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Precancerous and non-cancer disease endpoints of chronic arsenic exposure: the level of chromosomal damage and XRCC3 T241M polymorphism

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Abstract

Genetic variants are expected to play an important role in arsenic susceptibility. Our previous study revealed deficient DNA repair capacity to be a susceptibility factor for arsenicism. T241M polymorphism in *XRCC3* (a homologous recombination repair pathway gene) is widely studied for its association with several cancers. We have investigated the association of XRCC3 T241M polymorphism with arsenic-induced precancerous and non-cancer disease outcomes. The present study evaluated the association of T241M polymorphism with arsenic-induced skin lesions, peripheral neuropathy (neurodegenerative changes), conjunctivitis and other ocular diseases. A case-control study was conducted in West Bengal, India, involving 206 cases with arsenic-induced skin lesions and 215 controls without arsenic-induced skin lesions having similar arsenic exposure. XRCC3 T241M polymorphism was determined using conventional PCR-sequencing method. Chromosomal aberration assay, arsenic-induced neuropathy and ocular diseases were also evaluated. The data revealed that presence of at least one Met allele (Met/Met or Thr/Met) was protective towards development of arsenic-induced skin lesions [OR= 0.45, 95% CI: 0.30 – 0.67], peripheral neuropathy [OR=0.49; 95%CI: 0.30–0.82] and conjunctivitis [OR=0.60; 95%CI: 0.40–0.92]. A significant correlation was also observed between protective genotype and decreased frequency of chromosomal aberrations. Thus the results indicate the protective role of Met allele against the arsenic-induced skin lesions, chromosomal instability, peripheral neuropathy and conjunctivitis.

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Keywords

arsenic susceptibility; chromosomal instability; neuropathy; respiratory diseases; XRCC3 T241M polymorphism

1. Introduction

An estimated 26 million people in West Bengal, India have been chronically exposed to inorganic arsenic, a class I human carcinogen, by drinking water contaminated with arsenic above 10 µg/L [1]. A number of epidemiological studies report an increased risk of cancer in arsenic exposed populations [2–4]. The most common consequence caused by ingestion of arsenic-contaminated water is the development of various typical skin lesions like raindrop pigmentation on chest, back, and legs, hyperpigmentation known as ‘melanosis’ or bilateral–palmar plantar thickening known as ‘hyperkeratosis’. The latter is considered a premalignant lesion ultimately leading to development of Bowen’s disease, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) [5,6]. Besides skin lesions, which are recognized as the most sensitive end points of chronic arsenicism, malignancies in several other organs including lung, bladder, liver and kidney also are reported [7]. In addition to these cancer outcomes, noncancer outcomes of chronic arsenic toxicity include weakness, gastrointestinal problems, respiratory problems, sensorimotor peripheral neuropathy, lung disease, and splenomegaly [8–11]. Among several ocular diseases, conjunctivitis was more prevalent in arsenic exposed population in West Bengal [12–14].

Only 15–20% of people with similar arsenic exposure in West Bengal exhibit arsenic-induced skin lesions. This inter-individual variability suggests a major role of underlying genetic variability as a cause for differential susceptibility to arsenic toxicity. Although, the precise mechanism of arsenic-induced carcinogenicity is still under investigation, some key players have already been identified; including chromosomal aberrations, aneuploidy, oxidative stress, altered growth factors, altered DNA methylation, cell proliferation and DNA repair [15].

Reactive oxygen species (ROS) mediated oxidative damage has been shown to be involved in a number of diseases in addition to cancer, including neuro-degenerative disorders (e.g. Parkinson’s disease and Alzheimer’s disease), cardiovascular disease and several ocular diseases [16]. Arsenic enhances generation of ROS which may contribute to arsenic-induced clastogenesis. Direct attack by ROS on DNA can result in single and double strand breaks (DSBs). DSBs are potentially lethal DNA damage and are recombinogenic leading to chromosomal aberrations [17]. DSBs are processed by recombination mechanisms including non-homologous end-joining (NHEJ) and homologous recombination repair (HRR). NHEJ often results in loss of DNA sequence and chromosomal rearrangements. Error prone repair by NHEJ can induce permanent changes leading to cancer or to severely impaired cellular functioning eventually causing cell death by triggering apoptosis or irreversible cell growth arrest [18]. HRR uses an intact homologous region of the sister chromatid for usually error free repair of a DSB [19]. Thus, impairment of HRR may lead to erroneous rejoining of broken DNA strands by NHEJ resulting in genomic instability. Chromosomal instability induced by arsenic includes chromosomal aberrations [20–22]. Arsenic and its metabolites also can suppress DNA repair gene expression [23] and induce centrosome amplification [24]. These factors probably interact with individual differences in DNA repair capacity and contribute to the association of inefficient DNA repair capacity and enhanced chromosomal aberrations with arsenic susceptibility [25].

XRCC3 (X-ray repair cross-complementing group 3) is a key HRR gene. Cells mutated for *XRCC3* are much more prone to DNA damage and subsequent chromosomal breakage [26]. Cells deficient in *XRCC3* are sensitive to X-rays [27]. Among several *XRCC3* single nucleotide polymorphisms (SNPs), a nucleotide transition from C to T in exon 8 results in an amino acid substitution of Met for Thr at codon 241 (T241M). Variant gene allele *XRCC3* 241Met showed increased chromosomal deletions after X-irradiation [28]. However, the T241M allele is proficient in HRR, but is associated with centrosome amplification and a lack of apoptosis [29]. This SNP is extensively studied for its potential association with risk for several cancers.

Arsenic is a well established clastogen. Thus, alterations in the recombination repair response to DSBs resulting from arsenic exposure may play a role in susceptibility to arsenic-induced diseases. In the current study, we investigated the association of *XRCC3* codon 241 polymorphism (T241M) with arsenic-induced skin lesions, chromosomal instability and its involvement in peripheral neuropathy, respiratory diseases and conjunctivitis.

2. Materials and Methods

2.1. Study sites and subject selection

Arsenic level in ground water was found to be severe in several districts of West Bengal. The three most affected districts viz. North 24 Parganas, Nadia and Murshidabad were selected for the present study. The study subjects were selected from different villages including four villages from four administrative blocks of North 24 Parganas, two villages from Haringhata block Nadia and 5 villages from 5 different blocks of Murshidabad district. In brief, trained volunteers were sent to the villages to request the villagers to attend the medical camp, and also to identify individuals with skin lesions and to ask them to participate in the research study with informed consent. The response rate was about 90% among the participants. Information regarding age, gender, tobacco usage (both smoking and chewing), occupation, food habits, source of drinking water and medical history were obtained from questionnaire-generated data. Informed consent of the study participants was obtained before sample collection. The detailed procedures of field survey, strategy for genetically unrelated case-control selection, as well as sample collection have been described in our previous studies [30]. After clinical diagnosis by dermatologists, the arsenic exposed individuals were divided into 2 groups, individuals with cutaneous signs of arsenicism including raindrop pigmentation, palmer and planter hyperkeratosis, hypo- and hyper-pigmentation and ulcerative lesions, and individuals without sign of arsenic-related skin lesions. The present study includes a total of 421 genetically unrelated arsenic-exposed individuals, including 206 individuals with (cases) and 215 individuals without skin lesions (control). Individuals ranging from 15–60 years with at least 10 years of exposure were included as our study participants. The study protocols adhered to the tenets of the Declaration of Helsinki II and was approved by the Institutional Review Board.

2.2. Arsenic exposure assessment

We have assessed arsenic content in drinking water as well as urine of all selected participants. Flow injection-hydride generation-atomic absorption spectrometry (FI-HG-AAS) was performed using a Model Analyst-700 spectrometer equipped with a Hewlett-Packard (Houston, TX) Vectra computer with GEM software, Perkin-Elmer EDL System-2, arsenic lamp (lamp current 380 mA).

2.3. XRCC3 genotyping (T241M, rs 861539)

DNA extraction from blood mononuclear cells was carried out using standard protocol [31]. Polymerase chain reaction (PCR) was performed in a 25 µl reaction volume using standard buffer, MgCl₂ (1.5 mM), deoxyribonucleotides (200 µM) and Taq polymerase supplied by Takara (Otsu, Shiga, Japan) with the following primers: XRCC3 (Forward), 5'-TTC AGA CGG TCG AGT GAC AG -3'; and XRCC3 (Reverse) 5'-CCG CAT CCT GGC TAA AAA TA' (Metabion, Martinsried, Germany) to generate a 359 base pair 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 sec denaturation at 94°C, 30 sec annealing at 60°C, 30 sec extension at 72°C, and finally 5 min incubation at 72°C. PCR products were analyzed by polyacrylamide gel (6%) electrophoresis followed by bi-directional DNA sequencing [30].

2.4. Chromosomal aberrations assay

Lymphocyte culture was carried out following standard protocol as described earlier at 72 h, since arsenic delays cell cycle [22, 32]. Two hour before harvesting; colchicine was added to block the cells in metaphase. Cells were harvested and slides were stained with Giemsa stain (pH 6.8). Code number was assigned to each slide and they were scored blindly. Well spread metaphases with 46 ± 2 chromosomes were scored for CA (50–100 metaphases per sample) [33]. Both chromosome type (ring, dicentric, trisomic, centric fusion and chromosomal deletion and exchanges) and chromatid type of aberrations (chromatid break, deletion, and exchanges) were scored. Data were expressed both as total CA per cell and percentage of aberrant cells.

2.5. Arsenic-induced health effects

In the epidemiological survey, three major diseases were identified other than skin lesions i.e. (i) peripheral neuropathy, (ii) respiratory diseases and (iii) eye problems.

2.5.1. Identification of arsenic-induced neurological abnormalities—

Neurologists from our team recorded the criteria for neurological problems viz 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 in our participants. Care was taken to eliminate neuropathy from other possible sources such as alcoholism, nutritional deficiency neuropathies, tick paralysis, paralytic shell fish poisoning, uremia and so on [10].

2.5.2. Respiratory diseases—Ingestion of inorganic arsenic causes respiratory tract irritations including cough, chest sounds in lungs and shortness of breath (SOB). SOB along with crepitations and ronchi were found in interstitial lung disease due to arsenicosis. Cases with seasonal cough or chronic bronchitis were excluded from the study.

2.5.3. Identification of arsenic-induced eye diseases—We found recurrent cases of eye problems in our exposed study population. Hence, a systematic survey was conducted to determine whether chronic arsenic ingestion led to the development of any specific eye problems. Irritation, watering and redness in both eyes were often reported by the study participants. Conjunctivitis due to chronic arsenic ingestion had been reported in only a few populations previously [12,13]. An experienced ophthalmologist meticulously screened the study participants for conjunctivitis and other eye diseases. The cases having history of mucopurulent discharge (characteristic of bacterial conjunctivitis), severe watering and photophobia (characteristic of viral conjunctivitis), and severe itching and ropy discharge

(characteristic of allergic conjunctivitis) were exempted. Other symptoms screened included pigmentation in the sclera, pterygium, pinguecula and conjunctival congestion.

2.6. Statistical analyses

Mann-Whitney test was performed to compare the central tendencies of continuous independent variables (like age, arsenic content in water, urine, nail and hair) between cases and controls, as the data distribution for these parameters was not normally distributed. Chi-square test was used to compare the distribution of gender and tobacco usage between the two groups. For chromosomal aberration (percentage of aberrant cells, and CA/cell), mean and standard deviation were calculated. Odd's Ratio (OR), 95% confidence Intervals (CI) and two-tailed p values were estimated for assessing the risk of the variant genotype towards the development of skin lesions and other health effects. All the statistical analyses were performed using GraphPad InStat Software (Graphpad Software Inc., San Diego, CA).

3. Results

3.1. Demographic features

Descriptive characteristics of the total study population are summarized in Table 1. Age, gender and socio-demographic characteristics are similar in arsenic-exposed groups with (cases) and without (control) skin lesions. No significant differences were found between the groups in age, gender distribution or tobacco usage. Both the cases and control were exposed to arsenic at a similar extent, as evident by the arsenic content in water, urine, nail and hair. However, arsenic-induced health effects including peripheral neuropathy, respiratory problems and eye diseases were found to be significantly higher in cases compared to control individuals.

3.2. T241M polymorphism is associated with decreased incidence of arsenic-induced skin lesions

The allele and genotype frequencies in the control and case groups are shown in Table 2. For T241M polymorphism, both populations are in Hardy-Weinberg equilibrium. The distribution of the T allele (Met), encoding the T241M polymorphism, was significantly associated with the decreased risk for the development of skin lesions [OR=0.52, 95% CI: 0.37–0.72]. Odds ratios for the CT, TT as also combined (CT/TT) genotypes were calculated taking the CC genotype as reference (Table 2). Since, no additional protection was offered by TT genotype over the CT genotype, we have combined the genotypes for the variant allele (CT/TT combined), and subsequently evaluated the effect of this combined genotype on the other parameters (chromosomal aberration and health effects) with CC as the reference genotype.

3.3. The variant allele is associated with lower level of chromosomal aberrations

Table 3 shows the distribution of chromosomal aberration (CA) in cases and controls with different XRCC3 codon 241 genotype. A statistically significant decrease in CA (both parameters) was observed in individuals carrying at least one variant allele (C/T and T/T genotypes combined) compared to those having C/C genotype in either of the two study groups as well as in the combined study population.

3.4. Protection is offered by the variant allele towards the prevalence of conjunctivitis and peripheral neuropathy

Genotype-phenotype correlation (T241M with arsenic-induced health effects) of the combined population (cases and controls taken together) is detailed in Table 4. In this case, we have subdivided the combined study population with respect to their genotype only.

Individuals carrying at least one variant allele were found to be less prone towards arsenic-related conjunctivitis [OR=0.60; 95% CI: 0.40–0.92] as well as peripheral neuropathy [OR=0.49; 95% CI: 0.30–0.82]. However, the variant allele was not associated with resistance to respiratory diseases.

4. Discussion

XRCC3 is structurally related to and interacts with RAD51 playing an important role in HRR of DSB. HRR restores integrity of broken DNA through recombination with the homologous region of the second chromatid contained in a diploid cell [34]. This conscription of the undamaged copy of DNA involves XRCC3 which assembles and stabilizes RAD51. RAD51 polymerizes onto DNA to form a nucleofilament and searches for homologous DNA [35]. Cells with mutant *XRCC3* are hypersensitive to DNA strand breaks and show chromosomal instability [26]. This instability may be a consequence of the essential role of XRCC3 in centrosome maintenance [36]. Two polymorphisms in XRCC3 have different phenotypes. XRCC3 (D213N) is defective in HRR and causes an increase in spontaneous apoptosis, whereas XRCC3 (T241M) is proficient in HRR but causes an increase in centrosome amplification with no increased apoptosis [29]. XRCC3(D213N) is not associated with increased cancer risk whereas XRCC3(T241M) has been associated with increased cancer risk in some studies [29]. The differential effects of these two polymorphisms on cancer risk have been attributed to the difference in apoptosis induction. The increased apoptosis seen with XRCC3 (D213N) has been suggested to remove cells that would otherwise develop into cancers.

The T241M substitution in XRCC3 is non-conservative and not in the ATP-binding domains, the only domains functional in HRR that have been identified so far [37]. The fact that this polymorphism is associated with centrosome amplification suggests that it is in a domain that plays a role in centrosome stabilization. Thus, T241M might affect cancer risk by increasing centrosome amplification that leads to chromosomal aberrations. That this polymorphism may have functional consequences is supported by the SIFT prediction, which highlighted the possible damaging role of this substitution [38].

The T241M polymorphism is widely studied for its association with susceptibility to various types of cancer and DNA damage induced responses with mixed results. T241M was found to modulate the risk towards the development of melanoma skin cancer [39], bladder cancer [40,41] and also breast cancer in an Anglo-Saxon population [42]. In contrast, no association was observed with breast cancer in a Canadian population [43], gastric cancer [44] or benzene exposure induced hematotoxicity in Chinese populations [45], or with radiation-induced G2 phase delay in peripheral blood lymphocytes of American non-cancer patients [46]. However, a meta-analysis study including 24,975 cancer patients and 34,209 controls having Met/Met genotype showed an overall very small cancer risk for non-melanoma cancer, under a recessive genetic model [47].

Intriguingly, in our studies of the West Bengal population, the T241M allele, present as either Thr/Met or Met/Met genotype, was shown to impart decreased risk towards susceptibility to precancerous and non-cancerous skin lesions associated with arsenicism. Consistent with our findings, the Met allele was associated with decreased risk of BCC as well as with multiple BCC in a European population [48], with both SCC and BCC in the Nurses' Health Study [49], and also in female smokers with decreased risk of lung cancer [50]. In addition to skin cancer, chronic arsenic exposure gives rise to several non-cancer health effects, including non-malignant skin lesions, conjunctivitis, peripheral neuropathy and respiratory diseases [14]. Oxidative DNA damage is thought to play a major role in several ocular and neurological disorders [16, 51]. In addition to conjunctivitis, we also

observed conjunctival congestion, conjunctival melanosis, pinguecula, pterygium, and pigmentary changes in our arsenic exposed population.

The current and long-term arsenic exposure history, as measured by water and urine, and nail and hair, respectively, clearly reflect that both the case and control study populations are exposed to a similar extent. The two study populations are also matched with respect to other probable confounders like age, gender and tobacco usage. Despite this, controls are at significantly lower risk for development of each of the three disease outcomes. Concomitantly, the cases have a significantly lower distribution of individuals with the protection genotypes (CT/TT). These observations suggest that the over-representation of the protection genotypes in the controls might be the key factor behind their apparent resistance to developing non-cancer disease outcomes compared to the cases.

Genome stability is of primary importance for the survival and proper functioning of all organisms. HRR serves as a high-fidelity repair mechanism for the repair of DSBs and thus eliminates chromosomal aberrations before cell division [18]. We showed that individuals with arsenic-induced skin lesions have compromised DNA repair capacity compared to those without skin lesions [25]. Thus, one might hypothesize that the T241M polymorphism might impart a better DNA repair efficacy. However, in a functional study, the homologous directed recombination defect of XRCC3 deficient cells was complemented by the T241M variant to a similar extent as the wild type [52]. Thus, improved HRR in cells bearing T241M is unlikely. An alternative is that the increased centrosome amplification seen with XRCC3 (T241M) enhances the centrosome amplification [29] and mitotic catastrophe-associated apoptosis induced by arsenic [24,29]. This enhanced apoptosis, which occurs in arsenic exposed individuals, might eliminate cells with unrepaired DSBs, which ultimately reduces the incidence of CA [53]. In our present study the reduction of CA in the variant genotypes (CT & TT) compared to wild type (CC) fully support this earlier observation.

XRCC3 does not appear to be a major risk factor, but being a low-penetration susceptibility gene, its association with several cancers may be an outcome of interaction with other genes or environmental exposures. The apparent inconsistencies in association of *XRCC3* (T241M) with cancer risk is likely a consequence of the dual roles of XRCC3 in both HRR and centrosome maintenance. Thus, the inconsistencies in observation may be attributed to interaction between XRCC3 (T241M) and polymorphic alleles of other DNA repair genes, or this polymorphism might be in linkage disequilibrium with another gene in cancer association. In the present study, the most likely effector acting in combination with XRCC3 (T241M) is arsenic exposure which, like XRCC3 (T241M), induces centrosome amplification resulting in elimination of damaged cells by apoptosis, similar to the effect of XRCC3 (D213N).

5. Conclusion

In conclusion, our study assesses the potential contribution of XRCC3 (T241M) to modifying disease outcome of chronic arsenicism. To the best of our knowledge, we are the first group to relate this T241M polymorphism with arsenic-induced skin lesions, chromosomal instability, peripheral neuropathy and conjunctivitis. Further research of functional interaction of this allele with arsenic exposure may elucidate potential mechanisms of arsenic susceptibility.

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Abbreviations

CA	chromosomal aberration
CI	confidence interval
DSB	double strand break
FI-HG-AAS	flow injection-hydride generation-atomic absorption spectrometry
HRR	homologous recombination repair
OR	odds ratio
ROS	reactive oxygen species
SNP	single nucleotide polymorphism
XRCC3	X-ray repair cross-complementing group 3

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Table 1

Descriptive characteristics of the study participants.

Parameters	Control [N (%)]	Case [N (%)]
Total subjects	215	206
Male	100 (46.51)	104 (50.49)
Female	115 (53.49)	102 (49.51)
Age in years [Mean $\pm$ SD]	36.76 $\pm$ 12.31	38.49 $\pm$ 11.76
Overall tobacco usage	71 (33.02)	78 (37.86)
Smoking (bidi / cigarette)	45 (20.93)	50 (24.27)
Tobacco chewing	16 (7.44)	17 (8.25)
Both	10 (4.65)	11 (5.34)
Non-tobacco users	144 (66.98)	128 (62.13)
Occupation		
Cultivation	72 (33.48)	72 (34.95)
Business	13 (6.04)	14 (6.79)
Daily wage earners	14 (6.51)	10 (4.85)
Service	6 (2.79)	6 (2.91)
Teacher	5 (2.32)	5 (2.43)
Student	5 (2.32)	2 (0.97)
Unemployed	7 (3.25)	7 (3.39)
Housewife	93 (80.86)	90 (88.24)
Arsenic content [Mean $\pm$ SD]		
Drinking water ($\mu\text{g/l}$)	138.08 $\pm$ 171.82	151.74 $\pm$ 202.20
Urine ($\mu\text{g/l}$)	291.83 $\pm$ 332.63	306.97 $\pm$ 425.30
Nail ($\mu\text{g/g}$)	2.59 $\pm$ 1.63	3.43 $\pm$ 2.85
Hair ($\mu\text{g/g}$)	1.76 $\pm$ 1.24	2.06 $\pm$ 1.55
Arsenic-induced health effects		
Peripheral neuropathy	21 (9.77)	74 (35.92) *
Respiratory disease	13 (6.05)	43 (20.87) *
Eye problems	31 (14.42)	125 (60.68) *

*
p<0.001 (Chi-square test, 2-sided p value)

Table 2Association of *XRCC3* T241M polymorphism with arsenic-induced skin lesions.

<i>XRCC3</i> T241M Polymorphism	Control ^a [N (%)]	Case ^a [N (%)]	Odds ratio (95% CI) ^b	p value
C allele	303(72.49)	344(83.49)	1.00 (reference)	
T allele	115(27.51)	68 (16.50)	0.52(0.37–0.72)	0.0002
CC	107(51.19)	144(69.9)	1.00 (reference)	
CT	89	56	0.47 (0.31–0.71)	0.0004
TT	13	06	0.34 (0.12–0.93)	0.033
CT/TT	102(48.8)	62(30.1)	0.45(0.30–0.67)	0.0001

^aCase =206, control =215. Discrepancy in the number of genotyped data in control is due to PCR failure even after multiple independent efforts.

^bCI: confidence interval.

Table 3

Association of *XRCC3* T241M polymorphism with frequency of chromosomal aberrations (CA/cell) and percentage of aberrant cells in cases and control group

Genotype (<i>XRCC3</i> T241M Polymorphism)	CA/cell (mean $\pm$ SD)	% Aberrant cells (mean $\pm$ SD)
Control group		
CC	0.085 $\pm$ 0.02	8.074 $\pm$ 2.41
CT/TT	0.075 $\pm$ 0.02 **	7.212 $\pm$ 1.95 **
Case Group		
CC	0.105 $\pm$ 0.03	9.873 $\pm$ 2.91
CT/TT	0.096 $\pm$ 0.02 *	8.990 $\pm$ 2.37 *
Total study population		
CC	0.097 $\pm$ 0.03	9.017 $\pm$ 2.84
CT/TT	0.083 $\pm$ 0.02 ***	7.870 $\pm$ 2.28 ***

*
p<0.05;

**
p<0.01;

p<0.0001,

Mann-Whitney test (two tailed p value)

Table 4

Association between *XRCC3* T241M genotype and arsenic-induced health effects in combined study population

Parameters	CC genotype N (%)	CT/TT genotype N (%)	OR [95% CI] ^a
Peripheral neuropathy			
Present	69 (27.5)	26(15.85)	0.49[0.30–0.82] **
Absent	182 (72.5)	138(84.15)	
Conjunctivitis			
Present	105 (41.83)	50(30.49)	0.60[0.40–0.92] *
Absent	146 (58.17)	114(69.51)	
Respiratory Diseases			
Present	35 (13.94)	20(12.2)	0.85[0.47–1.54]
Absent	216 (86.06)	144 (87.8)	

^aOR and 95% CI have been calculated for (CT and TT genotypes combined), for each disease outcome, taking CC as the reference group.

*
p<0.05;

**
p<0.01