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ORIGINAL ARTICLE

Joint association of carrying *HLA-B*13:01* gene and human herpesvirus-6 with occupational trichloroethylene hypersensitivity syndrome

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Abstract

Purpose Occupational trichloroethylene hypersensitivity syndrome (OTHS) clinically manifests as generalized severe rash resembling drug-induced hypersensitivity syndrome (DIHS) and afflicts predominantly *HLA-B*13:01* gene carriers after their exposure to trichloroethylene. Meanwhile, OTHS may also be associated with human herpesvirus such as herpesvirus-6 (HHV6) and cytomegalovirus (HCMV) reported to participate in the pathology of DIHS. This study explored the association of carrying HHV6 and HCMV, and the joint association of carrying *HLA-B*13:01* and HHV6 and HCMV with OTHS.

Methods We recruited 30 OTHS patients and 40 trichloroethylene-exposed healthy workers as cases and controls, respectively. *HLA-B*13:01* was genotyped and HHV6 and HCMV DNA were detected in the DNA extracted from whole-blood sample of each participant with PCR techniques. Positive rates of *HLA-B*13:01* gene and HHV6 and HCMV DNA and their association with OTHS were then analyzed.

Results The OTHS cases showed significantly higher positive rates of *HLA-B*13:01* gene and HHV6 DNA, but not HCMV DNA, than the controls (83.3% vs. 25.0% and 56.7% vs. 10.0%, respectively, both $P < 0.001$). Positive rate of HHV6 DNA was significantly higher in *HLA-B*13:01* carriers than in non-carriers in the cases (68.0% vs. 0, $P = 0.005$), but not in the controls. Carrying *HLA-B*13:01* and HHV6 had an interactive effect on OTHS (OR = 91.80, $P < 0.001$).

Conclusions Carrying *HLA-B*13:01* and HHV6 may be associated with OTHS; furthermore, carrying *HLA-B*13:01* and HHV6 may be jointly associated with OTHS.

Keywords Trichloroethylene · Hypersensitivity syndrome · Human leukocyte antigen · Human herpesvirus-6 · Human cytomegalovirus

Introduction

Occupational trichloroethylene hypersensitivity syndrome (OTHS) usually afflicts a few workers after their respiratory and/or skin exposure to trichloroethylene, an industrial toxicant commonly used as organic solvent and degreasing agent in many countries for about a century (Xu et al. 2009), and it manifests as generalized severe rash mostly accompanied by fever, hepatitis, and lymphadenopathy, resembling serious drug hypersensitivities referred to as drug reaction with eosinophilia and systemic symptoms (DRESS) or drug-induced hypersensitivity syndrome (DIHS) (Kamijima et al. 2007). This disorder has been reported to occur widely in the world, including the United States, Spain, Japan, Korea, Singapore, Thailand, Philippines, and China (Kamijima et al. 2007), though the incidence was predicted to be only

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about 1% (Kamijima et al. 2007). Moreover, about 7% of the patients died of secondary hepatic encephalopathy, severe infection, and multiple organ failure (Hua et al. 2010), and those survived usually had a very long course and could relapse after cessation of the glucocorticoid therapy.

Currently, OTHS is mainly deemed as a T cell-mediated, Type IV hypersensitivity reaction based on the known clinical features: (a) the latency is about 2–5 weeks, (b) no dose–response relationship has been observed between trichloroethylene exposure and disease severity, (c) skin lesions are not confined to the area contacting trichloroethylene, (d) the glucocorticoid therapy is relatively effective, and (e) re-exposure to trichloroethylene leads to rapid recurrence (Kamijima et al. 2007; Zhang et al. 2017), also on the pathological observation that T cells were found in elevated level in affected skin tissues and peripheral blood of the patients (Chen et al. 2008). Moreover, OTHS was recently linked to *HLA-B*13:01* (Li et al. 2007), an allele of the human leukocyte antigen B gene. The *HLA-B*-encoded molecules exist in all nucleated cells and participate in the immune response by presenting endogenous antigens, and involvement of HLA is one of the prominent features of the mechanism of Type IV reaction (Pavlos et al. 2015). Carrying *HLA-B*13:01* was considered to be a risk factor of OTHS in trichloroethylene-exposed workers (Li et al. 2007). Asian workers exposed to trichloroethylene are more susceptible to OTHS, probably because their *HLA-B*13:01* frequency is much higher than the Europeans and Africans (Cao et al. 2001; Williams et al. 2001).

Another related factor that has been previously examined in OTHS patients is the reactivation of human herpesviruses such as herpesvirus-6 (HHV6) and cytomegalovirus (HCMV) (Huang et al. 2006; Kamijima et al. 2013; Watanabe et al. 2010), because OTHS resembles DIHS in various aspects and reactivation of commonly seen herpesviruses has been extensively explored in DIHS (Ishida et al. 2014; Tohyama et al. 2007). However, viral reactivation usually observed more than 2 weeks after symptom onset may not mediate the occurrence of DIHS or OTHS, it is possibly the result of general immune dysregulation or suppression as observed in organ transplant patients (Fishman 2013; Lautenschlager and Razonable 2012). The association of OTHS with carrying HHV6 and HCMV, which should be differentiated from viral reactivation and examined before or around OTHS onset, has not been reported in the literature as far as we know, though human herpesviruses were shown to play a key role in shaping the immune environment for occurrence of DIHS (White et al. 2015). Further, it is unknown whether carrying the susceptible gene *HLA-B*13:01* and HHV6 and HCMV may be jointly associated with OTHS. So, in this study, we genotyped *HLA-B*13:01*, detected HHV6 and HCMV by the DNA, and analyzed their independent and joint association with OTHS.

Methods

Study population

This study's protocol was approved by the Ethics Committee of Shenzhen Prevention and Treatment Center for Occupational Diseases (SPTCOD). All study participants or their guardians gave their written consent in accordance with the Declaration of Helsinki.

We investigated 30 OTHS patients who were consecutively admitted to SPTCOD from January 2014 to December 2017. Diagnosis of OTHS was made by a panel of at least three occupational physicians according to the diagnostic criteria GBZ 185-2006 (People's Republic of China 2007). The main points of diagnosis are that (a) being exposed to trichloroethylene in the present or previous job, (b) the latent period is 5–40 days or longer, but usually less than 80 days, (c) skin lesions manifest as acute dermatitis, exfoliative dermatitis in most patients and multiform erythema, Stevens–Johnson syndrome or toxic epidermal necrolysis in the rest, (d) accompanying symptoms include fever, hepatitis, and lymphadenopathy, (e) only a few of the workers suffer from OTHS in the same workplace with trichloroethylene exposure, (f) excluding similar diseases or symptoms caused by other etiologic factors such as drugs, pathogenic microbes, and autoantigens. We collected detailed information on demographic characteristics, occupational exposure history and medical conditions, and draw 2 mL EDTA-2K anticoagulated venous blood sample from each of the 30 cases just after their hospitalization (10.56 ± 5.07 days from symptom onset).

To compare measured values of the cases with those of exposed control subjects, 40 healthy workers were recruited who had been occupationally exposed to trichloroethylene for at least 6 months from the same factories where the cases worked. Their basic information and blood samples were collected in the same way as the cases. The diagnostic criteria described above were applied to the controls as to the cases and specified for each subject in the list of Online Resource 1.

Laboratory indices examination

White blood cells and lymphocytes in the blood samples of the subjects were automatically counted using an XE-5000 Blood Analyzer (Sysmex, Kobe, Japan). Serum was separated from 2 mL coagulated blood samples of each subject by centrifugation at $2564 \times g$ for 8 min, then serum alanine aminotransferase activity and total protein concentration were measured using an AU5800 Chemistry Analyzer (Beckman Coulter, California, US).

DNA extraction

We extracted DNA from 200 µL of the whole-blood sample of each subject with a QIAamp DNA blood mini Kit (QIAGEN, Shanghai, China) following the manufacturer's instruction. DNA concentration was quantified using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA). The extracted DNA were subsequently used as templates in the following PCR tests of genotyping *HLA-B*13:01* and detecting HHV6 and HCMV DNA.

*HLA-B*13:01* genotyping

*HLA-B*13:01* was genotyped with the duplex allele-specific real-time PCR, which is based on the multiplex PCR previously described by Liu et al. (2016). The specific primers and TaqMan probes were synthesized and supplied by Sangon Biotech Co. Ltd. (Shanghai, China) and their detailed information was listed in Online Resource 2. A StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA) was used to perform the PCRs. The reaction mixture with approximately 2 ng of DNA template contained 10 µL of 2 × Premix EX Taq™ (TaKaRa, Dalian, China), 0.4 µL of 50× ROX Reference Dye (TaKaRa, Dalian, China), 625 µM primers, 250 µM probes, and PCR-grade water in a final volume of 20 µL. Thermal cycling was initiated with 95 °C for 31 s, followed by 40 cycles at 95 °C for 5 s, and 64 °C for 35 s. Data were analyzed using the amplification-based threshold, and the adaptive baseline algorithms were provided by the software analysis system of the StepOnePlus machine. For quality assurance, each test included one negative and one positive *HLA-B*13:01* standard samples and all samples were tested twice independently.

HHV6 and HCMV DNA detection

Status of carrying HHV6 and HCMV was determined by detecting HHV6 and HCMV DNA from whole-blood DNA samples of the subjects, respectively. PCR was performed using A StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). For HHV6 DNA detection, the forward primer 5′-TCGACTCTCACCCTACTGAACGAG-3′ and the reverse primer 5′-TGACTAGAGAGCGACAAATTGGAG-3′, previously reported by Lyall and Cubie (1995), were synthesized and supplied by Sangon Biotech Co. Ltd. (Shanghai, China). The PCR mixture contained 0.25 µM of each primer, 10 µL of 2 × Premix EX Taq™ HS (TaKaRa, Dalian, China), 100 ng DNA template and PCR-grade water in a final volume of 20 µL. Thermal cycling was initiated with 95 °C for 30 s, followed by 45 cycles at 95 °C for 5 s, and 60 °C for 35 s. As for HCMV DNA detection, the forward primer 5′-GCCCAGGTAGGCCGTTAC-3′ and the reverse primer 5′-ATCTGCTGTCCGTCAAAGAT-3′, previously reported by Bilenoğlu et al. (2015) were synthesized and supplied by Sangon Biotech Co. Ltd. (Shanghai, China). The PCR mixture contained 0.20 µM of each primer, 10 µL of 2× Premix EX Taq™ HS (TaKaRa, Dalian, China), 100 ng DNA template and PCR-grade water in a final volume of 20 µL. Thermal cycling was initiated with 95 °C for 30 s, followed by 45 cycles at 95 °C for 3 s, and 60 °C for 35 s. PCR products were identified by electrophoresis on 2% agarose gel and those containing amplified fragments of 163 bp and of 138 bp were subsequently sequenced for further confirmation of HHV6 and HCMV DNA, respectively.

Statistical analysis

Numerical variables were compared between the OTHS cases and controls using Mann–Whitney *U* test or *t* test according to their distributions. Comparison of rates was performed with χ^2 test or Fisher's exact test accordingly. Logistic regression was used to analyze the independent effect of carrying *HLA-B*13:01* gene and of carrying HHV6 and their interactive effect on OTHS. All statistical tests were two-tailed with a significance level of $P < 0.05$ and performed using the Stata statistical software (Stata statistical software, release 12.0; Stata Corp, College Station, TX, USA).

Results

General characteristics and laboratory indices of the study subjects

As shown in Table 1, the averages of age and percentages of Han, smokers and drinkers were all comparable between the cases and controls. The percentage of females in the cases was significantly higher than in the controls ($P = 0.036$). The median concentration of trichloroethylene exposure was comparable between the cases and controls, while the median length of trichloroethylene exposure in the cases was significantly shorter than that in the controls ($P < 0.001$). The latency period in the OTHS cases was 27.9 days in average. Medians of white blood cell count and lymphocyte count and alanine aminotransferase activity were all significantly higher in the cases than in the controls ($P < 0.001$, $P = 0.015$, $P < 0.001$, respectively), while mean of total protein concentration in the cases was significantly lower than in the controls ($P < 0.001$).

Association of carrying *HLA-B*13:01* and HHV6 and HCMV with OTHS

The positive rate of *HLA-B*13:01* gene in the OTHS cases was significantly higher than that in the controls ($P < 0.001$)

Table 1 Comparison of general characteristics and laboratory indices between the OTHS cases and controls

Variables	OTHS cases ($n=30$)	Controls ($n=40$)	P
Age [year, median (IQR)]	24.5 (20.0–37.8)	28.0 (25.2–30.0)	0.443[a]
Sex (female, %)	33.3	12.5	0.036[b]
Race (Han, %)	86.7	90.0	0.717[c]
Smoking (yes, %)	36.7	42.5	0.622[b]
Drinking (yes, %)	3.3	5.0	0.999[c]
Trichloroethylene exposure concentration $_{TWA}$ [mg/m^3, median (IQR)]	15.2 (4.6–23.0)	17.2 (10.6–24.9)	0.296[a]
Trichloroethylene exposure period [day, median (IQR)]	31.0 (24.5–34.0)	555.0 (187.5–1822.5)	< 0.001[a]
Latency period (day, mean ± SD)	27.93 ± 11.69	–	–
White blood cell count [$\times 10^9$/L, median (IQR)]	12.01 (8.10–16.13)	5.66 (4.88–6.68)	< 0.001[a]
Lymphocyte count [$\times 10^9$/L, median (IQR)]	2.78 (1.81–4.69)	1.96 (1.78–2.34)	0.015[a]
Alanine aminotransferase activity [U/L, median (IQR)]	482.50 (244.25–769.00)	20.00 (15.00–29.50)	< 0.001[a]
Total protein concentration (g/L, mean ± SD)	62.40 ± 9.98	76.43 ± 3.64	< 0.001[d]
*HLA-B*13:01* gene (positive, %)	83.3	25.0	< 0.001[b]
Human herpesvirus-6 DNA (positive, %)	56.7	10.0	< 0.001[c]
Human cytomegalovirus DNA (positive, %)	30.0	15.0	0.130[b]

OTHS occupational trichloroethylene hypersensitivity syndrome, *IQR* interquartile range, *TWA* time-weighted average, *SD* standard deviation

[a]Mann–Whitney *U* test

[b]χ^2 test

[c]Fisher's exact test

[d]*t* test

(Table 1). This result was stable even after adjustment for sex with logistic regression (OR = 13.50, $P < 0.001$). The positive rate of HHV6 DNA in the cases was significantly higher than in the controls ($P < 0.001$) (Table 1). The positive rate of HCMV DNA in the cases was also higher than that in the controls, but the difference was not statistically significant (Table 1).

Joint association of carrying *HLA-B*13:01* and HHV6 with OTHS

The positive rate of HHV6 DNA was significantly higher in the cases carrying *HLA-B*13:01* than in the cases not carrying *HLA-B*13:01* ($P = 0.005$) (Table 2). However, the result in the controls was quite different. The positive rate of HHV6 DNA in the controls carrying *HLA-B*13:01* was comparable to that in the controls carrying no *HLA-B*13:01* (Table 2).

Although carrying HCMV was not related to OTHS in the present study, we still compared positive rate of HCMV DNA between *HLA-B*13:01* carriers and non-carriers to explore the association of HCMV with *HLA-B*13:01*. As shown in Table 2, positive rate of HCMV DNA in the cases (or controls) carrying *HLA-B*13:01* was not significantly different from that in the cases (or controls) carrying no *HLA-B*13:01*.

Table 2 Comparison of positive rates of HHV6 and HCMV DNA in *HLA-B*13:01* gene carriers with non-carriers

	OTHS cases ($n=30$)		Controls ($n=40$)	
	Carrying *HLA-B*13:01*	Not carrying *HLA-B*13:01*	Carrying *HLA-B*13:01*	Not carrying *HLA-B*13:01*
HHV6 DNA				
Positive	68.0% (17)	0% (0)	10.0% (1)	10.0% (3)
Negative	32.0% (8)	100.0% (5)	90.0% (9)	90.0% (27)
P*	0.005		0.999	
HCMV DNA				
Positive	36.0% (9)	0% (0)	20.0% (2)	13.3% (4)
Negative	64.0% (16)	100.0% (5)	80.0% (8)	86.7% (26)
P*	0.109		0.609	

OTHS occupational trichloroethylene hypersensitivity syndrome, *HHV6* human herpesvirus-6, *HCMV* human cytomegalovirus

*Fisher's exact test

Logistic regression was used to further examine the effects of carrying *HLA-B*13:01* gene and HHV6 on OTHS. As shown in Table 3, carrying *HLA-B*13:01* and HHV6 had independent effects on OTHS (OR = 9.17, $P = 0.001$ and OR = 5.71, $P = 0.015$, respectively); moreover, carrying *HLA-B*13:01* gene and HHV6 had an interactive effect on OTHS (OR = 91.80, $P < 0.001$).

Table 3 Effects of carrying *HLA-B*13:01* and HHV6 on OTHS

Variable	Coefficient (95% CI)	OR (95% CI)	*z*	*P**
Carrying *HLA-B*13:01*	2.22 (0.94–3.48)	9.17 (2.57–32.64)	3.42	0.001
Carrying HHV6	1.74 (0.34–3.14)	5.71 (1.40–23.21)	2.44	0.015
Interaction (*HLA-B*13:01* × HHV6)				
0 × 0	Reference	Reference		
0 × 1	0 (Empty)	1 (Empty)		
1 × 0	1.57 (0.22–2.92)	4.80 (1.25–18.48)	2.28	0.023
1 × 1	4.52 (2.29–6.75)	91.80 (9.86–854.70)	3.97	< 0.001

For carrying *HLA-B*13:01*, yes = 1, no = 0; for carrying HHV6, yes = 1, no = 0

OTHS occupational trichloroethylene hypersensitivity syndrome, *HHV6* human herpesvirus-6, *CI* confidence interval

*Logistic regression

Discussion

OTHS is a severe immune dermatitis syndrome with inexplicit pathogenesis. It was proposed to be mainly a type IV hypersensitivity reaction based on clinical manifestation and laboratory examination (Chen et al. 2008; Kamijima et al. 2007; Zhang et al. 2017), which was also supported by the strong association with *HLA-B*13:01* gene identified in previous studies (Dai et al. 2015; Li et al. 2007; Watanabe et al. 2010). In agreement with the previous studies, we also found that OTHS cases had significantly higher positive rate of *HLA-B*13:01* gene than the controls, confirming the solid link between OTHS and *HLA-B*13:01*. To date, three nonmutually exclusive models have been proposed to explain how the *HLA-B* encoded molecules take part in the immunopathogenesis of drug-specific hypersensitivity reactions, which could also be applied to that of OTHS. They are the hapten/prohapten model, the pharmacologic interaction (*p–i*) model, and the altered peptide repertoire model, which have been extensively recorded in the literature (Pavlos et al. 2015; White et al. 2015), and briefly illustrated in Online Resource 3a. Li et al. (2007) proposed that OTHS might be induced through the altered peptide repertoire model, in which the trichloroethylene (or/and its metabolites) occupies a position in the peptide-binding groove of the *HLA-B*13:01* encoded protein, thereby changing the chemistry of the binding cleft and the peptide specificity of HLA binding. The endogenous peptides presented in this context are recognized as "foreign" by the immune system and thereby elicit a T cell response.

In drug hypersensitivity setting, human herpesviruses have been demonstrated to play a key role in shaping the immune environment for occurrence of the hypersensitive reaction (White et al. 2015). Because OTHS resembles DIHS in clinical manifestation and laboratory indices (Kamijima et al. 2007), they may also be similar in mechanism of pathology. Therefore, herpesviruses such as commonly seen HHV6 and HCMV might also participate in occurrence of OTHS. Presently, the association of OTHS with carrying HHV6 and HCMV has not been examined, though HHV6 and HCMV reactivation was previously reported in OTHS patients (Huang et al. 2006; Kamijima et al. 2013; Watanabe et al. 2010). So, we detected HHV6 and HCMV in OTHS cases and controls and found that the cases had significantly higher positive rate of HHV6 DNA than the controls, suggesting that carrying HHV6 may be associated with OTHS. Carrying HCMV was not considered to be associated with OTHS by the present study, because the increase of positive rate of HCMV DNA in the cases relative to the controls was not statistically significant. Until now, it is still unclear how herpesviruses such as HHV6 may participate in occurrence of OTHS. A possible explanation is based on the heterologous immunity model proposed in DIHS (Pavlos et al. 2015; White et al. 2015). The model in general refers to the situation where T cells elicited by one epitope (e.g., from a pathogen) cross-recognize a different epitope (e.g., from another pathogen or from a neo-antigen) (briefly illustrated in Online Resource 3b). In OTHS setting, memory T cells that derive from earlier stimulation by herpesvirus such as HHV6 peptides before OTHS onset may cross-react with endogenous peptides mediated by the culprit trichloroethylene (or/and its metabolites).

Subsequently, we further explored the joint association of carrying *HLA-B*13:01* gene and HHV6 with OTHS, because we observed certain consistency between positive results of *HLA-B*13:01* gene and HHV6 DNA only in the cases. We found that *HLA-B*13:01* carriers had significantly higher positive rate of HHV6 DNA than noncarriers in the case, but not in the controls, suggesting that carrying *HLA-B*13:01* gene and HHV6 may be jointly associated with OTHS. Moreover, carrying *HLA-B*13:01* and HHV6 had an interactive effect, apart from their independent effects, on OTHS. Based on the associations of carrying *HLA-B*13:01* gene and HHV6 with OTHS and the potential explanation, the joint association is not hard to understand. The HHV6-peptides-stimulated T cells

may cross-recognize endogenous peptides mispresented by *HLA-B*13:01*-encoded molecules, and attach corresponding self-tissues, leading to the occurrence of OTHS. This may explain how carrying *HLA-B*13:01* gene and HHV6 were jointly associated with OTHS.

The HLA super family, rich in highly homologous genes, is the most complex and polymorphic genetic system in the human genome (Saito et al. 2000). Multiple polymorphic loci are involved in generating certain *HLA* alleles, raising a great challenge for genotyping. The duplex allele-specific real-time PCR uses two pairs of allele-specific primers and two TaqMan probes designed from the specific bases in *HLA-B*13:01* to increase the specificity of the assay. It can detect *HLA-B*13:01* even from 0.016 ng of DNA template, and the result is in complete concordance with the gold standard sequence-based typing (Liu et al. 2016), indicating that the duplex PCR is highly specific and accurate. Positive rates of *HLA-B*13:01* in the cases and controls were generally comparable to those reported in Li et al.'s study (2007).

We determined HHV6 and HCMV carriers by directly detecting corresponding virus DNA from whole-blood DNA samples of the subjects instead of virus antibodies, because detection of the virus antibodies may be affected by immune state which differed greatly between the OTHS cases and controls, and rheumatoid factor and some other autoantibodies in a few subjects may be falsely detected as the virus antibodies due to cross-reaction (De Paschale et al. 2010). The PCR methods used for detecting HHV6 and HCMV DNA in the present study amplify active virus DNA as well as latent virus DNA, so the positive rates of HHV6 and HCMV were, respectively, higher than the reactivation rates of HHV6 and HCMV previously reported in OTHS patients (Kamijima et al. 2013).

Trichloroethylene exposure concentration was not significantly different between the cases and controls, supporting that exposure dosage is unrelated to occurrence of OTHS (Kamijima et al. 2007). OTHS is an idiopathic disorder that usually has a latency period of 2–5 weeks after first exposure to trichloroethylene according to previous epidemiological studies (Kamijima et al. 2007; Zhang et al. 2017); for those exposed to trichloroethylene for more than 3 months and showing no sign of characteristic symptoms despite of exposure dosage, they are basically tolerant to OTHS. We randomly selected such tolerant heathy workers who had been occupationally exposed to trichloroethylene for at least 6 months as comparing control group to magnify the effects of carrying *HLA* gene and HHV6 on OTHS, thereby obtaining them with a relatively small sample size. The controls were selected to match the cases on demographic characteristics as far as possible, but we could not recruit enough female control workers during the implementation of the study, resulting in a higher percentage of female in the cases. Nonetheless, sex turned to be not a confounding factor during the analyses.

In conclusion, we examined the association of carrying HHV6 and HCMV with OTHS, and the joint association of carrying *HLA-B*13:01* and HHV6 with OTHS for the first time. Findings from this study help to understand the pathogenesis of OTHS and provide clues for further investigation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethics approval All procedures performed in the study involving human participants were in accordance with the ethical standards of the Ethics Committee of Shenzhen Prevention and Treatment Center for Occupational Diseases and with the 1975 Declaration of Helsinki and its later amendments or comparable ethical standards.

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