | Disorders of cell cycle regulation, induction of apoptosis, the release of cytochrome C from mitochondria, and caspase-dependent cell death |
| Q99572       | 3.6E-02 | 1.46        | P2RX7     | P2X purinoceptor 7 | Degradation of differentiated keratinocytes, release of pro-inflammatory mediators, proliferation of tumor cells, formation of reactive oxygen and nitrogen species |
| O14908       | 2.8E-03 | 1.45        | GIPC1     | PDZ domain-containing protein GIPC1 | Impaired proliferation, cell surface polarity, cytokinesis and cell migration |
phosphorylation, p-value: 9.3E-09 and FDR: 1.12E-07), module 2 with five nodes includes HBG1, HBB, HBD, HBA1, and HP, and module 3 with six nodes includes TPM4, MYH11, ACTN1, TPM2, FLNA, and TPM1 (KEGG Pathway Enrichment: Regulation of actin cytoskeleton, p-value: 0.001 and FDR: 0.004) were recovered from the PPI network.

Protein nodes in the first module are associated with mitochondrial electron transport chain (ETC) that includes 4 mitochondrial complexes. Significant alterations in the protein expression of mitochondrial respiratory chain complexes I, II, III, and IV in the SM-exposed patients compared to healthy controls are in line with previous studies [23–26]. There have been reports of mitochondrial dysfunction as a result of SM exposure [25, 26]. For example, Sourdeval et al. found that SM causes mitochondrial malfunction, which is followed by an increase in reactive oxygen species (ROS) formation and cellular OS [25]. Similar results were reported by Gould et al. for 2-chloroethyl ethylsulfide (CEES), and half mustard [26]. Another study found that mustards increase ROS production by changing cytochrome P450 activity by uncoupling microsomal electron transport at the flavoenzyme reductase [27]. Another mustard derivative, 11β: aniline mustard, alters the ETC at complex I, increasing ROS and OS generation while inhibiting oxygen consumption [28]. Another study reported chemical casualties had a higher pro-oxidant–antioxidant balance (PAB) score in comparison with the healthy control, indicating that they were exposed to OS [29].

ROS, particularly mitochondrial-derived ROS, play a role in a variety of signaling pathways, including eliciting antioxidative signaling, initiating DNA damage responses, affecting iron homeostasis, stimulating apoptosis, and signaling for cell survival and proliferation [30–32]. Thus, mitochondria may act as reservoirs of therapeutic targets in SM-exposed patients.

Altered expression and activity of mitochondrial complexes and increased OS in skin diseases similar to those exposed to mustard, including atopic dermatitis and atopic eczema, have also been reported. Previous research has found that nonlesional atopic dermatitis (ADNL) skin had higher expression of genes associated to OS [33]. Leman et al. discovered that increased complexes I and II activity in ADNL keratinocytes (KCs). Enhanced tricarboxylic acid cycle (TCA) turnover was discovered through metabolomics analysis. Mitochondria are important regulators of reactive oxygen species
(ROS) production. In ADNL KCs, increased aerobic metabolism resulted in OS. ADNL human epidermal equivalents had a higher mitochondrial function and an increased OS response when compared with the controls. In addition, they discovered that glycolysis in ADNL KCs complements but does not replace mitochondrial metabolism. As a result, aerobic metabolism dominates in ADNL, causing OS [34].

Of note, patients participating in this study, in addition to skin complications caused by SM, also have respiratory problems and hypoxia. Hypoxia affects the mitochondrial shape, such as cristae structure, as well as protein expression, such as HIF proteins [35, 36]. HIF proteins regulate intermediate metabolism by inducing many target genes, such as raising the expression of glycolysis-related proteins and lowering oxygen-dependent pathways via modifying the ETC complex expression and activity [35] that is consistent with enhanced glycolysis and decreased tricarboxylic acid cycle (TCA) turnover was discovered through metabolomics analysis in SM-exposed patients [37, 38]. Subsequently, HIF-1 cooperates with STAT3 on the Haptoglobin (Hp) promoter to increase Hp gene expression (node in the second module). Hp is a significant acute phase protein that binds to extravascular hemoglobin (Hb) and eliminates the harmful Hb quickly via macrophages' CD163 scavenger receptor [39]. As a result, Hp maintain tissues from oxidative damage caused by Hb [40, 41]. Increased level of Hp was shown in plasma SM-exposed patients in comparison with the control group [42]. Patients with skin illnesses, such as psoriasis, have considerably higher Hp mRNA expression in epidermal keratinocytes than controls [43]. Keratinocyte-derived Hp may be involved in the skin's downregulation of inflammatory reactions.
In addition to ROS production, mitochondria also are involved within the function of melanocytes and pigments [44]. Hair disorders and pimples are a part of the wide selection of symptoms of mitochondrial disease. Skin problems including scaling, itching and erythema are reported in some mitochondrial diseases like mitochondrial encephalomyopathy and lactic acidosis [45].

Nodes (Tropomyosin 1, Tropomyosin 2, Tropomyosin 4, Myosin Heavy Chain 11, Filamin A and Actinin Alpha 1) in module 3 are important in the protection of the cellular cytoskeleton that all of them increased in SM-exposed patients compared with the control group. Studies showed that in the short term (several hours after SM exposure), changes in these proteins could cause blisters [46, 47], while increased level of these proteins several years after SM exposure is unclear. It can be due to ROS-induced apoptosis or cell proliferation [35].

In addition to the proteins of three modules mentioned above, some of the top 10 proteins were described in the following part. ISG15 proteins are induced by interferon type I and have many functions. It also acts as an extracellular cytokine and modifier of intracellular proteins [48]. Non-conjugated extracellular ISG15 acts as a cytokine to modulate immune responses [49, 50]. Inflammation-induced interferon type 1 can even appear as ulcerative skin lesions within the armpits, groin, and neck [51]. ISG15 appears to act as a carcinogenic protein as well as a tumor suppressor protein [52]. The presence of dermatitis and melanoma of the skin may not be unrelated to elevated levels of this protein. Previous studies have reported that TSR2 overexpression can suppress NF-κB transcription activity in Hep-2 cells [53]. Suppression of NF-κB induces apoptosis [54, 55]. In our study, a rise within the level of Pre-rRNA-processing protein TSR2 homolog was observed within the skin of chemical operations victims. Given the role that these proteins play in carcinogenesis and apoptosis, it will be expected that these proteins also are involved within the development of melanoma. RPS15 proteins, independent of their major role in ribosomal assembly and protein translation, also accomplish several extra-ribosomal functions, including regulating apoptosis, cell cycle arrest, cell proliferation and migration, invasion, and repair of DNA [56]. RPS15 may be associated with expression disorders in some sorts of cancer, including esophageal, lung and skin cancers [57–59]. In an exceedingly, Wang et al. indicated that RPS15 is over-expressed in gastric cancer tissues [60]. In our study, a rise in RPS15 ribosomal proteins was observed in chemical warfare victims exposed to SM. COX6C is found within the inner membrane of the mitochondria and is vital within the process of apoptosis [61]. Previous studies have shown that COX6C can cause chronic kidney disease (CKD), diabetes, carcinoma, melanoma and other diseases [62, 63]. Increased expression of COX6C in various invasive organs has been reported to always be observed in damaged tissue cells [64]. In our study, the level of COX6C proteins was significantly increased. Activation of P2RX7 stimulates several pathways, including the discharge of pro-inflammatory cytokines like IL-1 and IL-6, modulation of cell surface receptors, ROS and RNS formation and necrobiosis [65]. Immuno-histochemical studies indicate that P2RX7 within the upper layer of human skin is involved in the death of differentiated keratinocytes [66]. P2RX7 in Allergic dermatitis in humans is related to up-regulation within the basal epidermal layer of inflamed skin of patients with atopic eczema [67]. Increased P2RX7 levels are reported in human melanoma and in several melanoma cell lines [68]. In our study, we also observed a rise in the extent of P2RX7 protein within the skin of chemical operations victims. GIPC1 is involved in the transport of assorted membrane proteins and regulates various cellular processes including cell proliferation, cell surface polarity, cytokines and migration [69].

WWC3 proteins are involved in the regulation of cell proliferation and migration [70]. WWC3 proteins preventing the proliferation of malignant cells are capable of inducing the expression of apoptotic molecules. Within the presence of WWC3, the activity of caspase-3 and caspase-7 increases and WWC3 proteins act as an autophagy regulator in Non-Small Cell carcinoma. [71]. Down-regulation of WWC3, which is related to increased cell proliferation and high metastasis [72]. In our study, WWC3 proteins within the skin of chemical operations victims were associated with a significant down-regulation. MGMT is expressed as a repairing enzyme in any kind of human tissue, the quantity of which is expressed in several tissues. For instance, It has the highest expression within the liver; Excessive expression in epidermis, fibroblasts and melanocytes; It has relatively high within the lungs, kidneys and colon, and lowest within the pancreas, hematopoietic cells, lymph tissues, and brain [73, 74]. In animal models, increasing MGMT levels is to guard against the event of tumors including carcinoma [75, 76]. Therefore, a significant down-regulating MGMT protein in chemical operations victims is expected to be involved in the development of carcinoma. Delta-5-desaturase (FADS1) and delta-6-desaturase (FADS2) are two enzymes, which accountable for the synthesis of highly unsaturated fatty acids Linolic acid (LA) and Omega-6 fatty acid. Among the two essential fatty acids present within the skin, LA plays a crucial role in regulating the function of the skin barrier [77]. Atopic eczema is related to abnormal fatty acid metabolism. Because linolic acid (LA) deficiency in humans and rodents ends up in certain abnormalities within the skin of patients
with atopic eczema [78]. In patients with atopic dermatitis, changes within the regulations of some transcription factors like SREBP-1c and PPAR-a have also been observed [79]. These transcription factors play a serious role in the regulation of FADS1 and FADS2 expressions [78]. In our study, FADS2 was related to a significant down-regulation within the skin of chemical operations victims.

Conclusion
According to what has been observed from skin protein changes in chemical veterans exposed to SM, it can be concluded that the increase and decrease in expression of some proteins are significant with skin problems such as itching, scaling, eczema, and atopic dermatitis. Distinct proteins are associated with OS, inflammatory pathways, apoptosis, and cell proliferation.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12920-022-01328-3.

Additional file 1. The list of differentially abundant proteins.
Additional file 2. The list of GO analysis and the KEGG pathway.

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Author contributions
HB and MGH conceived of the presented idea. SHP and VJ carried out the experiment. ASH and SM carried out data gathering. MA performed the experiment. BFNMGH helped to revise the manuscript. All authors discussed the results and contributed to the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated during and/or analysed during the current study are not publicly available due to confidentiality agreements, supporting data can only be made available to bona fide researchers subject to a non-disclosure agreement but are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
This study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Institutional Review Board and the Ethical Committee of Baqiyatallah University of Medical Sciences (Approval ID: IR.BMSU.REC.1398.114). Written informed consent was obtained from all study participants.

Consent for publication
Not applicable.

Competing interests
Authors have no conflict of interest to declare.

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