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Polymorphisms in the TNF-α and IL10 Gene Promoters and Risk of Arsenic-Induced Skin Lesions and Other Nondermatological Health Effects

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In West Bengal, India, at present, more than 26 million people are exposed to arsenic through drinking water. Among them, only 15–20% manifest arsenic-induced noncancerous, precancerous, and cancerous skin lesions, indicating that genetic variants play important role in arsenic susceptibility. Chronic arsenic exposure has been associated with impairment of immune systems in the exposed individuals. Because cytokines are important immune mediators, alteration in expression of these gene products may lead to arsenic-specific disease manifestations. The aim of the present work was to investigate the association between the *TNF*-α −308G>A (rs1800629) and *IL10* −3575T>A (rs1800890) polymorphisms and arsenic-induced dermatological and nondermatological health outcomes. A case-control study was conducted in West Bengal, India, involving 207 cases with arsenic-induced skin lesions and 190 controls without skin lesions having similar arsenic exposure. The polymorphisms were determined using conventional PCR-sequencing method. ELISA was done to determine the serum levels of the two cytokines tumor necrosis factor α (TNF-α) and interleukin 10 (IL10). Associations between the polymorphisms studied and nondermatological health effects in the study subjects were determined from our epidemiological survey data. Individuals with GA/AA (−308 *TNF*-α) and TA/AA (−3575 *IL10*) genotypes were at higher risk of developing arsenic-induced skin lesions, ocular, and respiratory diseases. Also the −308 *TNF* A allele corresponded to a higher production of TNF-α, and −3575 *IL10* A allele corresponded to a lower production of IL10. Thus, the polymorphisms studied impart significant risk toward development of arsenic-induced dermatological and nondermatological health effects in the chronically exposed population of West Bengal, India.

Key Words: **arsenic; IL10; polymorphisms; skin lesions; susceptibility; TNF-α.**

The authors certify that all research involving human subjects was done under full compliance with all government policies and the Helsinki Declaration.

More than 70 countries around the world are affected by drinking arsenic-contaminated ground water (Mondal *et al.*, 2010). In West Bengal, India, more than 26 million individuals are chronically exposed to arsenic by drinking heavily contaminated ground water, which has arsenic content much above the maximum permissible limits (MPL) laid down by World Health Organization (WHO) of 10 μg/l (Chakraborty *et al.*, 2009). Ingestion of arsenic causes various types of benign skin lesions including raindrop pigmentation, hyperpigmentation, hyperkeratosis, as well as squamous cell carcinoma (SCC), basal cell carcinoma, and Bowens disease (IARC, 2004; Rahman *et al.*, 2003). Skin lesions are hallmarks of chronic arsenic toxicity. Among arsenic-exposed individuals, only 15–20% show arsenic-induced skin lesions (symptomatic), the remainder being asymptomatic. This difference in skin lesion incidence suggests that underlying genetic variability plays an important role in disease outcome. Chronic arsenic exposure is also associated with lung, liver, kidney, bladder cancer, and other noncancerous outcomes including conjunctivitis, peripheral neuropathy, respiratory problems, gastrointestinal problems, lung diseases, splenomegaly, anemia, and vascular diseases (Baidya *et al.*, 2006; Ghosh *et al.*, 2007; Guha Majumder, 2003; Mukherjee *et al.*, 2003).

Cytokines are important immune mediators, which are released by cells in response to specific stimuli and alter the behavior of same or other cells. Regulation of cytokine levels has been shown to be under genetic control through study of genetic polymorphisms in coding and promoter sequences and of certain allelic variants of cytokine genes that are associated with lower or higher cytokine production *in vitro* and *in vivo* (Gibson *et al.*, 2001; Wilson *et al.*, 1997). In recent years, single nucleotide

polymorphisms (SNPs) in the promoter regions of cytokine genes have been associated with several diseases, including infectious diseases (Hohler *et al*., 1998), T-cell–mediated diseases of the skin (Arkwright *et al*., 2001), and lymphoproliferative malignancy (Tsukaszki *et al*., 2001). Interleukin 10 (*IL10*) and tumor necrosis factor alpha (*TNF*-α) are good candidate genes to study the role of SNPs in the promoter regions because they code for immunoregulatory cytokines that are critical mediators of inflammation, apoptosis, and maintaining helper T cells that participate in cell mediated immunity/helper T cells that provide help for B cells and are essential for production of antibodies balance (Khatri and Caligiuri, 1998). Both IL10 and TNF-α have also been associated with different skin diseases (Ettehadi *et al*., 1994; Kingo *et al*., 2003). Recent findings suggest that −3575 T/A change in the *IL10* promoter and −308 G/A change in *TNF*-α have been associated with several immune-mediated diseases and cancers (Lan *et al*., 2006; Purdue *et al*., 2007; Rothman *et al*., 2006; Wilson *et al*., 1997).

We have been studying the relationship between health effects and cytogenetic damage, genetic variants, apoptosis, and macrophage functions in people exposed to arsenic through drinking water in West Bengal (Banerjee *et al*., 2007, 2008, 2009; De Chaudhuri *et al*., 2008; Ghosh *et al*., 2006, 2007). During our survey, we have found that arsenic-exposed people were more susceptible to opportunistic infections, suggesting that their immune systems might be impaired. Our studies confirmed that impairment of macrophage functions and increased death of immune cells by apoptosis contributed to immune system impairment (Banerjee *et al*., 2008, 2009). Because cytokines are important immune mediators, which control immune functions in humans, we have investigated the association of *IL10* and *TNF*-α polymorphisms (−3575 T/A *IL10* and −308 G/A *TNF*-α) with arsenic susceptibility in people chronically exposed to arsenic through drinking water in West Bengal, India.

MATERIALS AND METHODS

Study sites and sample selection. Three districts of West Bengal—North 24 Parganas, Nadia, and Murshidabad with severe arsenic contamination were chosen for this study. Drinking water in these areas had arsenic content much above the MPL limits set by WHO (10 μg/l). A total of 397 arsenic-exposed people, with at least 10 years of exposure, were chosen as study subjects. The study population was divided into 207 cases (i.e., symptomatic, individuals with skin lesions) and 190 controls (i.e., asymptomatic, no skin lesion individuals). During our survey, we carried out a detailed pedigree analysis for every subject, rejecting the selection of parent-offspring or siblings from same family to avoid genetic overmatching. The details of field survey and sample selection have been described (De Chaudhuri *et al*., 2008; Ghosh *et al*., 2007). Briefly, trained volunteers were sent to villages for door-to-door survey to identify individuals with arsenic-induced skin lesions and also to request the villagers to join the medical camps irrespective of the presence or absence of arsenic-induced skin lesions. An interview was performed based on a structured questionnaire that elicited information about demographic factors, lifestyle, occupation, diet, smoking, medical, and residential histories. A dermatologist identified the characteristic arsenic-induced skin lesions in the symptomatic individuals and also confirmed that the asymptomatic individuals did not have any of the arsenic-induced skin lesions. Then, specialists in the fields of neurology, ophthalmology, and respiratory diseases examined each participant to diagnose nondermatological health effects. Samples were collected only from those subjects who provided informed consent to participate. This study was conducted in accord with the Helsinki II Declaration and approved by the Institutional Ethics Committee.

Arsenic exposure assessment. All the study participants were provided with acid-washed (nitric acid-water [1:1]) polypropylene bottles for collection of drinking water. First morning voids (approximately 100 ml) were collected in precoded polypropylene bottles for arsenic determination. Immediately after collection, the samples were stored in salt-ice mixture and brought to the laboratory where they were kept at −20°C until arsenic estimation was performed by flow injection-hydride generation-atomic absorption spectrometry. The urine samples were filtered and diluted with deionized water as required. The urine samples were then quantified for arsenic using a mixture of trivalent, pentavalent arsenic, monomethyl arsenic acid, and dimethyl arsenic acid as the standard. Concentration of arsenic in the samples was determined from the standard curve obtained. Freeze-dried urine standard (certified value: 0.137 ± 0.011 mg/l) NIES CRM No. 18 from the National Institute of Standards and Technology was used to calibrate the instrument and as standard reference, and arsenic measurement was done employing the atomic absorption spectrometer (Perkin Elmer Analyst 700) instrument.

TNF-α (−308G/A) and IL10 (−3575T/A) promoter polymorphism genotyping. Blood samples were collected from all study participants by vein puncture method, and DNA extraction from blood was carried out using standard protocol (Sambrook *et al*., 1989). SNPs at positions −308 in TNF-α and −3575 in IL10 were determined by conventional PCR-sequencing method. PCR was performed in a 25-μl reaction volume using standard buffer, $MgCl_2$ (1.5mM), deoxyribonucleotides (200μM), and Taq polymerase supplied by Takara (Otsu, Shiga, Japan) with the following primers—for TNF-α, PCR was carried out with the following primers: TNF-α (forward), 5′-GCCCCTCCCAGTTCTAGTTC-3′ and TNF-α (reverse), 5′-AAAGTTGGGGACACACAAGC-3′ (Metabion, Martinsried, Germany) to generate a 248 bp product. Cycling was performed in Eppendorf Mastercycler (Hamburg, Germany) as follows: a pre-PCR step of 5 min denaturation at 94°C, followed by 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 58°C, 30 s extension at 72°C, and finally 5 min incubation at 72°C. For IL10, PCR was carried out with the following primers: IL10 (forward), 5′-GCTTTGGGCTTCTTGATGAG-3′ and IL10 (reverse), 5′-AAGAGCCCATGAAGAGAGG-3′ (Metabion) to generate a 414 bp product. Cycling was performed as follows: a pre-PCR step of 5 min denaturation at 94°C, followed by 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 54°C, 30 s extension at 72°C, and finally 5 min incubation at 72°C. All PCR products were analyzed by polyacrylamide gel (6%) electrophoresis, stained with ethidium bromide, and photographed under UV. Bidirectional sequencing was done in an ABI prism 3100 DNA sequencer (Applied Biosystem, Foster City, CA) using Big Dye Terminator, pretreated with Exo-SAP (Amersham Life Sciences, UK). Samples with ambiguous chromatograms were subjected to a second, independent round of amplification, followed by DNA sequencing, and obtained chromatograms were analyzed with Chromas 2.32 (Technelysium Pty Ltd, Tewantin, Australia).

Cytokine quantification. Serum levels of cytokines TNF-α and IL10 were measured in a subset of the total population under study. A total of 100 individuals were randomly chosen for measurement of serum TNF-α and IL10 levels. The individuals were matched with respect to age-sex-tobacco usage status. They were divided into four groups of 50 each, having GG or GA/AA genotype at −308 position of *TNF*-α and TT or TA/AA genotype at −3575 position of *IL10*. Serum samples were collected from coagulated blood. TNF-α and IL10 concentrations were measured by ELISA using TNF-α and IL10 ELISA kits from Thermo Scientific (Pierce Biotechnologies), according to manufacturer's instructions.

Identification of eye problems. Ophthalmologists examined the study participants for conjunctivitis and other eye diseases. Cases having history of mucopurulent discharge (characteristic of bacterial conjunctivitis), history of

severe watering and photophobia (characteristic of viral conjunctivitis), and history of severe itching and ropy discharge (characteristic of allergic conjunctivitis) were excluded from the study. Other symptoms found included pigmentation in the sclera, pterygium, pinguecula, and conjunctival congestion.

Identification of neurological symptoms. Neurologists examined the arsenic-exposed individuals for symptoms of peripheral motor and sensory neuropathy and for other neurological abnormalities as well. The criteria recorded for neurological problems were pain and paresthesias in stocking and glove distribution, numbness, weakness, muscle cramp, anesthesia, or hypoesthesia (no or reduced sensation) to touch, pain, temperature, pressure, vibration, calf tenderness, and deep tendon reflexes. Criteria for the exclusion of neurological problem, not due to arsenic exposure, were followed according to Mukherjee *et al.* (2003). Electrophysiological studies nerve conduction velocity and electromyograph test were performed clinically to confirm the probable as well as the doubtful cases.

Identification of respiratory problems. Pulmonologists recorded respiratory tract irritations including cough, hoarseness of voice, and irritation of throat that resulted in laryngitis. Dyspnea along with crepitations and ronchi were noted and recorded. Individuals with history of seasonal cough or bronchial asthma or family history with chronic bronchitis were excluded from the study.

Statistical analysis. Mann-Whitney test was performed to calculate statistically significant difference of age, arsenic content in water, urine, and serum levels of TNF-α and IL10 between the study populations. Chi-square test was used to compare the distribution of gender and tobacco usage between two groups. Odds ratio (OR), 95% confidence intervals, and two-tailed *p* values were calculated for assessing the risk of the variant genotype toward the development of skin lesions and health effects. Microsoft Excel and GraphPad InStat Software (Graphpad Software Inc., San Diego, CA) were used for the purpose.

TABLE 1
Demographic Characteristics of the Study Participants

Parameters	Exposed individuals Without skin lesions	Exposed individuals With skin lesions	*p* Value[a]
Total subjects (*N*)	190	207	
Male *N* (%)	91 (47.9)	106 (51.2)	
Female *N* (%)	99 (52.1)	101 (48.8)	
Age (mean ± SD)	38.5 ± 11.8 (18–70)	39.3 ± 12.1 (16–70)	0.84
Tobacco usage *N* (%)			
Tobacco user	69 (36.3)	82 (39.6)	
Tobacco nonuser	121 (63.7)	125 (60.4)	
Occupation *N* (%)			
Male			
Farmer	55 (60.4)	64 (60.4)	
Dailywage earner	23 (25.3)	27 (25.4)	
Business	3 (3.3)	5 (4.7)	
Teacher	1 (1.1)	2 (1.9)	
Student	6 (6.6)	6 (5.7)	
Service	3 (3.3)	2 (1.9)	
Female			
Housewife	90 (90.9)	93 (92.1)	
Student	2 (2.0)	2 (1.9)	
Farmer	7 (7.1)	6 (5.9)	
Concentration of arsenic in			
i) Water (μg/l)	143.2 ± 114.2	160.7 ± 159.4	0.97
ii) Urine (μg/l)	623.6 ± 540.3	660.9 ± 535.6	0.87

[a]*p* Values when compared with the exposed individuals without skin lesions by Mann-Whitney test.

RESULTS

Demographic Characteristics of the Study Participants

A total of 397 arsenic-exposed individuals from three districts of West Bengal (Nadia, North 24 Parganas, and Murshidabad) with severe arsenic contamination were chosen for this study. The exposed group was further divided into 207 individuals with arsenic-induced skin lesions (symptomatic individuals) and 190 individuals without any skin lesions (asymptomatic individuals) with similar arsenic exposures through drinking water. Descriptive characteristics of the symptomatic (cases) and asymptomatic individuals (controls) are summarized in Table 1. The results show that there are no significant differences in the arsenic contents of urine or drinking water between the symptomatic and asymptomatic groups. The average daily intake of arsenic from water is 2.01 μg/kg/day in the symptomatic group and 1.99 μg/kg/day in the asymptomatic group. There was no significant difference in average daily intake of arsenic between two groups. Water is the main source of arsenic intake in the study population. In our earlier study, we recruited 234 individuals from the same population for arsenic exposure assessment from different routes. In that study, we have estimated arsenic from different dietary sources. It was found that rice was the only staple food in this population, and except rice, there was no other food that would have been a significant source of arsenic to this population. Results showed that median exposure from rice was 0.84 μg/kg/day (Mondal *et al.*, 2010). The average ages of asymptomatic and symptomatic individuals are 38.5 ± 11.8 and 39.3 ± 12.1 years, respectively. The majority of the male individuals are farmers, and females are housewives, by occupation. Table 1 also shows that there are no significant differences in the age or gender distribution patterns, tobacco usage, or socioeconomic status between the study groups.

Association of Polymorphisms with Arsenic-Induced Skin Lesions

The genotype frequencies in the control and case groups are shown in Table 2. Our results show that for *TNF-α* (−308G>A) SNP, the presence of an "A" allele (GA/AA genotype) was significantly overrepresented (OR = 3.04 [1.78–5.21]) in the exposed individuals with skin lesions. Again, we have found that −3575T>A polymorphism in *IL10* gene promoter was also associated with arsenic-induced skin lesions. Here also, we have found that the presence of A allele (TA/AA genotype) was significantly (OR = 2.03 [1.26–3.28]) overrepresented in the exposed individuals with skin lesions. Both the groups (symptomatic and asymptomatic individuals) in our study population were matched with respect to age, sex, tobacco usage, and socioeconomic status (all of which might act as potential confounders). Because both the groups were well matched, adjustments for potential confounders were not

TABLE 2
Association of *TNF*-α (−308G>A) and *IL10* (−3575 T>A) Polymorphisms with Arsenic-Induced Skin Lesions

Genotype	Exposed individuals Without skin lesions *N* (%)	Exposed individuals With skin lesions *N* (%)	OR (95% CI)	*p* Values
TNF-α (−308G>A)				
GG	168 (88.4)	148 (71.6)	1.0 (Ref)	< 0.001
GA/AA	22 (11.6)	59 (28.5)	3.04 (1.78–5.21)	
IL10 (−3575 T>A)				
TT	157 (83.2)	145 (71.1)	1.0 (Ref)	0.0046
TA/AA	33 (16.8)	62 (28.9)	2.03 (1.26–3.28)	

Note. CI, confidence interval.

done. Power calculation was done using the OpenEpi, Version 2, open source calculator—PowerCC. For *TNF* (−308G>A) change, statistical power obtained is 99.11%, whereas for *IL10* (−3575T>A), statistical power obtained is 81.14%.

Association of Polymorphisms with Serum Cytokine Levels

Table 3 shows the levels of TNF-α and IL10 in the study populations corresponding with the *TNF*-α (−308GG or GA/AA) and *IL10* (−3575TT or TA/AA) genotypes. Higher TNF-α serum levels were associated with the *TNF*-α GA/AA genotypes. However, lower serum IL10 levels were associated with *IL10* TA/AA genotypes.

Association between Serum Levels of Cytokines and Arsenic-Induced Skin Lesions

Table 4 shows the association between the serum levels of cytokines and arsenic-induced skin lesions. We have divided our study population into three groups based on the serum levels of TNF-α and IL10. Group A included individuals with serum levels of the cytokines of 1–5 pg/ml, group B included individuals with serum levels of cytokines of > 5–10 pg/ml, and group C consisted of the same with cytokine concentration of greater than 10 pg/ml. Results show that severity of skin lesions increased with increase in serum levels of TNF-α and decreased with increase in serum levels of IL10 in the symptomatic individuals. "Mild," "moderate," and "severe" explain degree of severity of skin lesions in the symptomatic individuals.

TABLE 3
Relationship of *TNF*-α −308 G/A and *IL10* −3575T/A Polymorphisms with Secretion of TNF-α and IL10

	TNF-α (pg/ml)		IL10 (pg/ml)	
	Mean ± SD		Mean ± SD	
Study participants	GG	GA/AA	TT	TA/AA
Total study population	6.3 ± 6.4	15.3 ± 8.7*	8.2 ± 8.4	5.92 ± 5.3**
Asymptomatic	6.3 ± 6.8	15.0 ± 8.5*	9.2 ± 11.9	5.9 ± 7.2#
Symptomatic	6.3 ± 6.2	15.1 ± 9.1*	8.5 ± 5.7	4.9 ± 2.1*

*$p < 0.0001$ when compared with the GG group by Mann-Whitney test.
**$p = 0.0058$ when compared with the TT group by Mann-Whitney test.
#$p = 0.03$ when compared with the TT group by Mann-Whitney test.

Genotype-Phenotype Correlations

Genotype-phenotype correlations of conjunctivitis, peripheral neuropathy, and respiratory diseases with *TNF*-α and *IL10* polymorphisms are shown in Tables 5 and 6. Individuals with GA/AA genotype at −308 position of *TNF*-α had higher risk of developing conjunctivitis (OR = 5.15 [3.04–8.72]) and respiratory problems (OR = 2.30 [1.34–3.94]) compared with the individuals with GG genotype (Table 5). Peripheral neuropathy was equally present in both groups. Individuals carrying TA/AA genotype at −3575 position of *IL10* had higher risk of developing conjunctivitis (OR = 3.75 [2.32–6.07]) compared with the carriers of TT genotype (Table 6). Neither peripheral neuropathy nor respiratory problems displayed greater association with either *IL10* genotype group.

DISCUSSION

Although more than 26 million people in West Bengal are exposed to very high levels of arsenic, only 15–20% have arsenic-induced skin lesions, the hallmark signs of chronic arsenic exposure. This observation suggests that genetic variability plays a critical role in susceptibility toward arsenic toxicity. Chronic arsenic exposure was shown to affect immune systems of the exposed individuals (Banerjee *et al.*, 2009; Soto Pena *et al.*, 2006). Because cytokines are important immune mediators, we tested SNPs in the promoter regions of two cytokine genes for association with dermatological symptoms of arsenic toxicity. We chose polymorphisms in the promoter regions of the *TNF*-α (−308G>A) and *IL10* (−3575 T>A), which have been associated with a number of immune-mediated diseases, skin diseases, and cancers. To our knowledge, no association study relating these polymorphisms and arsenicosis has been reported. We also determined the association of these

TABLE 4
Association between Serum Levels of Cytokines and Severity of Arsenic-Induced Skin Lesions.

Groups of symptomatic individuals	TNF-α	IL10
Group A (1–5 pg/ml)	Mild	Severe
Group B (> 5–10 pg/ml)	Moderate	Moderate
Group C (> 10 pg/ml)	Severe	Mild

polymorphisms with nondermatological health effects and with serum levels of the respective cytokines in the arsenic-exposed individuals of West Bengal.

TNF-α is a multifunctional gene that produces proinflammatory cytokine TNF-α, which is associated with cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. The −308 G/A polymorphism in the promoter region of this gene has been widely studied and is associated with several diseases including different types of cancers. In a study by Duarte *et al.* (2005), it was found that *TNF-α* G −308 A polymorphism was associated with an increased risk of invasive cervical cancer. Results showed that women carrying the A allele presented a twofold increased risk of developing invasive cervical cancer compared with those homozygous for the G allele. In another case-control study by Akkiz *et al.* (2009), it was found that *TNF-α* G −308 A polymorphism was associated with an increased risk of hepatocellular carcinoma (HCC) in a Turkish population. Again, it was found that the AA/GA genotypes were significantly overrepresented in patients with oral SCC in German and Greek patients (Yapijakis *et al.*, 2009). In a recent study of the *TNF-α* G −308 A polymorphism, a significant association was found between the A allele and both gastric cancer and duodenal ulcer (Partida-Rodríguez *et al.*, 2010). In a study by Kesarwani *et al.* (2009) where other *TNF-α* promoter region polymorphisms were also studied, haplotype analysis revealed that *TNF-α* −308G was significantly associated with prostate cancer risk (OR = 2.22, $p = 0.013$). A modest association was found between cutaneous malignant melanoma and GG genotype in the British population (Howell *et al.*, 2002). Our results show that GA/AA genotype was significantly overrepresented in the arsenic-exposed individuals with skin lesions (symptomatic) compared with the individuals devoid of any such skin lesions (asymptomatic) (3.04 [1.78–5.21]).

TABLE 5
Frequency Distribution of Health Effects in Different Genotypic Groups of TNF-α in the Study Populations

	Total study population			
Parameters	GG *N*	GA/AA *N*	OR (95% CI)	*p* Values
Eye problems				
Present	92	55	**5.15**	
Absent	224	26	**(3.04–8.72)**	**< 0.0001**
Peripheral neuropathy				
Present	69	25	1.59	
Absent	247	56	(0.92–2.74)	0.11
Respiratory Problems				
Present	59	28	**2.30**	0.0039
Absent	257	53	**(1.34–3.94)**	

Note. CI, confidence interval. Bold figures indicate that the individuals with the particular genotype are at higher risk of developing the particular diseases.

TABLE 6
Frequency Distribution of Health Effects in Different Genotypic Groups of IL10 in the Study Populations

	Total study population			
Parameters	TT *N*	TA/AA *N*	OR (95% CI)	*p* Values
Eye problems				
Present	89	58	**3.75**	**< 0.0001**
Absent	213	37	**(2.32–6.07)**	
Peripheral neuropathy				
Present	70	24	1.12	
Absent	232	71	(0.65–1.91)	0.68
Respiratory problems				
Present	67	20	0.935	0.88
Absent	235	75	(0.53–1.64)	

Note. CI, confidence interval. The bold figures indicate that the individuals with the particular genotype are at higher risk of developing the particular diseases.

Because these two genotypes (GA/AA) are associated with arsenic-induced skin lesions, we decided to measure the serum levels of TNF-α in randomly selected 50 individuals who have GA or AA genotype and 50 individuals who have GG genotype. Individuals in both the groups were matched with respect to age, gender, and tobacco usage. Interestingly, we found that individuals having GA or AA genotype showed increased serum TNF-α levels relative to those with GG genotype. Our studies are in agreement with previous reports that individuals with GA or AA genotype had higher TNF-α levels (Suarez *et al.*, 2005; Wilson *et al.*, 1997) than those with GG genotype.

SNPs in the *IL10* promoter region also have been widely associated with various disease outcomes including immune-mediated disease and cancers (Bidwell *et al.*, 1999; Haukim *et al.*, 2002; Purdue *et al.*, 2007). In some of these cancers (e.g., cutaneous malignant melanoma, noncardiac gastric cancer, and renal cell carcinoma), genotypes associated with low IL10 expression were a risk factor for disease development or disease progression, whereas in others (e.g., cervical cancer, cardiac gastric cancer, HCC after hepatitis B virus infection, posttransplant SCC of the skin, and multiple myeloma), genotypes or haplotypes associated with high IL10 expression were a risk factor (Bidwell *et al.*, 1999). In a population-based case-control study, conducted in New South Wales, Australia, it was found

that *IL10* −3575T>A polymorphism was associated with elevated risk of non-Hodgkin's lymphoma (Purdue *et al.*, 2007). *IL10* −3575A allele (both TA/AA genotypes) was significantly overrepresented in patients having diffused large B cell lymphoma. In another study by Lan *et al.* (2006), it was also found that −3575 AA genotype was significantly associated with an increased risk of B cell lymphomas. Rothman *et al.* (2006) also found that *TNF-α* −308 and IL10 −3575 polymorphisms provided significant risk of developing non-Hodgkin's lymphoma. We have found that the TA/AA genotype at −3575 was significantly overrepresented in the symptomatic individuals (OR = 2.03 [1.26–3.28]). Thus, we conclude that the −3575T>A polymorphism in the *IL10* promoter might contribute to susceptibility toward formation of premalignant skin lesions in the arsenic-exposed population, which may progress into arsenic-induced skin cancers.

Because the individuals having TA/AA genotype were at higher risk of developing arsenic-induced skin lesions, we randomly chose 50 individuals from each group (TA/AA or TT) and measured IL10 levels in their sera. We found that individuals with TA/AA genotype (at −3575) showed lower IL10 levels than those with TT genotype, which is consistent with previous findings where homozygosity for the haplotype with A at both −3575 and −2763 was associated with lower IL10 production (Gibson *et al.*, 2001).

The actions of cytokines may be profoundly regulated by the presence of other cytokines, particularly in the case of TNF-α and IL10, which are mutually regulated and have complex and predominantly opposing roles in systemic inflammatory responses. Several pathologies have been associated with differential expression of these two cytokines (López *et al.*, 2006; Suarez *et al.*, 2005). Previous studies indicate that elevated TNF-α levels were associated with common inflammatory skin diseases (Ettehadi *et al.*, 1994) and lower serum levels of IL10 were found in psoriatic skin lesions (Kingo *et al.*, 2003). We have also found that severity of skin lesions increase with increase in serum levels of TNF-α and decrease with increase in serum levels of IL10 in the symptomatic individuals, which support previous findings. It might be that lower production of IL10, a potent downregulator of TNF-α and other proinflammatory cytokines, might increase the risk of developing arsenic-induced precancerous, cancerous, and noncancerous skin lesions in the symptomatic individuals by less efficiently suppressing the proinflammatory cytokine production in them. Our observations of higher TNF-α levels in individuals with GA/AA genotype and lower IL10 levels with TA/AA genotypes, which were significantly overrepresented in the symptomatic individuals, further support this hypothesis.

When the association of these SNPs and nondermatological health effects was tested, it was found that the individuals carrying the TNF-α A allele (GA/AA genotype) had significantly higher risk of developing conjunctivitis or respiratory problems and also had higher TNF-α in their sera. These associations are consistent with the proinflammatory properties of TNF-α. The increased risk of respiratory diseases in the individuals carrying GA/AA genotype may be due to the increased TNF-α levels resulting in increased inflammation of the respiratory tract. Higher incidences of ocular problems in the individuals with GA/AA genotype also might be due to the increased inflammatory conditions in the eyes due to increased TNF-α levels. We found that the *IL10* −3575 TA/AA genotype was associated with higher risk of developing eye diseases and with decreased production of IL10. The decreased production of anti-inflammatory cytokine IL10 is likely related to the increased production of the proinflammatory cytokine TNF-α and thus helps explaining the increased inflammatory conditions associated with the main ocular problem, conjunctivitis, in our study population. Our findings are supported by previous studies where promoter polymorphisms in *IL10* have been associated with ocular diseases (Atan *et al.*, 2005). Moreover, IL10 has been used as a therapeutic agent in various inflammatory conditions of the eye like autoimmune uveoretinitis (Rizzo *et al.*, 1998) and experimental autoimmune uveitis (Broderick *et al.*, 2005), which further supports that lower IL10 production is associated with different inflammatory conditions of the eyes. However, none of the variant alleles were associated with other diseases in our study participants. This might be due to the fact that both the TNF-α and the IL10 are low-penetrating genes, and their associations with different disease outcomes might be as a result of interactions with other genes and environmental factors.

Although this is a pilot study, the results justify further larger cohort-based molecular epidemiological surveys, which will be able to delve deeper into the molecular mechanisms by which these genetic polymorphisms bring about the ultimate arsenic susceptible phenotype in a subsection of the exposed individuals. Haplotype analyses would certainly be important in this regard as they would be able to elucidate the effect of diversity of the genes under study with higher resolution than would any single marker but were, however, beyond the scope of the present study. In a population-based case-control study by Purdue *et al.* (2007), analyses of SNPs in *IL10* (−3575T>A, −1082A>G, −819C>T, and −592C>A) demonstrated that carriers of *IL10* −3575A and −1082G variants had significantly elevated levels of diffuse large cell B lymphoma. Another study also supports this notion as both AGCC and TATA haplotypes (for similar analyses of SNPs in *IL10*) were found to be associated with increased B-cell lymphoma (Lan *et al.*, 2006). Another point that deserves special mention, but has not been covered in the present manuscript, is that there is a possibility that the contribution of *TNF-α* SNP (−308G>A) to arsenic-induced skin effects may be confounded by the linkage disequilibrium within nearby human leukocyte antigen (HLA) genes. Association of −308A (*TNF-α*) with HLA genes has been found with many diseases (mainly autoimmune diseases) previously. Individuals of European ancestry are known to exhibit linkage disequilibrium between −308A and HLA-DR3 alleles in subacute cutaneous lupus erythematosus patients (Werth *et al.*, 2000). Contrastingly, in another study,

Werth *et al.* (2002) found no increase in the association of −308A and HLA-DR3 in Caucasians with dermatomyositis compared with the controls. Higher TNF-α serum levels have been found in Caucasians with the HLA-DR3 allele because of the linkage disequilibrium with −308A, and individuals homozygous for −308A have higher serum TNF-α levels than −308G homozygotes (Bouma *et al*., 1996; Jacob *et al*., 1990). Wilson *et al.* (1993) found strong association between the *TNF2* (A) allele and *HLA A1*, B8, and DR3 alleles and concluded that the above associations were due to linkage disequilibrium because of the close proximity of *TNF-α* gene to those genes in the major histocompatibility complex. Stable interindividual production rates for *TNF-α* have been demonstrated (Molvig *et al*., 1988), and in addition, production rate has been shown to correlate with DR alleles. DR2-positive individuals produce low levels, whereas DR3- and DR4-positive individuals produced high levels of *TNF-α* (Jacob *et al*., 1990). Thus, further refinement of the observations of this pilot study in larger population-based surveys, taking care of this potential confounder, is expected to shed further light on the causal relationship between the promoter polymorphism of the genes studied and susceptibility to arsenic toxicity.

To conclude, we might point out that both *TNF-α* (−308G>A) and *IL10* (−3575 T>A) SNPs render individuals susceptible toward developing arsenic-induced skin lesions, which have the potential to develop into cancerous skin lesions. The risk alleles were associated with differential expression of TNF-α and IL10, which might be partially responsible for the development of the arsenic-induced skin lesions, ocular, and respiratory diseases in individuals chronically exposed to arsenic through drinking water. Thus, these polymorphisms may act as biomarkers to determine arsenic susceptibility in future. This work has a far-reaching impact in monitoring the harmful effects of arsenic to which human systems are often exposed. To our knowledge, this is the first attempt to find the association between these polymorphisms and arsenic-induced dermatological and nondermatological health effects in a population chronically exposed to arsenic through drinking water.

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