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Association Between Polymorphisms of DNA Repair Gene XRCC1 and DNA Damage in Asbestos-Exposed Workers[1]

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Objective To compare the asbestos-induced DNA damage and repair capacities of DNA damage between 104 asbestos-exposed workers and 101 control workers in Qingdao City of China and to investigate the possible association between polymorphisms in codon 399 of XRCC1 and susceptibility to asbestosis. **Methods** DNA damage levels in peripheral blood lymphocytes were determined by comet assay, and XRCC1 genetic polymorphisms of DNA samples from 51 asbestosis cases and 53 non-asbestosis workers with a similar asbestos exposure history were analyzed by PCR/RFLP. **Results** The basal comet scores (3.95±2.95) were significantly higher in asbestos-exposed workers than in control workers (0.10±0.28). After 1 h H_2O_2 stimulation, DNA damage of lymphocytes exhibited different increases. After a 4 h repair period, the comet scores were 50.98±19.53 in asbestos-exposed workers and 18.32±12.04 in controls. The residual DNA damage (RD) was significantly greater (P<0.01) in asbestos-exposed workers (35.62%) than in controls (27.75%). XRCC1 genetic polymorphism in 104 asbestos-exposed workers was not associated with increased risk of asbestosis. But compared with polymorphisms in the DNA repair gene XRCC1 (polymorphisms in codon 399) and the DNA damage induced by asbestos, the comet scores in asbestosis cases with Gln/Gln, Gln/Arg, and Arg/Arg were 40.26±18.94, 38.03±28.22, and 32.01±11.65, respectively, which were higher than those in non-asbestosis workers with the same genotypes (25.58±11.08, 37.08±14.74, and 29.38±10.15). There were significant differences in the comet scores between asbestosis cases and non-asbestosis workers with Gln/Gln by Student's t-test (P<0.05 or 0.01). The comet scores were higher in asbestosis workers with Gln/Gln than in those with Arg/Arg and in non-asbestosis workers exposed to asbestos, but without statistically significant difference. **Conclusions** Exposure to asbestos may be related to DNA damage or the capacity of cells to repair H_2O_2-induced DNA damage. DNA repair gene XRCC1 codon 399 may be responsible for the inter-individual susceptibility in DNA damage and repair capacities.

Key words: Asbestos; Asbestosis; DNA damage; XRCC1; DNA repair; Polymorphisms; Comet assays

INTRODUCTION

Asbestos is an important environmental carcinogen and remains the primary occupational concern in many countries. The most important pulmonary disease associated with asbestos fiber exposure is asbestosis (diffuse interstitial pulmonary fibrosis). Exposure levels to asbestos fibers have been greatly decreased in the workplace during the past decades. However, the potential period between exposure and manifestation of the disease is about 20-40 years long, which is the reason why the incidence of this disease is still increasing. The mechanisms of the pathological events leading to asbestosis are still poorly understood. Previous studies have shown that asbestos is a potent DNA mutagen in mammalian cells and induces multilocus deletions[1-4]. Our previous report showed that exposure to asbestos could significantly decrease plasma activity of GSTs, and GSTM1 genotypes could affect the activity of GSTs in control workers, which is not so obvious in asbestos-exposed workers[5].

Alkaline single cell gel electrophoresis (SCGE) technique, also known as the comet assay, is a sensitive, simple and rapid technique for screening genotoxicity, capable of detecting DNA single- and double-strand breaks, alkali-labile sites and incomplete excision repair sites, and genomic structural discontinuities[6-7]. Due to its sensitivity in

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detecting genetic damage at the individual cell level and its potential application to different cells, the assay has been adopted as a useful tool in short-term genotoxicity and human biomonitoring studies[8-9]. Some studies reported that asbestos fiber induces DNA damage of V79 cells and lung cells[4,10].

Identification of polymorphisms in DNA repair genes now makes it possible to examine gene-environment interaction at the genotypic level. Molecular epidemiology has a great potential in the assessment of susceptibility factors that might predispose to cancer. Individual susceptibility that contributes to the development of asbestos-associated diseases has also been identified[11-12]. DNA repair gene polymorphisms, which may alter the efficiency of DNA repair, may contribute to cancer susceptibility[13]. X-ray cross-complementing group 1 (XRCC1), a DNA repair protein involved in single-strand breaks and base excision repair (BER) pathway, is responsible for the efficient repair of DNA damage caused by active oxygen, ionization, and alkylating agents[14]. Three polymorphisms of DNA repair gene XRCC1 have been identified at codons 194 (Arg to Trp), 280 (Arg to His), and 399 (Arg to Gln)[15]. In particular, XRCC1 Gln 399 polymorphism resulting in single base substitution, which could affect binding to PARP (poly(ADP-ribose) polymerase), may lead to deficiency of DNA repair. In cells lacking XRCC1 activity, an increased SCE frequency has been observed[11]. In addition, defects in XRCC1 Gln 399 are associated with increased smoking-related cancers, including head and neck cancer, and lung cancer[16-18]. However, Stern *et al.*[19] and Duell *et al.*[20] reported a possible reduction in bladder cancer risk among subjects with XRCC1 Gln 399 allele and found that the 399 Gln/Gln genotype is related to increased sister chromatid exchange frequencies among smokers.

In the present study, we used comet scores by SCGE assay in peripheral blood lymphocytes, a commonly used marker for DNA damage, to determine the association of XRCC1 genotype with asbestos-induced DNA damage, and compared their repair ability in lymphocytes between asbestos-exposed workers and control workers. Though asbestos is the major cause of asbestosis, only a small fraction of asbestos-exposed workers suffer from asbestosis during their lifetime. Genetic and other environmental factors might be important determinants of risk. In order to examine the association between DNA repair genotype XRCC1 and asbestosis incidence, we conducted a case-control study in asbestos-exposed workers.

MATERIALS AND METHODS

Study Design

A total of 205 subjects were selected (104 workers from an asbestos producing plant and 101 control workers from non-asbestos plants in Qingdao, China). Information on smoking history, alcohol-drinking history, medical history, and occupational exposure was gathered by interview with the subjects. We obtained a DNA sample from lymphocytes adequate to analyze genotypes for XRCC1 codon 399 in 51 non-asbestosis and 53 asbestosis workers. All subjects gave informed written consent for participation in the study. To explore the effects of genotypes on DNA damage, DNA damage rate and XRCC1 genotype in asbestosis and non-asbestosis workers were analyzed.

Sampling and Sample Preparation

Blood samples were collected by venipuncture and transferred to the laboratory within a few hours. One sample (5 mL) in a heparinized tube was used for comet assay. Another sample (5 mL) containing no anticoagulant was used for genotypic characterization.

Anticoagulated fresh peripheral blood samples were isolated for lymphocytes using Ficoll-Paque (Shanghai Huajing, China)[21]. Lymphocytes were adjusted in PBS at 5×10^6/mL and divided into two parts. One was used directly with comet assay for the baseline of DNA damage level; the other part was washed with 5 mL of ice-cold PBS three times before 40 μmol/L H_2O_2 was applied in 0.5 mL of PBS, and then incubated for 1 h at 37℃. After solutions were removed, cells were washed with cold PBS and centrifuged (500×g, 7 min) (Eppendorf, Germany) and re-suspended at approximately 5×10^6 cells/mL PBS for SCGE. The rest of H_2O_2-stimulated cells were continually cultured for 4 h for DNA repair and SCGE.

DNA was isolated from peripheral blood lymphocytes containing no anticoagulant by the standard sodium dodecyl sulfate/proteinase K method for subsequent molecular analysis.

Single Cell Gel Electrophoresis (SCGE or Comet Assay)

SCGE assay was performed as previously described[6,22] with slight modifications. Briefly, anticoagulated fresh peripheral blood samples isolated for lymphocytes were adjusted in PBS at 5×10^6, then 10 μL of each subject's cell suspension was mixed with 35 μL of 20 mmol/L EDTA / Ca^{2+} and Mg^{2+} free PBS and embedded in low melting agarose (Promega, USA). After incubation with lysis

solution (2.5 mol/L NaCl, 100 mmol/L EDTA, 10 mmol/L Tris, 1% Triton X-100) for at least 1 h at 4℃, slides were placed in an electrophoresis tank allowing DNA to unwind for 20 min in fresh alkaline electrophoresis buffer (1 mmol/L EDTA, 300 mmol/L NaOH). Electrophoresis was conducted at room temperature for 15 min at 24 V and 300 mA (DYY-2C Electrophoresis, Beijing Liuyi, China). The slides were then neutralised three times with Tris buffer (0.4 mol/L, pH 7.5) and stained with ethidium bromide (Sigma, USA). All the steps were performed under yellow light in order to prevent additional DNA damage. The slides were observed under a fluorescent microscope (Leica Microsystems, Wetzlar, Germany). DNA damage was divided into four groups based on the length and fluorescence intensity of the comet tail: type 1, no tail (intact nuclei); type 2, comet with short tail (tail length less than the head diameter); type 3, comet with tail length longer than the head diameter and with low fluorescence intensity; and type 4, comet with tail length longer than the head diameter and with high fluorescence intensity (Fig. 1). Types 1-4 were weighed by special factors of 0-3[22], respectively, and comet scores were calculated as 0×ntype1 +1×ntype2 +2×ntype3 + 3×ntype4 (n is the number of different comet types per 100 counted cells). Data were expressed as $\bar{x} \pm s$ (the comet scores). For each subject, at least 100 individual lymphocytes were evaluated. For the analysis of repair kinetics, the residual DNA damage was calculated as follows: percent residual DNA damage at 4 h after H_2O_2 -induction (%RD) = 100 × (DNA damage at 4 h after H_2O_2 stimulation-DNA damage before H_2O_2)/(DNA damage at 1h after H_2O_2 stimulation-DNA damage in control cells before H_2O_2 -induction)[23].

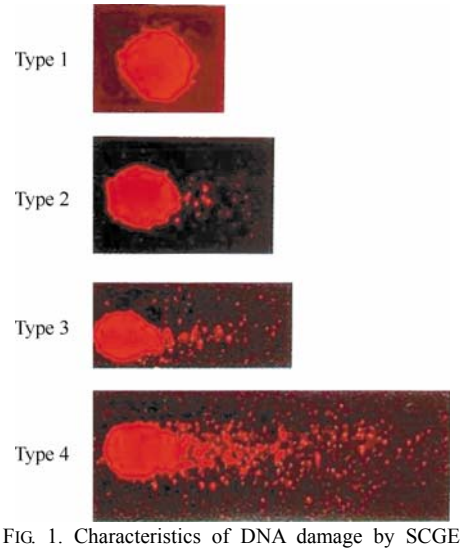

FIG. 1. Characteristics of DNA damage by SCGE assay.

Genotyping Analysis of XRCC1 Codon 399

XRCC1 polymorphism was determined by PCR-RFLP assay[20]. Primers used for exon 10 (codon 399) were 5′-CCCCAAGTACAGCCAGGTC-3′ and 5′-TGTCCCGCTCCTCT CAGTAG-3′ (synthesized by Shanghai Shengong, China). Amplification was carried out with denaturation at 95℃ for 30 s, annealing at 60℃ for 30 s, and extension at 72℃ for 30 s (GeneAmp PCR System, Perkin Elmer, USA). PCR products were digested with MspI (Takara, Japan) at 37℃ overnight and analyzed on 2% agarose gel. Arg/Arg individuals had 94 and 148 bp fragments; Arg/Gln individuals had 94, 148, and 242 bp fragments; and Gln/Gln individuals had only 242 bp fragments.

Statistical Analysis

Student's *t*-test was used to test the differences in DNA damage level (comet scores). DNA damage level was further compared using stratified analysis. A goodness-of-fit χ^2 test was used to determine whether the polymorphisms were in Hardy-Weinberg equilibrium between asbestosis and non-asbestosis workers. Odds ratios (ORs) were used as estimates of the relative risk, and 95% confidence intervals (CI) were calculated by multivariate logistic regression to adjust for age, gender, alcohol-drinking, and smoking status to assess the relationship between genotypes and asbestosis risk. All statistical analyses were performed using SPSS11.0.

RESULTS

Demographic Characteristics

The study population consisted of 104 asbestos-exposed workers, and 101 control workers. Among asbestos-exposed workers, 51 had asbestosis, and 53 had not. No statistically significant differences between asbestos-exposed and control workers were found in terms of the mean age, sex, smoking, and alcohol drinking status. The age distribution in asbestosis and non-asbestosis workers was quite similar (Table 1).

DNA Damage Level by SCGE in Asbestos-Exposed Workers

DNA damage level was determined by increased total migration area and length of the stained DNA. After staining with ethidium bromide, nuclear DNA appeared to be a bright orange mass in the fluorescent microscope system. DNA from normal cells was primarily visualized as a round area that did not migrate in the gel to any great extent. The comet tail was due to the increased migration of DNA fragments to the anode.

TABLE 1

Age Distribution in Asbestos-exposed Workers and Controls

Age (y)	Asbestos-exposed Workers			Controls
	Asbestosis	Non-asbestosis	Total	
⩽60	3	13	16	19
61-70	25	31	56	53
⩾70	23	9	32	29
Total	51	53	104	101

H_2O_2 has been established as a potent and directly acting agent in oxidant-induced DNA SSB studies[24]. Therefore, induction and repair of DNA damage induced by H_2O_2 were analyzed by comet assay in asbestos-exposed workers and controls. Comet scores obtained before treatment (baseline damage), 1 h after stimulation, and 4 h after repair are presented in Table 2. The results showed that basal comet score (3.95±2.95) in the asbestos-exposed workers was significantly higher than that in the controls (0.10±0.28). After 1 h H_2O_2 exposure at 37℃, DNA damage of lymphocytes exhibited different increases, reaching statistically significant difference between the two groups (P<0.01). Compared with basal DNA damage level, the increased percentage of the comet score was 96.85% in asbestos-exposed workers and 99.83% in controls. After a 4 h repair period, the comet scores were 50.98±19.53 in asbestos-exposed workers and 18.32±12.04 in controls. The difference in the comet scores between the two groups was significant (P<0.01). The residual DNA damage (RD) was 35. 62% in asbestos-exposed workers and 27.75% in controls. The capacity of DNA repair in asbestos-exposed workers was lower than that in controls.

TABLE 2

Analysis of DNA Damage by Comet Assays in Asbestos-Exposed Workers and Controls ($\bar{x} \pm s$)

Group	Number of Subjects	Comet Scores					
		Basal DNA Damage	P	H_2O_2-induced	P	After Repair	P
Asbestos	104	34.73±2.95		136.35±35.94	<0.01	50.98±19.53	<0.01
Control	100	3.17±3.09	<0.01	58.54±24.73		18.32±12.04	

After stratification of asbestos-exposed workers by asbestosis and non-asbestosis, the baseline damage level and the levels of induction and repair induced by H_2O_2 are shown in Table 3. There was no significant difference in basal DNA damage level between the two groups, but more serious DNA damage by comet assays was observed in the asbestosis group after a 4 h repair period, the comet score of DNA damage being 56.87±21.42, significantly different from that in non-asbestosis group (44.26±15.24). The rate of DNA damage repair was 61.37%, suggesting that DNA repair capacity was decreased in asbestosis workers.

TABLE 3

Analysis of DNA Damage by Comet Assays in Asbestosis and Non-asbestosis Workers ($\bar{x} \pm s$)

Group	Number of Subjects	Comet Scores					
		Basal DNA Damage	P	H_2O_2-induced	P	After Repair	P
Asbestosis	51	37.15±19.67	＞0.05	147.01±30.75	0.001	56.87±21.42	0.001
Non-asbestosis	53	31.72±13.2		123.96±39.00		44.26±15.24	

Analysis of XRCC1 Genotype in Asbestos-exposed Workers

The XRCC1 genotype distributions in asbestos-exposed workers are summarized in Table 4. Chi-square analysis of the distribution of repair genotypes in non-asbestosis workers indicated that all the alleles were in Hardy-Weinberg equilibrium. Analysis of the 399 Gln polymorphism showed that the frequency of the mutant allele in asbestosis

workers was 0.57. The homozygous Arg/Arg genotype was present in 31.4%, the heterozygous genotype in 23.5%, and the homozygous Gln/Gln genotype in 45.1%. The prevalence of the 399 Gln genotype was significantly higher in asbestosis workers than in non-asbestosis workers (χ^2=6.62, df= 2, P=0.037). No significant difference was detected in distribution of the allele (χ^2 =1.88, df=1, P=0.17). Risk estimates for XRCC1 variant alleles in asbestosis and non-asbestosis workers are shown in Table 3. The variant allele genotypes of XRCC1 were not associated with an overall higher risk of asbestosis, after adjustment for age, sex, and smoking status (adjusted OR =0.95, 95% CI=0.38-2.38). This may be due to the small sample size for the GG genotype subgroup. We did not find any association between the XRCC1 genotype and asbestosis risk.

TABLE 4

Genotype and Allele Frequencies of Gln 399Arg Polymorphism of XRCC1 Gene in Asbestos-exposed Workers

Group	Genotype[a]			Allele[b]	
	Gln/Gln (%)	Gln/Arg (%)	Arg/Arg (%)	Gln (%)	Arg (%)
Asbestosis (n=51)	23(45.1)	12(23.5)	16(31.4)	56.9	43.1
Non-asbestosis (n=53)	12(22.6)	22(41.5)	19(35.8)	43.4	56.6

Note. [a]Significant difference in the genotype distribution between asbestosis workers and non-asbestosis workers (χ^2= 6.62, df=2, P= 0.037). [b]No significant difference was detected in the allele distribution between asbestosis and non-asbestosis workers (χ^2=1.88, df=1, P= 0.17).

Effect of XRCC1 Polymorphisms on DNA Damage in Asbestos-Exposed Workers

In order to assess the role of environmental and genetic factors in DNA damage, means of the comet scores were compared between subjects categorized by asbestosis and DNA repair genotype. Comet scores of baseline, H_2O_2-induced DNA damage and after its repair were compared between asbestosis and non-asbestosis workers for Gln/Gln, Gln/Arg, and Arg/Arg genotypes (Table 5). Stratification of asbestos-exposed workers by XRCC1 genotypes showed that the comet scores in asbestosis workers with Gln/Gln, Gln/Arg, and Arg/Arg were 40.26±18.94, 38.03±28.22, and 32.01±11.65, respectively, higher than those in the non-asbestosis workers with the same genotypes (25.58±11.08, 37.08±14.74, and 29.38±10.15). There were significant differences in the comet scores between asbestosis and non-asbestosis workers with Gln/Gln by Student's t-test (P<0.05 or 0.01). Comet score in workers with Gln/Gln was higher than that in those with Arg/Arg in the asbestosis workers, and in the non-asbestosis workers exposed to asbestos, but without statistically significant difference.

TABLE 5

Effect of XRCC1 Polymorphisms on DNA Damage in Asbestos-exposed Workers ($\bar{x} \pm s$)

Groups	XRCC1		Comet Scores		
	Genotype	n (%)	Basal DNA Damage	H_2O_2-induced	After Repair
Asbestosis	Gln /Gln	23(45.10)	40.26±18.94*	162.36±23.03**	65.45±25.94*
	Arg /Arg	16(31.4)	32.01±11.65	129.79±33.15	47.26±12.04
	Gln/Arg	12(23.5)	38.03±28.22	140.54±27.72	53.22±15.44
Non-asbestosis	Gln /Gln	12(22.6)	25.58±11.08	118.57±46.23	46.58±18.27
	Arg /Arg	22(41.5)	29.38±10.15	123.28±39.30	44.04±14.58
	Gln/Arg	19(35.8)	37.08±14.74	127.40±36.27	43.17±14.62

Note. *Compared with non-asbestosis, P<0.05. **Compared with non-asbestosis, P<0.01.

DISCUSSION

Understanding of the mechanisms of DNA damage and repair has grown tremendously in recent years[25-26]. Comet assay helps us obtain information on inter-individual differences in DNA repair kinetics by the measurement of DNA damage at multiple time points. Due to its good sensitivity for detecting genetic damage at the individual cell level, the assay has been widely used in people exposed to occupational carcinogens. In the present study, asbestos exposure had obvious effects on increasing DNA damage level.

Repair of DNA damage protects cells from injuries by environmental toxins and is necessary for

the maintenance of genomic stability. Failure of this system may lead to development of cancer and other diseases, such as occupational diseases. In considering interaction of DNA damages and DNA repair capability at an individual level, the effect of asbestos exposure was observed. It seems that the repair capacity of DNA damage in asbestos-exposed workers was lower than that in control group.

Markers of genetic susceptibility are promising tools, since they can identify high risk profiles in relation to a particular environmental exposure. This allows the study of gene-environment or multiple gene-environment interactions. Hereditary defects in DNA repair may be associated with disease risk. Although our results did not show a significant interaction between XRCC1 399 Gln alleles in asbestosis risk, our data might be validated in larger population-based studies.

In studies about examining biomarkers of DNA damage associated with the codon 399 polymorphism, Lunn *et al.*[27] found that levels of both AFB1-DNA adducts and glycophorin A variants are higher in subjects with the Gln allele. Duell *et al.*[20] reported that the mean sister chromatid exchange (SCE) frequency is higher in current smokers homozygous for the codon 399 Gln allele than in non-smokers homozygous for the Arg allele[20]. Higher levels of NNK-induced SCE have been observed in cells with the Arg/Gln or Gln/Gln genotypes relative to cells with the Arg/Arg genotype[28]. These studies on adducts and SCE provide only indirect evidence that the change in codon 399 amino acid might be associated with decreased repair capacity. We investigated the relationship between polymorphisms of DNA repair gene and asbestos-induced DNA damage. The results revealed that DNA repair gene XRCC1 codon 399 is related to asbestos exposure and comet scores, suggesting that XRCC1 polymorphisms may play an important role in asbestos-induced DNA damage. Our study also supports previous epidemiological studies that asbestos exposure may play a role in asbestos-induced DNA damage. Interestingly, when gene-exposure interactions are considered, the XRCC1 399 Gln/Gln genotype is associated with DNA damage level in subjects with occupational asbestos exposure, especially in asbestosis workers. But we did not find any marked relationships between XRCC1 genotype and asbestosis risk. The conflicting results may stem from the complexity of disease etiology, concentration of asbestos exposure, DNA repair genotypes, limitations related to small sample size, and other genetic factors. Unfortunately, since it was difficult to collect exact exposure data, we cannot evaluate gene-exposure interactions.

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