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Proteomics analysis identified serum biomarkers for occupational benzene exposure and chronic benzene poisoning

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Abstract

The study aimed to find novel effect biomarkers for occupational benzene exposure and chronic benzene poisoning (CBP), which might also provide clues to the mechanism of benzene toxicity.

We performed a comparative serological proteome analysis between healthy control workers with no benzene exposure, workers with short-term benzene exposure, workers with long-term benzene exposure, and CBP patients using 2D-DIGE and MALDI-TOF-MS. Two of the differentially expressed proteins were then selected to be validated by immune turbidimetric analysis.

A total of 10 proteins were found to be significantly altered between different groups. The identified deferentially expressed proteins were classified according to their molecular functions, biological processes, and protein classes. The alteration of 2 important serum proteins among them, apolipoprotein A-I and transthyretin, were further confirmed.

Our findings suggest that the identified differential proteins could be used as biomarkers for occupational benzene exposure and CBP, and they may also help elucidate the mechanisms of benzene toxicity.

Abbreviations: 2D-DIGE = two-dimensional difference gel electrophoresis, ALB = serum albumin, Apo-AI = apolipoprotein A-I, CBP = chronic benzene poisoning, DTT = DL-Dithiothreitol, F2 = prothrombin, FCN3 = ficolin-3, HP = haptoglobin, HPX = hemopexin, IAA = iodoacetamide, IARC = International Agency for Research on Cancer, IEF = isoelectric focusing, IgG = immunoglobulin G, IPG = immobilized pH gradient, MALDI-TOF-MS = matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, PMF = peptide mass fingerprinting, SDS = sodium dodecyl sulfate, SDS-PAGE = sodium dodecyl sulfate-Polyacrylamide gel electrophoresis, SERPINC1 = antithrombin-III, Tris = tris (hydroxymethyl)aminomethane, TTR = transthyretin, VTN = vitronectin.

Keywords: 2D-DIGE, apolipoprotein A-I, benzene exposure, chronic benzene poisoning, transthyretin

1. Introduction

Benzene belongs to a class of chemicals called volatile organic compounds. It is one of the most frequently used toxic *chemicals* in industry.[1–3] It is widely used as an industrial solvent in some developing countries, posing a huge threat to their occupational health.[4] Benzene exposure is most commonly found in industrial sectors including benzene production, the rubber tire manufacturing industry, and companies engaged in the bulk storage and transportation of benzene or petroleum products containing benzene.[5–7] It is well-known that chronic exposure to benzene leads to a range of hematotoxicity including leucopenia, thrombocytopenia, anemia, pancytopenia, and even leukemia.[8,9] Benzene was classified by the International Agency for Research on Cancer (IARC) as carcinogenic to human.[10] The adverse effects of benzene were described in a series of mechanistic studies in the literature, such as covalent binding,[11] immune suppression,[12] oxidative damage,[13] and chromosome aberrations.[14] Moreover, several newly identified mechanisms in last few years include DNA mutation,[15] and alterations in gene expression.[16] Although lots of epidemiological investigations and experimental studies have been implemented in the whole world, mechanisms underlying benzene-induced toxicity remain controversial and have not been clearly clarified. In addition, there is still a lack of sensitive effect biomarkers for occupational benzene exposure and CBP. Therefore, it is urgent to find novel biomarkers for occupational benzene exposure and CBP, as well as to provide important clues to the mechanism of benzene toxicity.

In many studies, serum has been used as an ideal biological specimen, and serum proteomics analysis has been developed for identifying potential biomarkers and for exploring mechanisms of diseases.[17] Based on the isoelectric points in the first

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dimension and the sizes in the second dimension, 2-dimensional difference gel electrophoresis (2D-DIGE) could resolve several thousand proteins.[18,19] A new method, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), has been recently developed to find novel biomarkers.[20] In the present study, we compared the expression of various proteins by analyzing their contents in the sera of healthy control workers with no benzene exposure, workers with short-term benzene exposure, workers with long-term benzene exposure, and CBP patients using 2D-DIGE coupled with MALDI-TOF-MS strategy. Differential proteins were classified by PANTHER (Protein Analysis through Evolutionary Relationships).[21] One serum protein significantly altered between the control group and benzene exposure group, and one between the control group and CBP group were further validated by Immune turbidimetric analysis. New insight into the molecular mechanism of benzene toxicity has been provided by this study, it also identified potential serum biomarkers for occupational benzene exposure and CBP.

2. Materials and methods

2.1. Instruments and reagents

The major instruments used in this experimental study include UltrafleXtreme MALDI-TOF/TOF mass spectrometer from Bruker Daltonics (USA). The Ettan IPGphor 3 Integrated Isoelectric Focusing Unit Electrophoresis System and Ettan DALT six Electrophoresis Unit from GE Healthcare (USA), and Beckman Coulter AU5800 automatic biochemical analyzer from Beckman Coulter (USA). The 2-D Quant Kit and the CyDye DIGE Fluor Labeling Kit were purchased from GE Healthcare (USA). The ProteoExtract Albumin/IgG removal kit was purchased from Merck KGaA (Germany). Research-grade acetonitrile (ACN), trifluoroacetic acid (TFA), Dimethyl formamide, ammonium bicarbonate, and DL-Dithiothreitol were purchased from Sigma-Aldrich (USA)

2.2. Subjects and serum sample collection

The study was conducted in accordance with the declaration of Helsinki (1997). The study protocol was approved by the Medical Ethics Committee of Shenzhen Prevention and Treatment Center for Occupational Diseases. Each subject gave their written informed consent before the study. All the serum samples were collected from January 2015 to September 2016 at Shenzhen Prevention and Treatment Center for Occupational Diseases. Occupational CBP patients were diagnosed according to the diagnostic criteria "Diagnosis of occupational benzene poisoning (2013 Edition)" issued by National Health and Family Planning Commission of the People's Republic of China (http://www.nhfpc.gov.cn/zhuz/pyl/201410/1f4b144cdc5b4c72a6f910209518daa3.shtml). Briefly, workers with occupational chronic benzene poisoning should have occupational exposure to benzene for more than three months, and show symptoms such as dizziness, headache, fatigue, insomnia, memory loss. The blood cell counts are tested every 2 weeks within 3 months, and one of the following conditions should be met: 1. the white blood cell count is mostly below 4×10^9/L or neutrophils count is below 2×10^9/L; 2. the platelet count is mostly below 80×10^9/L. For 2D-DIGE analysis, 4 screening groups of subjects (control group, short-term exposure group, long-term exposure group, and CBP group) were enrolled, and sex and age were matched between groups. There were 6 subjects in each screening group, 3 males and 3 females, aged 38.67±4.76, 38.83±3.43, 36.67±6.35, 37.50±5.82, respectively (Supplementary Table 1, http://links.lww.com/MD/D44). In the validation study, another four validation groups of subjects were enrolled to facilitate data analysis, the number of subjects was 149 in the control group, 61, 237, and 53 in the short-term, long-term exposure group, and CBP group, respectively (Table 1). Workers meeting the following conditions were selected into control group: non-smoker, with no benzene exposure before, without X-ray or other radiation exposure in the past 3 months, no contact history with other blood toxicity and genotoxic substances, without undergoing tumor and blood system diseases and other major disease history. Workers meeting the following conditions were selected into short-term exposure groups: clear exposure to benzene by inhalation or dermal for 3 to 12 months, the blood routine examination is within the clinical references, no smoking history. Workers meeting the following conditions were selected into long-term exposure groups: clear exposure to benzene by inhalation or dermal for more than 12 months, the blood routine examination is within the clinical references, non-smoker. Venous blood sample of each subject was collected with BD vacutainer venous blood collection tubes (BD, USA) containing coagulant. Approximately 4 mL of blood was collected for each person. All blood samples were centrifuged at 4000 rpm for 10 minutes, and the supernatant liquid for each sample was stored in aliquots at −80°C for further analysis. Each serum sample underwent no more than 2 freeze/thaw cycles prior to protein/peptide extraction and MS analysis.

Table 1

Serum concentrations of differentially expressed proteins tested by immune turbidimetric analysis.

Items	Control	SEB	LEB	CBP
Number	149	61	237	53
Gender (Male/Female)	91/58	47/14	159/78	15/38
Age (yr, Mean±SD)	30.09±7.82	31.96±9.32	37.25±8.31	41.55±6.13
Apolipoprotein A-I (g/L)	0.71±0.17	0.75±0.26	0.78±0.31	1.17±0.31*
Transthyretin (g/L)	0.19±0.065	0.17±0.043*	0.16±0.060*	0.17±0.062*

CBP = chronic benzene poisoning, LEB = long-term exposure to benzene, SEB = short-term exposure to benzene.
* means significantly different from the normal group, $P < .05$.

2.3. Sample preparation for 2-D gel electrophoresis

For 2-D gel electrophoresis, the serum samples were processed by using the ProteoExtract Albumin/IgG removal kit (Merck, Germany) and following the standard protocol provided by the manufacturer. Briefly, 60 μL of each serum was diluted 10-fold with binding buffer, and then the diluted serum sample was added to the resin column and allowed to pass by gravity-flow. The resin column was washed twice with 600 μL of binding buffer. The flow-through fraction was concentrated using a 3kDa cut off centrifugal filter device (Millipore, USA). The final serum protein concentration was determined with the 2-D Quant kit (GE Healthcare, USA) according to the manufacturer's instructions.

2.4. CyDye labeling

Protein extracts were minimally labeled (25 μg protein per 200 pmol dyes) with Cy2, Cy3 or Cy5 fluorescent dyes following the

standard protocol provided by the manufacturer (GE Healthcare). An internal standard sample was first generated by pooling equal amounts (25 μg) of all the samples together. The internal standard was labeled by Cy2 and used to minimize the gel-to-gel variation and assess the reproducibility. Then, each group was divided into two parts and one was labeled with Cy3 and another with Cy5, which were used to eliminate the effect of dyes in gel electrophoresis. The Cy3 and Cy5-labeled samples of different groups were mixed with 25 μg of Cy2-labeled internal standard and separated in a gel, and 12 gels were used for various labeled sample combinations (Supplementary Table 2, http://links.lww.com/MD/D44).

2.5. 2-D gel electrophoresis

Equal volume of 2 × lysis buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, 2% w/v DTT, 2% v/v IPG buffer pH 4–7) was added to each mixed labeled sample and incubated on ice for 10 minutes. Then, rehydration buffer (8Murea, 2% w/v CHAPS, 0.28% w/v DTT, 0.5% v/v IPG buffer pH 4 to 7, 0.002% w/v bromophenol blue) was added to make the total volume of the sample up to 450 μL. The first dimension was carried out on Ettan IPGphor Isoelectric Focusing System (GE Healthcare) using immobilized pH gradient (IPG) strips (pH 4–7, 24 cm, GE Healthcare). Two mL of mineral oil was added to cover each strip to reduce solvent evaporation. The IEF was performed at 20°C under the following 7 steps: step 50 V for 18 hours, step 300 V for 2 hours, step 500 V for 2 hours, step 1000 V for 2 hours, gradient 8000 V for 8 hours, step 8000 V for 8 hours, and step 500 V for 10 hours. After isoelectric focusing, the IPG strips were equilibrated for 15 minutes at room temperature in equilibration buffer (6 M urea, 75 mM Tris-HCl buffer pH 8.8, 30% v/v glycerol, 2% w/v SDS, 0.002% w/v bromophenol blue) containing 1% w/v DTT. Subsequently, the IPG strips were re-equilibrated for 15 minutes in equilibration buffer containing 4.5% w/v iodoacetamide. The equilibrated strips were loaded on the top of 12.5% SDS-PAGE gels with agarose sealing solution (25 mM Tris, 192 mM glycine, 0.1% SDS, 0.5% w/v agarose, and 0.02% bromophenol blue). The second dimension separation was carried out on an Ettan DALT 6 Electrophoresis Unit (GE Healthcare) with the following conditions: 1 W per gel for 1 hours, and subsequently 11 W per gel for 6 hours in the dark at 15°C.

2.6. Image acquisition and analysis

As soon as the 2-D gel electrophoresis was finished, the gels were scanned using a Typhoon Trio Variable Mode Imager (GE Healthcare) with the following settings: the Cy2, Cy3, and Cy5-labelled images for each gel were scanned with excitation/emission wavelengths of 488 (blue)/520, 532 (green)/580 and 633 (red)/670 nm, respectively. The resulted 36 maps were analyzed by DeCyder 2D v6.5 software (GE Healthcare). To analyze the differentially expressed protein spots, gel images were processed to the differential in-gel analysis (DIA) and the biological variation analysis (BVA) modules. Protein spots were detected and manually landmarked to the master gel to improve matching quality before the automatic matching. The differences in spot ratios were analyzed by one-way ANOVA analysis and the Student's *t* test. The spots considered statistically significant ($P < .05$) with an average ratio more than 1.5-fold were further analyzed.

2.7. Spot picking and in-gel digestion

A total of 1 mg of unlabeled proteins was used to run 2-DE using identical conditions as described above and stained with coomassie brilliant blue G-250 staining solution. The significant different protein spots detected by Decyder 2D v6.5 software analysis were manually excised by Coomassie Blue G-250 staining gel. The gel pieces were destained with 50% acetonitrile in 25 mM ammonium bicarbonate and then dehydrated in 100% acetonitrile. After the reagents were removed, each gel piece was digested overnight at 37°C with 0.01 mg/μL of sequencing grade trypsin (Promega, USA) in 15 μL digestion buffer containing 25 mM ammonium bicarbonate.

2.8. Mass spectrometry and database searching

Peptide mixtures were analyzed with an UltrafleXtreme MALDITOF/TOF mass spectrometer (Bruker Daltonics, USA). Briefly, a volume of 2 μL of extracted peptide mixtures was dripped on a polished steel MTP 384 target plate (Bruker Daltonics), air dried at room temperature and covered with 1 μL CHCA matrix (5 mg/mL in 50% CAN and 0.1% TFA). Standard peptide calibration mixtures were served as internal standard calibration. Protein identification by peptide mass fingerprint and MS/MS spectra was carried out using the BioTools software and MASCOT search engine 2.2. Confident matches in SwissProt database were defined by the sequence coverage by matching peptides, the MASCOT score and statistical significance ($P < .05$).The protein names, Mascot scores, the percent sequence coverage, theoretical pI, and theoretical molecular weight of these proteins were obtained from the SwissProt database.

2.9. Bioinformatics analyses of differentially expressed proteins

Differential proteins were classified by importing the proteins into the PANTHER-Gene List Analysis website (http://www.pantherdb.org/)[21] according to their molecular functions, biological processes and protein classes. STRING is a database to disclose possible connections among proteins and visualize the protein-protein interaction network. The differential proteins were further analyzed by the STRING-10.5 software (http://string-db.org). The active interaction derived from seven separate sources: Textmining, Experiments, Databases, Co-expression, Neighborhood, Gene Fusion and Co-occurrence. During the analysis, a network was constructed at a medium confidence level (0.411).

2.10. Immune turbidimetric method analysis

The proteins in the serum could combine with their corresponding antibody to form the antigen-antibody complex and result in certain turbidity. The concentrations of the target proteins can be obtained by measuring the turbidity and comparing the standard curve with the calibrator. Commercially available immune turbidimetric kits for human apolipoprotein A-I (Apo-AI) (Beckman Coulter, USA) and human prealbumin (transthyretin) (Beckman Coulter, USA) were employed to quantify the concentrations of the target proteins in each serum sample according to the operations of Beckman Coulter AU5800 automatic biochemical analyzer (USA) and immune turbidimetric kits manufacturer's instructions.

2.11. Statistical analysis

Data were analyzed using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL). Numerical variables with normal distribution were expressed as mean ± standard deviation. Means were compared between 2 groups with Student *t* test and means among 3 or more groups were compared with One-way ANOVA followed by Student Newman-Keuls post-hoc tests. $P < .05$ was considered statistically significant.

3. Results

3.1. Identification of the differentially expressed protein spots

Serum proteins of the 4 screening groups of subjects (control group, short-term exposure group, long-term exposure group, and CBP group) were resolved on 2D-DIGE. After typical 2D-DIGE gel images acquired (Supplementary Fig. 1, http://links.lww.com/MD/D44), comparative analysis of the protein profiles was carried out between the 4 groups by using the DeCyder software version 6.5 and the protein spots were annotated with Arabic numerals. Average ratio refers to the difference in protein concentration of spots between the 2 groups. Protein concentration of spots with at least 1.5-fold changes and statistically different with a *P* value $< .05$ between 2 groups were selected for MS identification. As the results, a total of 16 protein spots were selected for further examination (Fig. 1, Table 2). Proteins presented at the 16 spots were isolated and subjected to further analysis for their classification and characteristics. As showed in Table 2, a total of 10 proteins were identified as differentially expressed. Among the 16 spots, 4 spots (No. 2, 3, and 4) were identified as haptoglobin (HPT), and 3 (No. 5, 6, and 7) as hemopexin (HEMO), The spots No. 11, 12, and 13 were transthyretin (TTHY). Only 1 spot was identified as ficolin-3 (FCN3), Ig alpha-1 chain C region (IGHA1), prothrombin (THRB), serum albumin (ALBU), vitronectin (VTNC), antithrombin-III (ANT3), and apolipoprotein A-I (Apo-AI).

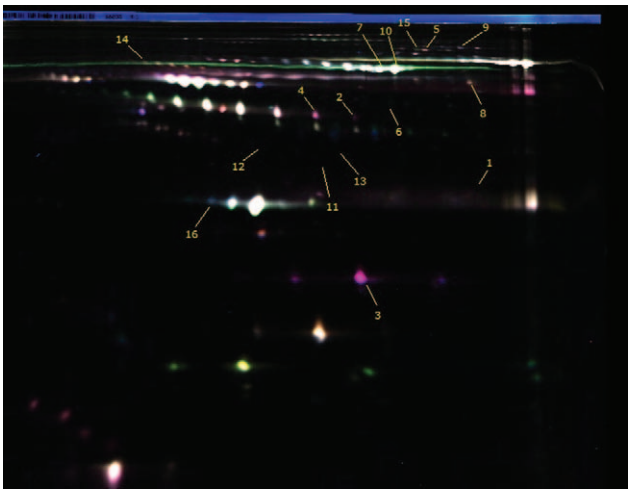

Figure 1. Differentially expressed proteins identified in 2-DE gel.

3.2. Functional classifications and protein-protein interaction

In order to seek the possible biological effects of occupational benzene exposure, the 10 identified proteins were subjected to the

Table 2

Ten differentially expressed proteins identified by 2D-DIGE and mass spectrometry.

No.	Protein Name*	Mascot Score†	Coverage‡	Protein MW§	Protein pI¶	Av. Ratio‖	*t* test
1	Ficolin-3	65	8	33,395	6.2	2.11**	0.0027**
2	Haptoglobin	338	11	45,861	6.1	2.92#/1.90**/2.18††	0.0039#/0.0075**/0.0018††
3	Haptoglobin	151	9	45,861	6.1	2.32‡‡	0.023‡‡
4	Haptoglobin	481	14	45,861	6.1	1.70#	0.032#
5	Hemopexin	225	9	52,385	6.6	−1.61††/1.57‡‡	0.023††/0.03‡‡
6	Hemopexin	202	9	52,385	6.6	−1.62††	0.032††
7	Hemopexin	192	10	52,385	6.6	−1.57#/1.82§§	0.019#/0.028§§
8	Ig alpha-1 chain C region	103	8	38,486	6.1	1.60‡‡/1.89§§	0.0011‡‡/0.013§§
9	Prothrombin	322	8	71,475	5.6	−1.69§§	0.027§§
10	Serum albumin	242	9	71,317	5.9	−1.64#/−3.03††/1.85‡‡	0.025#/0.00035††/0.01‡‡
11	Transthyretin	226	40	15,991	5.4	−2.21††	0.00089††
12	Transthyretin	332	25	15,991	5.4	−3.27‡‡	0.028‡‡
13	Transthyretin	366	34	15,991	5.4	−33.88#/16.46**	0.019#/0.047**
14	Vitronectin	428	12	55,069	5.5	1.56§§	0.047§§
15	Antithrombin-III	46	3	53,025	6.3	1.59§§	0.039§§
16	Apolipoprotein A-I	39	5	30,759	5.5	1.92††	0.0055††

* Protein name identified by MALDI-TOF-MS.
† Spots identified by MS/MS analysis, the MASCOT score is indicated.
‡ Sequence coverage achieved by MALDI-TOF-MS.
§ The theoretical molecular weight of matched protein in Da.
¶ The theoretical isoelectric point of matched protein.
‖ Average ratio calculated between two groups.
\# Parameters of short-term exposure to benzene against control.
** Parameters of long-term exposure to benzene against control.
†† Parameters of poisoning against control.
‡‡ Parameters of short-term exposure to benzene against poisoning.
§§ Parameters of long-term exposure to benzene against poisoning.

analysis with PANTHER database and sorted by PANTHER classification system according to their functional properties (Supplementary Fig. 2, http://links.lww.com/MD/D44). The identified differential proteins were found with important molecular functions including catalytic activity (37.5%), binding (37.5%) and transporter activity (25%) (Supplementary Fig. 2A, http://links.lww.com/MD/D44), and could contribute in various biological processes, such as localization (20%), cellular processes (16%), metabolic processes (12%), response to stimuli (12%), biological regulation (12%), cellular components or biogenesis (8%), multicellular organisms Process (8%), bioadhesion (4%), developmental process (4%), immune system process (4%) (Supplementary Fig. 2B, http://links.lww.com/MD/D44). Regarding the protein classes, most of the proteins belonged to transporter/carrier protein (23.1%), enzyme modulator (15.4%), hydrolase (15.4%), signaling molecule (15.4%) (Supplementary Fig. 2C, http://links.lww.com/MD/D44).

To figure out the associations between differential proteins, we combined differential proteins for building a regulatory network using STRING-10.5 software. The result (Supplementary Fig. 3, http://links.lww.com/MD/D44) showed that the differentially expressed proteins form a complex regulatory network containing 7 nodes and 26 edges with an average node degree of 5.78. The number of expected edges was 2, which was less than the actual 26 edges found. This result indicates that the network has significantly more interactions than expected, which means that these proteins are at least partially biologically connected as a group.

3.3. Validation of differentially expressed proteins

Immune turbidimetric analysis were used to validate differentially expressed proteins identified by proteomic analysis based on 2D-DIGE. Among the 10 proteins, Apo-AI and transthyretin (pre-albumin) were selected for validation and their concentration in the crude serum samples from the four validation groups (control group, short-term exposure group, long-term exposure group, and CBP group) were quantified by immune turbidimetric assay. The results demonstrated clearly that the concentrations of Apo-AI in sera of the CBP patients were significantly higher than in sera of the controls, and the concentrations of transthyretin (pre-albumin) in sera of the controls were significantly higher than in the other three groups (Table 1). This result is consistent with the results from 2D-DIGE and should prove that benzene exposure could alter the expression of these proteins in human serum.

4. Discussion

In this study, a well-designed proteomic analysis based on 2D-DIGE was adopted,[22] which is highly reproducible, with a wide linear range of protein detection sensitivity. This comparative serological proteomic method is a powerful technique that enables the simultaneous visualization of relatively large portions of the proteome. By the fluorescence labeling and internal standards, 2D-DIGE has the advantages of minimizing the effect of gel-to-gel variation, more accurate and sensitive proteomic quantization over traditional 2-DE proteomics. Four groups of subjects were employed for comparison if benzene exposed workers have altered serum protein contents. As a result, a total of 10 differentially expressed proteins were identified among the 16 varied spots resolved by 2D-DIGE, for 3 of these proteins appeared on more than one spot on the 2D-DIGE map. This phenomenon has also been found in other studies and may be explained by post-translational modifications and differential cleavage of the proteins.[23] The analysis with PANTHER demonstrated that the molecular functions of the identified differential proteins included catalytic activity, binding and transporter activity. They were involved in many biological processes including localization, cellular process, response to stimuli and metabolic processes. Based on the protein classes, most proteins were related to transporter/carrier protein, and the next prevalence were enzyme modulator, hydrolase, signaling molecule. Furthermore, by using the STRING database to retrieve the relationship among the differentially expressed proteins, we found that Apo-AI and transthyretin are involved in a variety of biological processes. They were important hubs for the coordination of protein regulation in the construction of a benzene exposure regulatory network. Apo-AI participates in endocytosis, receptor-mediated endocytosis, negative regulation of response to wounding, negative regulation of response to external stimulus, regulation of response to stress, blood coagulation, regulation of response to stimulus. Transthyretin is involved in response to external stimulus. These findings not only increase our understanding of potential biomarkers, but also reveal the complexity of the mechanisms of chronic benzene poisoning.

Two differentially expressed proteins, apolipoprotein A-1and transthyretin, were selected and retested by immune turbidimetric analysis to verify their changes in sera of the workers exposed to benzene. The consistent result not only confirmed that these 2 serum proteins were really regulated by benzene exposure, but also indicated that the differentially expressed proteins identified by 2D-DIGE were reliable. The reasons for selection of these 2 proteins for verification came from the following two aspects. First, a relatively low P value of t test was performed in comparison with the controls. Second, their representative functions could be helpful in elucidating the pathogenesis of benzene exposure.

Apo-AI is the main polypeptide of human plasma high-density lipoprotein (HDL) which constitutes approximately 70% of the apolipoprotein content of HDL particles. Apo-AI belongs to the apolipoprotein A1/A4/E family, it is synthesized in the liver and intestine. It is a protein that participates in the reverse transport of cholesterol from tissues to the liver for excretion by promoting cholesterol efflux from tissues and by acting as a cofactor for the lecithin cholesterol acyltransferase (LCAT).[24–26] Liang's and Huang's[27,28] research suggest that APOB100 is a potential biomarker for chronic occupational benzene exposure and may play a role in benzene-induced lipid metabolism disorders. APOB100 is a large amphipathic glycoprotein that is synthesized in the liver and the intestine and a major apolipoprotein of very-low-density lipoprotein (VLDL), intermediate-density lipoprotein, and low-density lipoprotein (LDL). Therefore, we hypothesize that Apo-AI may also play a role in benzene-induced lipid metabolism disorders. Apo-A1 has been shown to be a specific inhibitor of cytokine production by monocytes and macrophages in response to interaction with stimulated T-cells.[26] Apo-A1 is a constitutive anti-inflammatory factor, and the decrease in HDL-associated Apo-A1 level may be a signal of chronic inflammation progression. It was shown that Apo-A1 concentration in blood is up-regulated in a variety of malignant tumors of ovarian, liver, breast. Apo-A1 has been identified as a potential biomarker of ovarian cancer, colorectal cancer, and pancreatic cancer by using

MS.[29,30] However, controversial observations were also reported including down-regulation of Apo-A1 in different types of cancer[31,32] Apo-A1 has also anti-apoptotic and anti-oxidant functions. Oxidative damage has been thought one of the mechanisms by which benzene damages the hematopoietic system and the increase of Apo-AI is probably a protective mechanism activated in response to the oxidative damage resulting from CBP. In this study, an increase in Apo-AI was observed in CBP, consistent with Zhang's findings.[25] Thus, it could be a potential sensitive serum biomarker for CBP.

Transthyretin is a 15951Da tetrameric non-glycosylated serum protein. When electrophoresed on agar, its electrophoresis rate is 25% faster than serum albumin. It locates in front of albumin, so it is also called prealbumin. Transthyretin is one of the fast transporters made by liver cells. Its basic function is to transport thyroxine and retinol. It also has thymosin activity, which can promote the maturation of lymphocytes and increase the immunity of the body. Its daily decomposition rate is 33.1% to 39.5%, and its half-life is short, only 1.9 days. Therefore, this index can reflect the slight changes in liver synthesis and catabolism, and the extent of serum concentration reduction and the degree of liver parenchymal damage. Closely related, it is a sensitive indicator of early liver function damage.[33–35] Many circumstances are associated with reduced serum concentrations of transthyretin. The most common is the acute phase response, which may be due to inflammation, malignancy, trauma, or many other disorders.[35] Transthyretin in the blood also interacts with high-density lipoproteins by binding to Apo-A1. Further studies have also found that transthyretin is a novel plasma protease that can cleave C-side of Apo-A1.[36] This study found that transthyretin levels in sera of benzene exposed workers decreased, these results suggest that serum thyroxine transporter may be a potential biomarker for workers with benzene exposure or even CBP.

This study only validated 2 of the 10 screened proteins; the rest 8 proteins also need further studies to reveal their role in benzene exposure and CBP. The number of samples of benzene exposure and CBP is relatively small and need to be further expanded. Moreover, further trials are needed for practical use of the biomarkers.

5. Conclusions

In conclusion, 10 proteins were identified as differentially expressed in the sera of benzene exposure and CBP patients by using 2D-DIGE combined with MALDI-TOF-MS. Moreover, alterations of Apo-AI and transthyretin were validated by immune turbidimetric analysis. These proteins are related to oxidative stress and inflammation, which may help to elucidate the mechanism of benzene toxicity and provide potential effect biomarkers for occupational benzene exposure and CBP.

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Supervision: yanfang zhang.
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