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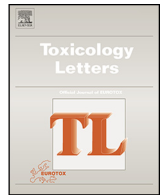

Serum proteomic analysis reveals potential serum biomarkers for occupational medicamentosa-like dermatitis caused by trichloroethylene

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HIGHLIGHTS

- Identify 8 proteins differentially expressed between OMLDT patients and controls.
- The altered expressions were validated by Western blot and ELISA analysis.
- TTR and PBP4 were significantly down-regulated in OMLDT patients.
- Haptoglobin was up-regulated in OMLDT patients.

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ABSTRACT

Trichloroethylene (TCE) is an industrial solvent with widespread occupational exposure and also a major environmental contaminant. Occupational medicamentosa-like dermatitis induced by trichloroethylene (OMLDT) is an autoimmune disease and it has become one major hazard in China. In this study, sera from 3 healthy controls and 3 OMLDT patients at different disease stages were used for a screening study by 2D-DIGE and MALDI-TOF-MS/MS. Eight proteins including transthyretin (TTR), retinol binding protein 4 (RBP4), haptoglobin, clusterin, serum amyloid A protein (SAA), apolipoprotein A-I, apolipoprotein C-III and apolipoprotein C-II were found to be significantly altered among the healthy, acute-stage, healing-stage and healed-stage groups. Specifically, the altered expression of TTR, RBP4 and haptoglobin were further validated by Western blot analysis and ELISA. Our data not only suggested that TTR, RBP4 and haptoglobin could serve as potential serum biomarkers of OMLDT, but also indicated that measurement of TTR, RBP4 and haptoglobin or their combination could help aid in the diagnosis, monitoring the progression and therapy of the disease.

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Abbreviations: TCE, trichloroethylene; OMLDT, occupational medicamentosa-like dermatitis induced by trichloroethylene; TTR, transthyretin; RBP4, retinol binding protein 4; Hp, haptoglobin; SAA, serum amyloid A protein; CLU, clusterin; Apo, apolipoprotein; 2D-DIGE, two dimension difference gel electrophoresis; MALDI-TOF, matrix-assisted laser desorption ionization time of flight tandem; MS/MS, mass spectrometry; ELISA, enzyme linked immunosorbent assay; DTT, DL-dithiothreitol; CHAPS, 3-[3-cholamidopropyl]-dimethylammonio-1-propanesulfenate; Tris, trashydroxymethyl amino methane; IPG, immobilized pH gradient; IEF, isoelectric focusing; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; CHCA, α-cyano-4-hydroxycinnamic acid; PMF, peptide mass fingerprint; LIFT, laser-induced forward transfer; PFF, peptide fragment fingerprint; ROC, receiver operating characteristic; AUC, area under the curve; TP, total protein; APPs, acute phase proteins; TBIL, total bilirubin; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma glutamyl transpeptidase; ALP, alkaline phosphatase.

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1. Introduction

Trichloroethylene (TCE) is a volatile organic solvent that has been widely used since the early 1900s in industry as metal degreaser, industrial intermediates, dry cleaning and food processing agent, and even in medicine as an anesthetic drug. It is estimated that more than 400,000 workers are exposed to TCE annually in the U.S., where approximately 50 million pounds of TCE are released annually into the environment (NIOSH, 1994; Goldman, 2010). Many studies have demonstrated that TCE exposure can cause severe toxic effect to the central nervous system, liver, kidney, immune system, reproductive system, etc. In 2011, the U.S. Environmental Protection Agency (EPA) released a final health assessment for TCE, characterizing it as a human carcinogen and non-cancer health hazard. TCE occupational exposure can lead to systemic skin disorders, named "occupational medicamentasa-like dermatitis by trichloroethylene" (OMLDT) accompanying with liver dysfunction (Xu et al., 2009; Chiu et al., 2013). Occurrences of OMLDT have been reported from USA, Japan, Spain, Singapore, China, Korea, Thailand, and the Philippines, while the number of OMLDT cases has increased since the mid-1990s in China (Kamijima et al., 2007), and 394 cases have been reported as of 2009 in Guangdong province, where an estimation of at least 20,000 new workers were exposed to TCE every year (Huang and Huang, 2010).

Extensive researches have been carried out to study OMLDT in recent years. However, the studies mainly focus on retrospective epidemiological investigations (Kamijima et al., 2008), genetic polymorphisms associated susceptibility (Li et al., 2006), and immunological mechanisms (Dai et al., 2005). Very few studies have focused on valid population studies, the dose–response relationship, period of exposure before disease onset, etiology and recurrence of OMLDT still remains unclear (Nakajima et al., 2003). OMLDT is often misdiagnosed, and proper treatment is delayed due to unavailable biological diagnostic methods. OMLDT has become an ongoing health problem because of TCE exposure in China (Xu et al., 2009).

Serum is an easily accessible body fluid that contains different types of proteins released by various diseased tissues. Proteome analysis of the serum provides insight into disease pathophysiology and mechanisms and helps to identify potential biomarkers with diagnostic and prognostic significance (Ray et al., 2011). In our previous study (Liu et al., 2009), we used serological proteomic analysis (SERPA) to screen and identify auto-antigens involved in TCE-induced autoimmune diseases. Six proteins were screened, and non-metastasis (NM23) protein was found to be a potential toxicological biomarker in TCE-induced autoimmune diseases. In this study, we compared the expression of proteins in the sera of OMLDT patients at three different stages using 2D-DIGE and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) strategy. Eight proteins were significantly altered among the healthy, acute-stage, healing-stage and healed-stage groups. The proteins TTR, RBP4 and haptoglobin were further validated by Western blot and ELISA. This study can provide potential serum biomarkers that may be used for the diagnosis and monitoring the progression of the disease.

2. Materials and methods

2.1. Subjects and serum collection

This study was conducted in accordance with the principles of the Declaration of Helsinki (World Medical Association, 1997). Informed consent was obtained from all subjects, and the Medical Ethics Committee of Shenzhen Center for Disease Control and Prevention approved the study protocol. The skin manifestations of the OMLDT patients enrolled in the study and the details for serum collection were described in our previous study (Hong et al., 2013). For 2D-DIGE analysis and Western blot validation experiments, 3 male cases with typical symptoms were enrolled (Tables 1 and 2, patients 1–3). For the ELISA, the 3 cases used in the 2D-DIGE study and an additional 15 cases (10 males and 5 females; 23.9 ± 8.7 years of age) were included (Tables 1 and 2, patients 4–18). The patients were hospitalized for treatment between March 2010 and November 2011. Eighteen age- and sex-matched normal subjects were also selected as the controls.

Table 1
Age and TCE exposure duration of OMLDT patients.

Patient no.	Age (year)	TCE exposure duration (day)
1[a]	18	14
2[a]	16	21
3[a]	19	36
4	26	51
5	18	46
6	20	25
7	39	32
8	27	30
9	21	32
10	31	20
11	22	37
12	39	44
13	19	25
14	17	29
15	17	42
16	43	13
17	14	31
18	25	34
Average	23.94 ± 8.70	31.22

[a] Patients whose serum specimens were included in the 2D-DIGE study and western validation experiments.

2.2. Depletion of high abundance serum proteins

The serum samples were processed using the ProteoExtract Albumin/IgG removal kit according to the manufacturer's instructions. Briefly, 40 μL of each serum was depleted by the albumin/IgG affinity resin columns, and the flow-through fraction was concentrated and desalted by ultrafiltration using a 3 kDa cut off centrifugal filter device (Millipore, USA). For the final centrifugation step, the binding buffer in the samples was displaced by 1× sample buffer, and the protein concentration of all depleted samples was determined with the 2-D Quant kit (GE Healthcare, USA).

2.3. CyDye minimal labeling

The depleted serum samples were minimally labeled (25 μg protein per 200 pmol dyes) with Cy2, Cy3 or Cy5 fluorescent dyes according to the manufacturer's instructions (GE healthcare). One half of the twelve test samples were labeled with Cy3 or Cy5, respectively. Cy2 was used to label an internal standard sample generated by pooling an aliquot of all twelve test samples. Equal portions (25 μg) of the internal standard were run on all six DIGE gels to assess the reproducibility and minimize the gel-to-gel variation. Three differently labeled samples were mixed in one gel, and an equal volume of 2× sample buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, 2% w/v DTT, and 2% v/v IPG buffer pH 3–10) was added to each mixed sample and incubated on ice for 10 min prior to bringing the total sample volume to 450 μL with rehydration buffer (8 M urea, 2% w/v CHAPS, 0.28% w/v DTT, 0.5% v/v IPG buffer pH 3–10, and 0.002% w/v bromophenol blue).

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