Original Articles

N-acetyltransferase and cytochrome P450 2E1 gene polymorphisms and susceptibility to antituberculosis drug hepatotoxicity in an Indian population

SWAPNIL MISHRA, SUNILBARAN DASCHAKRABORTY, PALLAVI SHUKLA, PRERNA KAPOOR, RAKESH AGGARWAL

ABSTRACT

Background. Antituberculosis drug hepatotoxicity (ATDH) is common in India. Isoniazid, a constituent of most antituberculosis drug regimens, is metabolized by N-acetyltransferase (NAT2) and cytochrome P450 2E1 (CYP2E1) enzymes. We therefore studied the association of some single-nucleotide polymorphisms (SNPs) in these enzyme genes with ATDH.

Methods. Allelic and genotypic frequencies at three SNP loci in the *NAT2* gene (rs1799929, rs1799930 and rs1799931) and one locus (rs2031920) in the *CYP2E1* gene were studied using restriction fragment length polymorphism in 33 patients who developed ATDH following an isoniazid-containing antituberculosis drug regimen and 173 healthy blood donors. After confirming adherence of the control data to the Hardy–Weinberg equilibrium model, genotype and allele frequencies in the two groups were compared.

Results. For SNP rs1799930 in the *NAT2* gene, 7 (21%), 21 (64%) and 5 (15%) patients, and 93 (54%), 62 (36%) and 18 (10%) controls had GG, GA and AA genotypes, respectively (p=0.003; odds ratio [OR] for GA *v.* GG=4.50 [95% CI 1.80–11.22] and for AA *v.* GG=3.69 [1.05–12.93]). Allele frequency for G nucleotides for this SNP was 0.53 among patients and 0.72 among controls (OR 2.24 [1.31–3.84], p=0.007). The allele and genotype frequencies of the other *NAT2* SNPs and the *CYP2E1* SNP showed no significant difference between cases and controls. All the 33 patients and 151 (87%) of 173 controls had mutant allele at one or more of the three *NAT2* SNP loci (p=0.03). The presence of two or more mutant alleles, a marker of slow acetylator status, was more frequent in patients (23/33 [70%]) than in controls (73/173 [42%]; OR 3.23 [95% CI 1.45–7.19], p=0.004).

Conclusion. In India, the risk of ATDH is increased in persons with 'A' allele at SNP rs1799930 in the *NAT2* gene, but is not associated with rs2031920 polymorphism in the *CYP2E1* gene.

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INTRODUCTION

Tuberculosis continues to be a major cause of disease burden and mortality around the world, particularly in developing countries. WHO estimates that, in the year 2009, nearly 9.4 million new cases of tuberculosis occurred worldwide and that 1.7 million people died from tuberculosis; these included 1.1 million new cases and 380 000 deaths among those with HIV infection.[1] In recent years, outcome of tuberculosis infection has markedly improved through the development of effective antituberculosis drugs (ATDs) and shorter-duration treatment regimens. However, the ATD treatment is associated with frequent occurrence of adverse events, in particular ATD-induced hepatotoxicity (ATDH).[2–8] The occurrence of these liver-related adverse events is one of the major reasons for discontinuation of ATDs, and is associated with a poorer outcome of tuberculosis.

The frequency of ATDH has varied widely between different studies, ranging from 1% to 36%.[2–8] This wide variation is related in part to variations in the nature and doses of ATD used, and in the definitions used for ATDH. However, a part of this variation is related to host factors that may influence the risk of an individual to develop such liver injury.

Several host factors have been studied for their influence on the risk of ATDH. Of these, older age, early childhood, female gender, alcohol use, baseline liver function test abnormalities, malnutrition, hypoalbuminaemia, infection with hepatitis B virus, hepatitis C virus or HIV, and prior liver transplant surgery have been shown to be associated with an increased risk of ATDH in some studies. However, other studies have failed to find such an association.[9] The most consistent association of risk of ATDH has been with genetic factors that influence the enzymes that metabolize ATDs, in particular isoniazid.[9,10]

Initial studies suggested that persons with rapid acetylation phenotype were more vulnerable to ATDH.[11,12] This was explained

by a faster rate of acetylation of isoniazid to acetylhydrazine, a toxic metabolite. However, subsequent studies showed that slow acetylators rather than rapid acetylators had a higher risk of ATDH,[10,13–17] and a propensity to develop more severe hepatotoxicity.[10] This was compatible with the knowledge that acetylation is also involved in the conversion of toxic acetylhydrazine to non-toxic metabolites. Similarly, some studies have shown an association between *cytochrome P450 2E1* (*CYP 2E1*) genetic polymorphism and susceptibility to ATDH[13] as well as its severity.[17]

Nearly one-fifth of the new cases of tuberculosis in the world occur in India.[1] In addition, the frequency of ATDH appears to be higher in Indian patients[4,5] than in those from western countries.[7,8] In a pooled analysis of data from four prospective Indian studies, Pande *et al*. calculated the risk of ATDH in Indian patients to be 11.5% (95% CI 9.5%–13.5%),[3] compared to a risk of 4.3% (3.4%–5.3%) in a meta-analysis of 14 studies from the West.[18] In India, ATDs are the most common cause of drug-induced liver injury.[6] Thus, it may be important to determine factors associated with ATDH in the Indian population.

We therefore studied *NAT2* and *CYP2E1* genetic polymorphisms in a group of patients with ATDH in comparison with healthy controls to determine whether these polymorphisms were associated with the occurrence of ATDH in a northern Indian population.

METHODS

Study subjects

This case–control study prospectively enrolled patients attending the outpatient clinics or inpatient services of our institution with evidence of symptomatic hepatic injury (jaundice, or prominent nausea and vomiting, or both) that appeared after the initiation of ATD treatment from 2008 to 2010. ATDH was defined as (i) an increase in serum alanine transaminase (ALT) or aspartate transaminase (AST) level above three times the upper limit of normal (ULN) after beginning the ATD treatment, using a slight modification, to make the diagnosis more stringent, of the criteria (above twice the ULN) laid down by an International Consensus Meeting;[19] (ii) absence of serological evidence of infection with hepatitis A, B, C or E viruses, and (iii) normalization of the serum transaminase level after discontinuation of isoniazid and other hepatotoxic drugs. All the patients had been receiving oral isoniazid (300 mg), rifampicin (600 mg or 450 mg depending on whether their body weight was ≥50 kg or <50 kg), pyrazinamide (20 mg/kg body weight) and ethambutol (800 mg) daily, at the time of development of ATDH.

All patients underwent routine laboratory investigations, including haemogram and liver function tests (serum bilirubin, AST, ALT, alkaline phosphatase, serum total protein and serum albumin levels). Abdominal ultrasonography was done in all patients to look for features of biliary obstruction and chronic liver disease; any patients with such features were excluded. Markers for infection with hepatitis viruses were tested using commercial enzyme immunoassays, and included immunoglobulin (Ig) M anti-hepatitis A virus (BioMérieux, Marcy I'Etoile, France), hepatitis B surface antigen (BioMérieux), anti-hepatitis C virus antibodies (Hepanostika HCV Ultra, Beijing United Biomedical, Shanghai, China) and IgM anti-hepatitis E virus antibody (MP Diagnostics, Singapore).

Patients with one or more of the following conditions were also excluded: (i) alcoholic liver disease or habitual alcohol consumption; (ii) evidence of chronic liver disease; (iii) abnormal serum ALT, AST or bilirubin levels before the start of ATD; (iv) administration of other potentially hepatotoxic drugs (e.g. methotrexate, phenytoin, valproate, fluconazole); and (v) those declining informed consent.

From each study subject, 2 ml of blood was collected in EDTA. From this, genomic DNA was extracted using commercial DNA extraction kits (QIAmp DNA Mini Kit; Qiagen, Valencia, CA, USA). Each study subject provided written informed consent. An institutional ethics committee approved the study protocol. Anonymized residual blood specimens from a group of healthy blood donors were used as controls.

Determination of NAT2 polymorphism

Polymerase chain reaction (PCR) with restriction fragment length polymorphism (RFLP) was used to genotype *NAT2* polymorphisms as reported previously.[20] In brief, PCR was done in a 50 μL reaction mixture that contained 20 ng of genomic DNA, 1.5 mM $MgCl_2$, 200 μM deoxynucleotide triphosphates, 0.2 μM each of forward (GGAACAAATTGGACTTGG) and reverse (TCTAGC ATGAATCACTCTGC) primers, and 1.5 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). The cycling conditions used were: initial denaturation at 94 ºC for 5 minutes, 35 cycles of denaturation at 94 ºC for 30 seconds, annealing at 57 ºC for 30 seconds and extension at 72 ºC for 90 seconds, followed by a final extension at 72 ºC for 5 minutes. After verification of amplification, the PCR products were digested separately with each of three restriction endonuclease enzymes, KpnI, TaqI and BamHI (New England Biolabs, Ipswich, MA, USA). The digested products were subjected to electrophoresis on a 2% agarose gel, and visualized using a UV transilluminator and gel documentation system (BioRad, Rishon Le Zion, Israel) after ethidium bromide staining. Table I shows the restriction sites and fragment sizes for the wild-type and mutant alleles for each single-nucleotide polymorphism (SNP).

Loss of KpnI, TaqI and BamHI restriction sites denotes the presence of NAT2*5, NAT2*6 and NAT2*7 alleles, respectively. Further, the presence of any two mutant alleles defines the slow acetylator genotype; in contrast, rapid acetylators have either no or only one such allele.[10]

Determination of CYP2E1 polymorphism

This was done using PCR with RFLP, as described previously.[13] The PCR conditions were similar to those for *NAT2*, except for the primers used (forward: 5´-TTCATTCTGTCTTCTAACTGG-3´, reverse: 5´-CCAGTCGAGTCTACATTGTCA-3´). The PCR products were digested using RsaI (Table I), and each individual was classified into one of three CYP2E1 genotypes, namely c1/c1, c1/c2, and c2/c2, based on the presence of wild (c1) and mutant (c2) alleles.[11]

Confirmation of RFLP results with sequencing

PCR products of a few specimens showing different alleles at each locus using the RFLP technique were sequenced in both directions using the dideoxynucleotide triphosphate chain termination method.

Sample size calculation

Sample size calculation was done using the Quanto software (version 1.2.4; available at *http://hydra.usc.edu/gxe/request.htm*) for SNP rs1799929. For this calculation, we assumed the minor allele frequency to be 30% (based on data from HapMap database [*http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap27_B36/#search*] and SNP database [*http://www.ncbi.nlm.nih.gov/*

snp?term=rs1799929], an unmatched study design with case-to-control ratio of 1:5, a relative risk associated with variant allele of 2.25, a log additive model (risk increasing with number of variant alleles), 2-sided alpha error of 0.05 and study power of 0.80. The required sample size was 31 cases and 165 controls.

Statistical analysis

For each SNP, data from control subjects were tested to confirm their compliance with the Hardy–Weinberg equilibrium model. Data for allele and genotype frequencies at each SNP locus in patients and controls were compared using the chi-square test.

RESULTS

Subjects

Thirty-three patients with ATDH were enrolled. Their mean (SD) age was 38 (13.5) years and 17 (52%) were men. The median treatment duration with ATDs before the diagnosis of liver injury was 4 (range 2–16) weeks. The median bilirubin level in patients with ATDH was 1.8 (range 0.4–25.0) mg/dl; of the 33 patients, 12 (36%) had discernible jaundice. The median serum ALT and AST levels were 188 (range 33–1200; ULN 40) IU/ml and 178 (118–997; ULN 40) IU/ml, respectively. For each patient, levels of at least one of the transaminases exceeded three-fold the ULN.

In addition, residual blood specimens from 173 healthy blood donors were studied. All these specimens tested negative for hepatitis B surface antigen, anti-hepatitis C virus antibody and antibodies to HIV.

All the cases and controls were of northern Indian origin.

NAT2 gene SNP data

Table II shows the results of genotype frequencies of each of the three SNP loci studied. For SNP rs1799930, both genotype and allele frequencies showed significant differences between patients with ATDH and healthy controls. The odds ratio for the association of heterozygous genotype (GA) as compared to homozygous wild-type genotype (GG) for the occurrence of ATDH was 4.50 (95% CI 1.80–11.22), and that for the association of homozygous mutant genotype (AA) as compared to GG genotype was 3.69 (95% CI 1.05–12.93).

TABLE I. Single nucleotide polymorphism (SNP) loci studied, enzymes used for restriction digestion, and band patterns expected for each (wild and mutant) allele

Gene	SNP ID	Restriction enzyme	Expected sizes of restriction digestion fragments	
			Wild-type allele	Mutant allele
NAT2	rs1799929;14240C>T	KpnI	658, 434	1092
	rs1799930;14349G>A	TaqI	170, 226, 316, 380	316, 380, 396
	rs1799931;14616G>A	BamHI	282, 810	1092
CYP 2E1	rs2031920; 3979C>T	RsaI	61, 352	413

NAT2 N-acetyltransferase 2 CYP 2E1 cytochrome P450 2E1

TABLE II. Genotype and allele frequencies at various single nucleotide polymorphism (SNP) loci studied in patients with antituberculosis drug-induced hepatotoxicity and healthy people

A. Genotype frequencies

Gene	SNP ID	Genotype	Patients *n*=33 (%)	Controls *n*=173 (%)	Odds ratio (95% CI) *v.* wild type	Overall p value
NAT2	rs1799929;14240C>T	WW	15 (45.5)	79 (45.7)	–	0.167
		MW	18 (54.5)	78 (45.1)	1.22 (0.57–2.58)	
		MM	0	16 (9.2)	0	
	rs1799930;14349G>A*	WW	7 (21.2)	93 (53.8)	–	0.003
		MW	21 (63.6)	62 (35.8)	4.50 (1.80–11.22)	
		MM	5 (15.2)	18 (10.4)	3.69 (1.05–12.93)	
	rs1799931;14616G>A	WW	25 (75.8)	152 (87.9)	–	0.102
		MW	8 (24.2)	19 (11)	1.83 (0.74–4.52)	
		MM	0	2 (1.1)	0	
CYP2E1	rs20319203979C>T	WW	31 (93.9)	168 (97.1)	–	0.459
		MW	2 (6.1)	4 (2.3)	2.71 (0.48–15.44)	
		MM	0	1 (0.6)	0	

B. Allele frequencies

Gene	SNP ID	Allele	Patients *n*=33 (%)	Controls *n*=173 (%)	Odds ratio (95% CI) *v.* wild type	p value
NAT2	rs1799929;14240C>T	W	48 (72.7)	236 (68.2)	–	0.467
		M	18 (27.3)	110 (31.8)	0.80 (0.45–1.45)	
	rs1799930;14349G>A*	W	35 (53.0)	248 (71.7)	–	0.003
		M	31 (47.0)	98 (28.3)	2.24 (1.31–3.84)	
	rs1799931;14616G>A	W	58 (87.9)	323 (93.4)	–	0.122
		M	8 (12.1)	23 (6.6)	1.94 (0.83–4.54)	
CYP2E1	rs20319203979C>T	W	64 (97.0)	340 (98.3)	–	0.484
		M	2 (3.0)	6 (1.7)	1.77 (0.35–8.97)	

NAT2 N-acetyltransferase 2 CYP2E1 cytochrome P450 2E1 W wild-type allele M mutant allele WW homozygous for wild-type allele MW heterozygous MM homozygous for mutant allele *p=0.003 for genotype frequencies and p=0.007 for allele frequencies

TABLE III. N-acetyltransferase 2 (NAT2) acetylator status in patients and controls

NAT2 acetylator status	Patients (*n*=33)	Controls (*n*=173)	Odds ratio (95% CI)	p value
Slow acetylator	23 (70%)	73 (42%)	3.23 (1.45–7.19)	0.019
Rapid acetylator	10 (30%)	100 (58%)	Referent	

For the other two *NAT2* gene SNP loci, no significant difference was found in either genotype frequencies or phenotype frequencies between the two groups.

Based on the *NAT2* genotype data, 23 (70%) of 33 patients and 73 (42%) of 173 controls had slow acetylator status, i.e. presence of two or more mutant alleles at the three *NAT2* SNPs studied. Ten (30%) patients and 100 (58%) controls were defined as rapid acetylators. Patients with ATDH had a significantly higher frequency of slow acetylator genotype than controls (Table III) with an odds ratio of 3.15 (1.41–7.02).

CYP2E1 polymorphism and ATDH

Of 33 patients with ATDH, 31 (94%) had c1/c1 genotype and 2 (6%) had either c1/c2 or c2/c2 genotype (Table II). In comparison, 168 of 173 (97%) controls had c1/c1 genotype and 5 (3%) had either c1/c2 or c2/c2 genotype. The genotype and allelic frequencies for this SNP locus were similar in the two groups.

Sequencing data

Dideoxynucleotide triphosphate sequencing confirmed the results of RFLP analysis at each of the four SNPs.

DISCUSSION

Our results show that SNPs associated with the *NAT2* gene, in particular SNP rs1799930 in which 'G' nucleotide at position 14349 is replaced by 'A' nucleotide, were associated with the occurrence of ATDH. The association of ATDH with mutant 'A' allele at SNP rs1799930 was observed on comparison of both allelic and phenotype frequencies. However, no association of *CYP2E1* genotypes or alleles with ATDH was found.

The *NAT2* gene product plays an important role in the metabolism of isoniazid. This enzyme is a primary metabolizer of isoniazid and converts most of isoniazid into acetyl-isoniazid, which then undergoes hydrolysis to produce acetylhydrazine. A small fraction of isoniazid is also metabolized by enzyme amidase.[21] Acetylhydrazine is then further metabolized by NAT2 to diacetylhydrazine, a non-toxic compound. Some acetylhydrazine is also oxidized into hepatotoxic intermediaries by CYP2E1.[10,13] Thus, variations in the NAT2 activity can influence the rates of both generation and removal of acetylhydrazine, and those in CYP2E1 can affect the levels of acetylhydrazine and its hepatotoxic oxidative derivatives.

Polymorphisms in the *NAT2* gene have been shown to alter the metabolism of ATD. The rs1799930 polymorphism in the *NAT2* gene, which was found to be significantly more frequent among patients with ATDH than healthy subjects in our study, is associated with a change in the amino acid sequence of the *NAT2* gene product. The mutant allele, also known as NAT2*6A, was first identified in 1991 by Vastis,[22] and is associated with replacement of an arginine residue at amino acid location 197 with glutamine. This amino acid change possibly alters the folding of the NAT2 protein, and has been shown to be associated with reduced activity of the NAT2 enzyme.[23] This slow acetylation may lead to accumulation of acetylhydrazine due to its slow downstream metabolism, leading to liver injury.

The rs1799931 polymorphism is associated with a glycine to glutamic acid change in amino acid 286 of the NAT2 protein; the mutant allele in this polymorphism is known as NAT2*7B. The third *NAT2* polymorphism studied, rs1799929 is a synonymous mutation which does not change the NAT2 protein *per se*, but is associated with another polymorphism (rs1801280) which is associated with a T to C nucleotide change that leads to replacement of an isoleucine at amino acid 114 by threonine, and characterizes NAT2*5A. Both NAT2*7B and NAT2*5A are associated with a slow acetylator phenotype.

These *NAT2* polymorphisms associated with slow acetylator phenotype have been shown to be associated with occurrence of ATDH in diverse populations. The association was first identified in a Japanese population study that included 77 patients with ATDH.[24] Similar findings have subsequently been reported from other parts of Asia, in studies that included 33 and 18 patients from Taiwan[10] and Korea,[17] respectively. All these studies compared the overall frequency of slow acetylator genotypes between patients and controls, rather than changes at individual loci. More recently, similar data have been reported from Brazil[25] and Turkey.[26] In these studies, comparisons at individual loci were done and the association with ATDH was the strongest for NAT2*6 slow acetylator allele. To date, only one study, conducted in Europe[27] has failed to find an association between the slow NAT2 acetylator status and occurrence of ATDH. A recent meta-analysis[28] has also confirmed the association between the slow NAT2 acetylator status and ATDH.

Our data extend these findings further and show that the 'A' allele at rs1799930 polymorphism or NAT2*6A allele is associated with ATDH in the Indian population too. Another recent study[29] also found a similar association of this allele with ATDH, making this finding more robust.

The relationship of the *CYP2E1* gene SNP that we studied with ATDH has been more variable in the previously published data. The *CYP2E1* c1/c1 genotype has a higher activity than that of the *CYP2E1* c1/c2 or c2/c2 genotype; the former may therefore be a risk factor for ATDH through an increased generation of oxidative metabolites of acetylhydrazine.[13] In a study from Taiwan, Huang *et al.* showed that those persons who are homozygous for wild-type *CYP2E1* had an increased risk of ATDH.[13] However, in another study from the same population, this genotype did not have an increased risk of ATDH, though they were more likely to develop severe hepatitis.[15] Other studies, including one from India, have failed to show an association between c1/c2 *CYP2E1* polymorphism and ATDH.[29,30] In our study, no association was found between c1/c2 *CYP2E1* locus genotype and occurrence of ATDH. In another Indian study, a different SNP site in *CYP2E1* was associated with ATDH,[29] but we did not study that SNP.

To study the effect of a mutant allele on the occurrence of a disease condition, two factors are important, namely the degree of extra risk that it confers for the disease and its frequency in the general population. If a mutant allele has a low population frequency, its effect is more difficult to assess and the fraction of a disease attributable to this allele can be expected to be smaller. The mutant 'A' allele at SNP rs1799930 had a high frequency in

our control subjects (28%). In view of its high frequency and strong association with the occurrence of ATDH, its overall contribution to the occurrence of ATDH in the Indian population is likely to be high. Of the remaining SNPs studied for which no significant difference was found between patients and controls, one (rs1799929) had a high frequency (32%) of the mutant allele. Failure to find an association for this allele indicates that the contribution of this allele to ATDH is likely to be low. On the other hand, failure to demonstrate an association of ATDH for the remaining NAT2 SNP (rs1799931) and the *CYP2E1* SNP (rs2031920) may not exclude a role for these in ATDH since the frequencies of these genes in our population was low (7% and 2%, respectively). To determine the role of these SNPs in causation of ATDH, much larger studies would be necessary.

In addition to *NAT2* and *CYP2E1*, polymorphisms in genes for two enzymes belonging to the glutathione S-transferase family, namely GSTM1 and GSTT1, have also been studied in patients with ATDH. In an Indian study, patients with ATDH had a higher frequency of homozygous 'null' mutation at the *GSTM1* gene than healthy controls.[31] However, in another study, no association between hepatotoxicity and GSTM1 or GSTT1 null genotypes was found.[32] We did not study polymorphisms in these genes.

Another limitation of our study is that we used a healthy control group of blood donors. Admittedly, it would have been preferable to use as controls those persons who complete the ATD treatment without developing ATDH. However, this limitation does not invalidate our results. The use of a control group such as ours would be expected to dilute the association between a genetic factor studied and disease condition. Thus, the true association between *NAT2* SNP and ATDH would be expected to be somewhat stronger than what we measured. Also, the use of healthy controls and cases with tuberculosis implies that observations noted in our study could arise if *NAT2* gene polymorphisms were associated with the occurrence of tuberculosis. However, in a detailed literature review, we did not come across any published data on the association of tuberculosis with polymorphisms in this gene. This, combined with data from other studies, suggests that the association that we observed was related to ATDH.

The accumulating data on the association of genetic polymorphisms with ATDH may have clinical implications. Attempts should be made to incorporate testing for *NAT2* gene polymorphism in clinical algorithms for treatment of tuberculosis. Similar algorithms have been developed on the basis of pharmacogenomic differences in the metabolism of other drugs, such as azathioprine. The use of such algorithms for ATD treatment would require the development of cheap and easy methods to detect the less frequent *NAT2* alleles, and to demonstrate that such testing is helpful and cost-effective at the population level. This can be done by undertaking large randomized trials where SNP testing is done in one group but not in the other group.

Currently, DNA tests on mycobacteria in clinical specimens to detect drug resistance are being introduced for use before drug therapy is started. Since the assays for human SNPs are based on similar DNA techniques, it should not be difficult to do these using the same laboratory facilities. Thus, if and when the decision to include these SNP tests in a clinical algorithm is taken, it should be possible to make these assays available widely at a low cost.

In conclusion, our data indicate that, in the Indian population where both tuberculosis and ATDH are common, genetic polymorphisms in the *NAT2* gene associated with slow acetylator status are at an increased risk for development of ATDH. However, we failed to find any association between CYP2E1 polymorphism and ATDH. Further work should be undertaken to define this association better and to find out whether determination of these genotypic markers before starting ATD treatment can help guide the treatment and monitoring of such patients, and reduce the frequency of ATDH in a cost-effective manner. This may enable completion of treatment in a larger proportion of patients with tuberculosis, and reduce relapse rates of this serious infection.

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Conflict of interest. None.

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As part of an Indo-US Collaboration, the National Institutes of Health, USA has sponsored a series of workshops since 2006 on various aspects of clinical research (with an emphasis on clinical trials), including biostatistics, study design and randomization issues, data management, research ethics, and regulatory aspects.

As a continuation of this series, three workshops are planned at SGPGI, Lucknow during 2014 as follows: (i) Workshop on 'Basic Biostatistics' on July 18-20, 2014, (iii) Workshop on 'Observational Studies' on September 19-21, 2014 (exact dates to be announced later) and (iii) Workshop on 'Scientific Paper Writing' in December 2014 (exact dates to be announced later).

The workshops are aimed at active biomedical researchers who hold faculty positions. Investigators involved in clinical research, who are in a position to lead clinical research studies, should find this workshop useful. This email is to seek your help in dissemination of information about the workshop. I request you to kindly circulate this information in your institution, particularly among those who have appropriate expertise and experience to benefit from the workshop.

Only a limited number of applicants will be accepted for each workshop. Applicants should download an application format (asks for summary of their experience and expertise in clinical researchin a structured format) from *https://sites.google.com/site/sgpginihcourses/* and email it **as an email attachment** to *sgpgi.courses@gmail.com.* The last date for applications for the three courses are February 28, May 31 and July 31, 2014, respectively. A selection committee will notify the successful applicants of acceptance about 4 weeks before each course.

There is no registration fee, and twin-shared guest house accommodation and boarding will be provided without any charge. However, participants need to fund their travel through their personal funds, their institutions or other sources. We may be able to fund travel for a few qualified applicants whose institution cannot cover their expenses; however, in view of limited funds, this will be possible only in exceptional cases.

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