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N-acetyltransferase 2 (*NAT2*) gene polymorphism as a predisposing factor for phenytoin intoxication in tuberculous meningitis or tuberculoma patients having seizures - A pilot study

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***Background & objectives*: Simultaneous administration of phenytoin and isoniazid (INH) in tuberculous meningitis (TBM) or tuberculoma patients with seizures results in higher plasma phenytoin level and thus phenytoin intoxication. N-acetyltransferase 2 (NAT2) enzyme catalyses two acetylation reactions in INH metabolism and *NAT2* gene polymorphism leads to slow and rapid acetylators. The present study was aimed to evaluate the effect of allelic variants of *N*-acetyltransferase 2 (*NAT2*) gene as a predisposing factor for phenytoin toxicity in patients with TBM or tuberculoma having seizures, and taking INH and phenytoin simultaneously.**

***Methods*: Sixty patients with TBM or tuberculoma with seizures and taking INH and phenytoin simultaneously for a minimum period of seven days were included in study. Plasma phenytoin was measured by high performance liquid chromatography. *NAT2* gene polymorphism was studied using restriction fragment length polymorphism and allele specific PCR.**

***Results*: The patients were grouped into those having phenytoin intoxication and those with normal phenytoin level, and also classified as rapid or slow acetylators by *NAT2* genotyping. Genotypic analysis showed that of the seven SNPs (single nucleotide polymorphisms) of *NAT2* gene studied, six mutations were found to be associated with phenytoin intoxication. For rs1041983 (C282T), rs1799929 (C481T), rs1799931 (G857A), rs1799930 (G590A), rs1208 (A803G) and rs1801280 (T341C) allelic variants, the proportion of homozygous mutant was higher in phenytoin intoxicated group than in phenytoin non-intoxicated group.**

***Interpretation & conclusions*: Homozygous mutant allele of *NAT2* gene at 481site may act as a predisposing factor for phenytoin intoxication among TBM or tuberculoma patients having seizures.**

Key words Adverse drug interaction - *NAT2* gene polymorphism - phenytoin intoxication - rapid acetylator genotypes - seizures - tuberculoma - tuberculous meningitis

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Central nervous system (CNS) tuberculosis (TB) is a serious, often fatal form of tuberculosis, predominantly affecting young children. Tuberculous meningitis (TBM) or tuberculoma accounts for 70 to 80 per cent cases of neurological tuberculosis[1]. Incidence of meningitis in patients with tuberculosis has been reported to vary from 7.4 to 11.8 per cent from various centres in India[2,3]. Seizures are a potential complication in 10-20 per cent of children, 10-15 per cent of adults and more than 50 per cent can develop seizures during their initial hospitalization[4,5]. Phenytoin is one of the most widely prescribed anti-convulsant drugs for treatment and prevention of seizures[6]. It is metabolized extensively by Cytochrome P450 2C9 (CYP2C9) and to small extent by CYP2C19[7]. The narrow therapeutic index, the wide inter-individual variability in the rate of phenytoin metabolism, clearance and saturation (zero-order) pharmacokinetics of phenytoin are responsible for the observed dose-related toxicity.

Isoniazid (INH) is one of the most effective antimycobacterial drugs. Two acetylation reactions in the metabolism of INH are catalyzed by *N*-acetyltransferase 2 (NAT2) which shows genetic polymorphism, resulting in two distinct phenotypes, *i.e.* slow and rapid acetylators[8]. Till date, *NAT2* loci have been shown to express 36 alleles, resulting from the existence of numerous single nucleotide polymorphisms (SNPs). Seven missense (G191A, T341C, A434C, G590A, A803G, A845C and G857A) and four silent (T111C, C282T, C481T and C759T) substitutions have been identified in the *NAT2* coding exon. Absence of any of these substitutions is considered as the wild-type allele (rapid acetylator). *NAT2* alleles containing the G191A, T341C, A434C, G590A, and/or G857A missense substitutions are associated with slow acetylator phenotype[9].

INH is also a microsomal enzyme inhibitor, and inhibits the metabolism of co-administered drugs such as acetaminophen, carbamazepine, diazepam, phenytoin, theophylline and warfarin resulting in their high plasma concentration[10]. In TBM or tuberculoma patients with seizures, INH and phenytoin are simultaneously administered. If these patients are slow acetylators, possibility of phenytoin intoxication becomes high[11].

Pharmacogenomics may enable the identification of responders, non-responders, or patients at an increased risk of toxicity in response to drug administration[12,13]. Phenotypic assessment of acetylator status by INH pharmacokinetic study alone is not sufficient to solve the problem. It is essential to find out frequencies of the *NAT2* allelic variants in patients taking INH and phenytoin simultaneously. Hence, *NAT2* genotyping is recommended in larger prospective trials to elucidate the role of *NAT2* genetic polymorphism in phenytoin intoxication in TBM or tuberculoma patients. Various studies[14-16] have shown difference in frequencies and distribution of various alleles of *NAT-2* gene among Indians. We have demonstrated a correlation between the INH levels and phenytoin toxicity in TBM or tuberculoma patients in our earlier study[17]. Therefore, the aim of this pilot study was to elucidate whether any allelic variant of *NAT2* gene acted as a predisposing factor for phenytoin toxicity in patients with TBM or tuberculoma taking INH and phenytoin simultaneously. The objectives were to determine the presence of phenytoin intoxication, to analyze seven [rs1801279 (191G-A), rs1041983 (282C-T), rs1799929 (481C-T), rs1799930 (590G-A), rs1208 (803A-G), rs1799931 (857G-A) & rs1801280 (341T-C)] alleles of *NAT2* gene and to investigate association of these alleles with phenytoin intoxication in these patients.

Material & Methods

Chemicals and drugs: Sucrose, magnesium chloride ($MgCl_2$), tris-HCl, triton-X100, sodium chloride (NaCl), ethylenediamine tetraacetic acid (EDTA), sodium dodecyl sulphate (SDS), glucose, citric acid, sodium citrate, chloroform, ethanol, agarose, glycerol, bromophenol blue, boric acid, ethidium bromide, glacial acetic acid and benzene were procured from Qualigens Fine Chemicals, Mumbai. Taq polymerase, sodium perchlorate, deoxynucleoside triphosphate were procured from Sigma Chemical Co (St. Louis, MO, USA.) Primer sequences were selected and synthesized from Sigma-Aldrich Pvt. Ltd in Bengaluru. Restriction enzymes with their respective buffers like *Msp*I (*Moraxella* species), *Fok*I (*Flavobacterium okeanokoites*), *Kpn*I (*Klebsiella pneumoniae OK8*), *Bam*HI (*Bacillus amyloliquefaciens*), *Dde*I (*Desulfovibrio desulfuricans*) and *Taq*I (*Thermus aquaticus*) were obtained from Genetix Biotech Asia Pvt. Ltd, New Delhi, India.

Study subjects: Sixty patients with TBM or tuberculoma attending the outpatients Neurology clinic, Neurology ward or Emergency ward of Nehru Hospital, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, from

January to December 2009 were randomly included in this pilot study after obtaining written informed consent as described earlier[12]. Study protocol was approved by Institutional Review Board of PGIMER, Chandigarh.

Blood samples (5 ml) from all patients with TBM or tuberculoma were collected in sterilized tubes exactly after 180 min of drug administration because peak plasma phenytoin level would reach in 1.5 to 3 h. Of the five ml blood collected, three ml was put in tubes containing 525 µl acid citrate dextrose (ACD) (0.48% w/v citric acid: 1.32% w/v sodium citrate: 1.47% w/v glucose) as anticoagulant for genotypic analysis and two ml in heparinized tubes for plasma phenytoin estimation by high performance liquid chromatography (HPLC) as described earlier[17]. Total plasma phenytoin concentration was measured by Winter-Tozer equation[18].

All patients were grouped into phenytoin intoxicated and phenytoin non-intoxicated as described earlier[17]. Therefore, the cut-off level of plasma phenytoin of 15 µg/ml was considered for grouping patients as phenytoin intoxicated and phenytoin non-intoxicated[19]. Patients with both clinical symptoms and a plasma phenytoin concentration more than 15 µg/ml were grouped as phenytoin intoxicated.

NAT2 genotyping and SNP selection: Genomic DNA was isolated from whole blood (3 ml)[20] and stored at -20° C till further use. *NAT2* polymorphism was studied using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP), nested PCR and allele-specific PCR. Standardized protocol for amplification of *NAT2* gene and RFLP for analysis of point mutations at 191(*Msp*I), 282 (*Fok*I), 481 (*Kpn*I) and 857(*Bam*HI) positions, nested PCR-RFLP for the analysis of point mutation at 590 (*Taq*I) and 803 positions and allelic-specific PCR for analysis of the mutational status at 314 site were performed as described earlier[14]. The digestion products were resolved on high efficiency agarose gel and the DNA bands were visualized by ethidium bromide staining[14] (Table I).

Estimating the frequency of rapid and slow acetylators: As per the band patterns given in Table I, the wild-type and mutant alleles were recorded; genotyping of *NAT2* gene in the study patients was done based on this observation. Patients with wild type for all of the *NAT2* polymorphisms were phenotyped as rapid acetylators and patients with homozygous mutants or heterozygous for more than one of the polymorphisms were phenotyed as slow acetylators.

Statistical analysis: Statistical analysis was performed on SPSS 16.0 software (SPSS, Inc., Chicago, USA). Differences between allelic frequencies between phenytoin intoxicated and non-toxicated groups were determined using Chi square test or Fisher's exact test. Odds ratios (OR) and 95% confidence interval (CI)

Table I. Restriction endonucleases used to detect various polymorphisms in *NAT2* gene

SNPs	Recognition site	Restriction endonuclease used	Size of fragments (bp) obtained after restriction endonuclease digestion in different genotypes		
G191A rs1801279	C'CGG	*Ms*pI	GG	GA	AA
			763,190,165,93	763, 283,190,165, 93	763,283,165
C282T rs1041983	GGATG(N)	*Fo*kI	CC	CT	TT
			429,337,288, 122,35	766,429,337,288, 122,35	766,288,122,35
C481T rs1799929	GGTAC'C	*Kp*nI	CC	CT	TT
			662,549	1211,662,549	1211
G590A rs1799930	T'CGA	*Ta*qI	GG	GA	AA
			109,88	197,109,88	197
A803G rs1208	C'TNAG	*Dd*eI	AA	AG	GG
			124,74	124,97,74,27	97,74,27
G857A rs1799931	G'GATCC	*Bam*HI	GG	GA	AA
			925,286	1211,925,286	1211

SNP, single nucleotide polymorphism
Source: Ref. 14

were obtained by summarizing data between phenytoin intoxicated and non-intoxicated patients. Correlations of *NAT-2* genotypes with mean plasma total and free phenytoin levels between phenytoin intoxicated and non-intoxicated groups were analyzed by Wilcoxon Rank Sum test.

Results

Basal characteristics of patients are shown in Table II. Of the 60 patients, 37 were males (mean age 29.23 ± 10.95 yr) and 23 females (mean age 29.33 ± 10.87 yr). Five patients were CSF culture and acid fast bacilli (AFB) positive, 45 patients were culture negative and AFB positive and 10 were culture and AFB negative. All patients included in the study revealed normal liver, kidney function tests and haematological parameters.

Phenytoin status: Of the 60 patients studied, 37 showed no signs and symptoms of phenytoin intoxication, and 23 patients exhibited phenytoin intoxication based on the citeria mentioned[17].

NAT2 genotyping: BLAST search (*https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE-BlastSearch*) of *NAT2* sequence revealed 99 per cent identity with Homosapien *NAT2* gene. *NAT2* gene product matched with the nucleotide number 13716-14409 of Homosapien *NAT2* gene (Accession No: NC_000008.11). *NAT2* gene SNPs C282T, G590A, T341C, G191A, A803G, G857A and C481T were studied and sequence variation was identified in each case (Figs 1-5). The observed genotype frequencies satisfied the Hardy-Weinberg equilibrium for all polymorphisms studied. Distribution of homozygous wild, heterozygous and homozygous mutant SNPs among the 60 patients is shown in Table III. C481T was found to be the most predominant, whereas the mutations A803G and G857A were the least observed. No case of G191A was observed. Of the 60 patients, 38 and 45 per cent were found to be heterozygous for C282T (frequency of 'T' allele = 31%) and T341C (frequency of C allele = 33%) polymorphisms, respectively. Analysis of C481T and G857A revealed that the percentage of homozygous mutants *i.e.* TT (20%) and AA (8%) were higher as compared to heterozygous *i.e.* CT (17%) and GA (5%), respectively. There were 37 rapid and 23 slow acetylator phenotypes as shown in Table IV. Among slow acetylators, homozygous mutants of C282T, C481T, G857A, G590A, A803G and T341C were 30, 52, 22, 48, 22 and 26 per cent, respectively while heterozygous were 30, 4, 4, 17, 0 and 39 pe cent, respectively. Among rapid acetylators, heterozygous of C282T, C481T, G857A, G590A and T341C were 43, 24, 5, 27 and 49 per cent, respectively. The results of *NAT2* genotyping among phenytoin intoxicated and non-intoxicated groups are shown in Table V. All patients with rapid acetylator status had plasma phenytoin level less than 15 µg/ml and showed no sign and symptoms of phenytoin toxicity, while all patients with slow acetylators status had plasma phenytoin level above 15 µg/ml and had signs and symptoms of phenytoin toxicity. The allelic frequencies for C282T, C481T, G857A, G590A, A803G and T341C were significantly associated with phenytoin intoxicated group as compared to non-intoxicated group [OR = 3.04 (95% CI, 1.36-6.78); OR = 8.59 (3.47-21.29); OR = 11.31 (2.37-53.83); OR = 8.32 (3.43-20.16); OR = 42.86 (2.44-751.89) and OR = 2.61 (1.19-5.73)], respectively. Mean plasma total and free phenytoin levels were significantly (P<0.05) higher in phenytoin intoxicated group compared to non-intoxicated group (Table VI).

Table II. Basal characteristics of phenytoin intoxicated and non-intoxicated patients

Basal characteristics	Phenytoin intoxicated, (N=23)	Phenytoin non-intoxicated, (N=37)
Mean age (yr)	30.3 ± 9.3	29.1 ± 11.6
Gender		
Male	17	20
Female	6	17
CSF analysis		
Protein level (Normal, 15- 50 mg/dl)	112.97 ± 75.25	115.03 ± 61.18
Sugar (Normal, 40-70 mg/dl)	25.98 ± 12.21	27.05 ± 10.24
Cells (Normal, 0-5 mononuclear cells per µl)	88.45 ± 65.21	89.75 ± 64.32
Adenosine deaminase (Normal, less than 10U/l).	16.08 ± 4.23	17.10 ± 4.12

Values are mean ± SD

Discussion

Seven SNPs of *NAT2* gene at 191, 282, 590, 857, 481, 803 and 314 positions were examined in patients with TBM or tuberculoma having seizures and taking INH and phenytoin simultaneously. *NAT2* genotyping

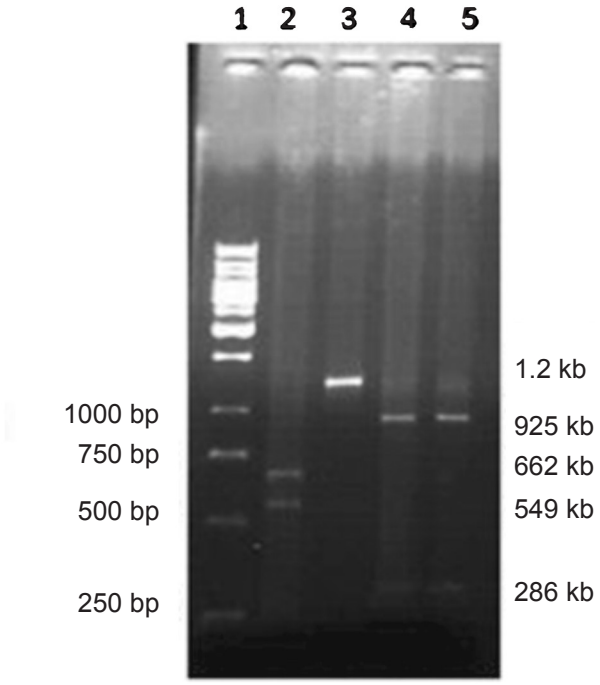

Fig. 1. Electrophoresis band pattern by *Kpn*I and *BamH*I digestion. Lane 1: 1kb DNA ladder, lane 2: *Kpn*I digestion (wild), lane 3: *Kpn*I digestion (mutant), lane 4: *BamH*I digestion (wild), lane 5: *BamH*I digestion (wild).

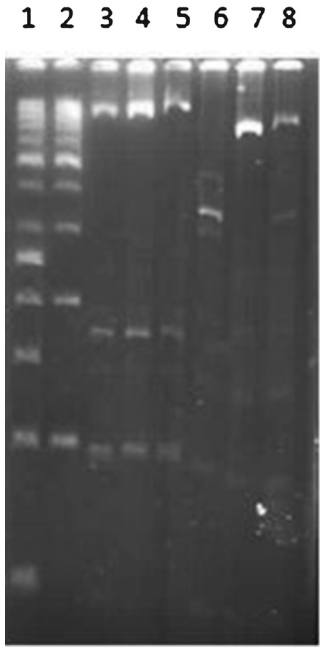

Fig. 2. Electrophoresis band pattern by *Msp*I and *Fok*I digestion. Lane 1: 50bp DNA ladder, lane 2: 100bp DNA ladder, lanes 3-5: *Msp*I digestion (wild), lane 6: *Fok*I digestion (wild), lane 7: *Fok*I digestion (mutant), lane 8: *Fok*I digestion (heterozygous).

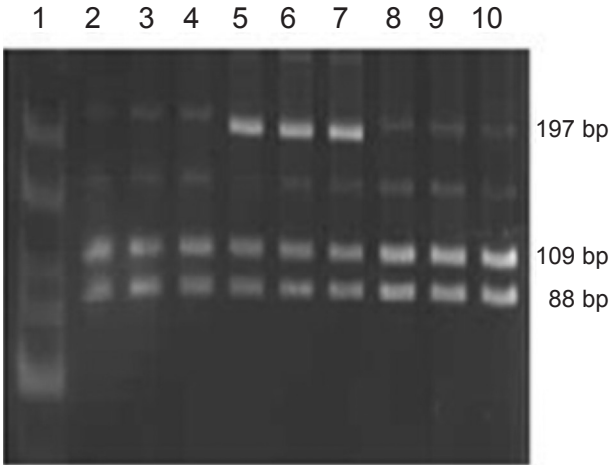

Fig. 3. Electrophoresis band pattern by *Taq*I digestion. Lane 1: 25-300bp DNA ladder, lanes 2-4: *Taq*I digestion (wild), lanes 5-7: *Taq*I digestion (heterozygous), lanes 8-10: *Taq*I digestion (wild).

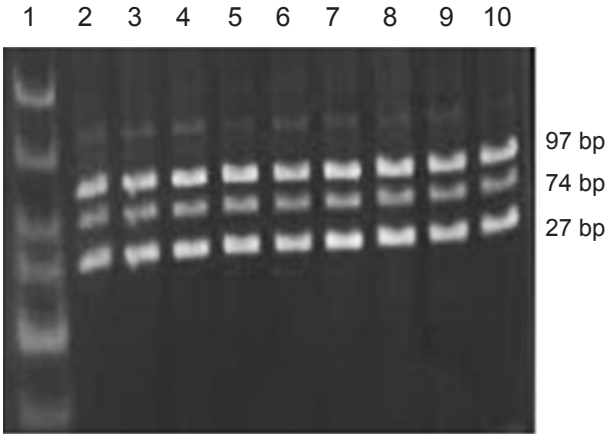

Fig. 4. Electrophoresis band pattern by *DdeI* digestion. Lane 1: 25-300bp DNA ladder, lanes 2-10: *DdeI* digestion (mutant).

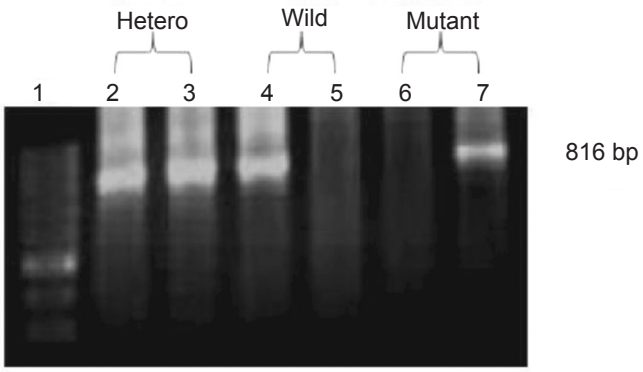

Fig. 5. Electrophoresis band pattern from allele-specific PCR for T341C site. Lane 1:100bp DNA ladder, lanes 2 and 3: heterozygous, lanes 4 and 5: wild, lanes 6 and 7: mutant.

Table III. Distribution of genotypes among tuberculous meningitis or tuberculoma patients (n=60)

SNPs	Genotype numbers			Allele frequency N (%)	
G191A	GG	GA	AA	G	A
	60	0	0	1.00 (120)	0.00 (00)
C282T	CC	CT	TT	C	T
	30	23	7	0.69 (83)	0.31 (37)
C481T	CC	CT	TT	C	T
	38	10	12	0.72 (86)	0.28 (34)
G857A	GG	GA	AA	G	A
	52	3	5	0.89 (107)	0.11 (13)
G590A	GG	GA	AA	G	A
	35	14	11	0.70 (84)	0.30 (36)
A803G	AA	AG	GG	A	G
	55	0	5	0.92 (110)	0.08 (10)
T341C	TT	TC	CC	T	C
	27	27	6	0.67 (81)	0.33 (39)

SNP, single nucleotide polymorphism

Table IV. Genotypic frequencies indicating acetylator phenotypes among tuberculous meningitis or tuberculoma patients (n=60)

SNPs	Genotype	Rapid acetylator (n =37)	Slow acetylator (n=23)
G191A	G191G	37 (100)	23 (100)
	G191A	0	0
	A191A	0	0
C282T	C282C	21 (56.8)	9 (39.1)
	C282T	16 (43.2)	7 (30.4)
	T282T	0	7 (30.4)
C481T	C481C	28 (75.7)	10 (43.5)
	C481T	9 (24.3)	1 (4.3)
	T481T	0	12 (52.2)
G857A	G857G	35 (94.6)	17 (73.9)
	G857A	2 (5.4)	1 (4.3)
	A857A	0	5 (21.7)
G590A	G590G	27 (73)	8 (34.8)
	G590A	10 (27)	4 (17.4)
	A590A	0	11 (47.8)
A803G	A803A	37 (100)	18 (73.9)
	A803G	0	0
	G803G	0	5 (21.7)
T341C	T341T	19 (51.4)	8 (34.8)
	T341C	18 (48.6)	9 (39.1)
	C341C	0	6 (26.1)

Values are number (%)

among patients showed 61.66 per cent were rapid acetylators and 38.34 per cent were slow acetylators. The distribution of acetylator status was found to be similar to that found among north Indian population[15]. This was in contrast with another study on south Indian population, where frequency of slow acetylators was higher than rapid acetylators[14]. Overall, 30-40 per cent rapid acetylators and 60-70 per cent slow acetylators have been reported in India[14,16,21]. The slow allele has been shown to be present in up to 90 per cent in Arab population, 40-60 per cent of Caucasians, 5-25 per cent East Asian[22-24] and 74 per cent in south Indians[14]. Percentage of slow acetylators was higher than rapid acetylators in most of the populations. The most common genotype among slow acetylators was found to be TT at 481 site. This result was consistent with another study among north Indian population[25]. Mutation frequencies observed in earlier studies were 30 per cent at 590 G-A, 25 per cent at 857 G-A and 50 per cent at 481C-T site among north Indian population[15]. In south Indian population mutation frequencies were 44 per cent at 282 C-T, 37 per cent at 590 G-A, 30 per cent at 341 T-C, 29 per cent at 803 A-G, 25 per cent at 857 G-A, 22 per cent at 481 C-T sites[14]. Mutation frequencies among Caucasian-American population were 45 per cent at 481 C-T, 28 per cent at 590 G-A, and 2 per cent at 857 G-A[26] and among African-American population were 30 per cent at 481 C-T, 22 per cent at 590 G-A, 2 per cent at 857 G-A and 9 per cent at 191 G-A[27].

Table V. Genotype and allelic frequencies of *NAT2* gene polymorphism among phenytoin intoxicated and non-intoxicated patients and their association with risk of phenytoin intoxication

Polymorphism	Genotypes	Phenytoin non-intoxication group (n=37)	Phenytoin intoxication group (n=23)	OR	95%CI
G191A	GG	37 (100)	23 (100)	1	
	GA	0	0	-	-
	AA	0	0	-	-
	G	74 (100)	46 (100)	-	-
	A	0	0	-	-
C282T	CC	21 (56.75)	9 (39.14)	1	
	CT	16 (43.25)	7 (30.43)	1.02	0.31-3.33
	TT	0	7 (30.43)	33.94	1.75-656.98
	C	58 (78.37)	25 (54.34)	3.04*	1.36-6.78
	T	16 (21.63)	21 (45.66)		
C481T	CC	28 (75.67)	10 (43.47)	1	
	CT	9 (24.33)	1 (4.34)	1.04	0.03-2.77
	TT	0	12 (52.19)	67.85	3.68-1250.58
	C	65 (87.83)	21 (45.66)	8.59*	3.47-21.29
	T	9 (12.17)	25 (54.34)		
G857A	GG	35 (94.59)	17 (73.91)	1	
	GA	2 ((5.41)	1 (4.34)	1.02	0.08-12.16
	AA	0	5 (21.75)	22.31	1.16-426.83
	G	72 (97.29)	35 (76.08)	11.31*	2.37-53.83
	A	2 (2.71)	11 (23.92)		
G590A	GG	27 (72.97)	8 (34.48)	1	
	GA	10 (27.03)	4 (17.39)	1.35	0.33-5.48
	AA	0	11 (48.13)	74.41	3.95-1399.27
	G	64 (86.48)	20 (43.47)	8.32*	3.43-20.16
	A	10 (13.52)	26 (56.53)		
A803G	AA	37 (100)	18 (78.26)	1	
	GA	0	0	2.02	0.03-103.26
	GG	0	5 (21.74)	22.29	1.16-425.23
	A	74 (100%)	36 (78.26%)	42.86*	2.44-751.89
	G	0	10 (21.74%)		
T341C	TT	19 (51.35)	8 (34.48)	1	
	TC	18 (48.65)	9 (39.14)	1.18	0.37-3.75
	CC	0	6 (26.38)	29.82	1.50-591.38
	T	56 (75.67)	25 (54.34)	2.61*	1.19-5.73
	C	18 (24.33)	21 (45.66)		

Values are denoted as no. (%)
OR, odds ratio; CI, confidence interval; *$P<0.05$

Table VI. Correlation of plasma total and free phenytoin levels among phenytoin intoxicated and non-intoxicated groups with *NAT-2* genotypes

Genotypes	Plasma phenytoin concentration, µg/dl (Mean ± SD)			
	Phenytoin intoxicated (n=23)		Phenytoin non-intoxicated (n=37)	
	Total	Free	Total	Free
C341C	29.35 ± 10.79	5.70 ± 2.29	0	0
C341T	21.94 ± 6.90	4.67 ± 1.34	8.20 ± 4.62	1.03 ± 0.55
T341T	24.39 ± 11.57	4.72 ± 1.97	7.64 ± 4.30	1.01 ± 0.60
A803A	26.23 ± 10.30	5.20 ± 1.95	7.90 ± 4.40	1.02 ± 0.57
A803G	0	0	0	0
G803G	18.44 ± 1.06	3.98 ± 0.17	0	0
G590G	22.76 ± 8.96	4.50 ± 1.85	8.30 ± 4.43	1.07 ± 0.57
G590A	27.11 ± 8.54	5.52 ± 1.80	6.73 ± 4.32	0.87 ± 0.57
A590A	24.82 ± 11.13	5.00 ± 1.84	0	0
G857G	24.73 ± 9.51	5.01 ± 1.80	7.94 ± 4.50	1.04 ± 0.58
G857A	17.00 ± 0.00	3.75 ± 0.00	7.36 ± 3.11	0.74 ± 0.29
A857A	25.70 ± 11.75	4.93 ± 2.07	0	0
C481C	22.21 ± 7.60	4.55 ± 1.39	8.12 ± 4.36	1.06 ± 0.57
C481T	20.60 ± 0.00	3.98 ± 0.00	7.24 ± 4.74	0.91 ± 0.57
T481T	27.14 ± 11.36	5.38 ± 2.13	0	0
C282C	27.14 ± 11.49	5.56 ± 1.97	8.43 ± 4.79	1.09 ± 0.63
C282T	25.20 ± 9.47	5.07 ± 1.66	7.24 ± 3.92	0.94 ± 0.49
T282T	20.18 ± 5.97	3.95 ± 1.40	0	0
G191G	24.61 ± 9.61	4.95 ± 1.80	7.90 ± 4.40	1.02 ± 0.57

Wilcoxon rank sum test between phenytoin intoxicated and non-intoxicated groups, $P < 0.05$

Also G857A variation is frequently present in Asians (12%) but rare in persons of European descent and Africans (1-2%)[28]; 191 G-A (R64Q) SNP is frequent in Africans and African-Americans, but virtually absent in Caucasian, Indian and Korean population. Similarly, 857 G-A (K268R) SNP is more frequent in south India and Korea than in other populations while 341 T-C (I114T) SNP is less frequent in Korea than in Europe, North America, India and Africa[28-30].

In the present study, genotype and allelic frequencies of *NAT2* gene polymorphism at seven sites were correlated with the risk of phenytoin intoxication. Among all, 481TT was found to be the most frequent genotype among the phenytoin intoxicated patients. SNPs lead to reduction in substrate affinity, catalytic activity and/or protein stability of recombinant *N*-Acetyltrasferase 2 alloenzyme. Reduced activity of NAT2 protein leads to less clearance of INH. Higher INH concentration leads to more inhibition of CYP enzymes and reduced clearance of anticonvulsant drug administered simultaneously leading to its toxicity[7,10]. This was supported by a consistent rise in mean plasma total and free phenytoin levels in the phenytoin intoxicated group. Distribution of allele variants of *NAT2* gene among a population decides the individual response to INH administration. Our study indicates an association of T481T allele of *NAT2* gene with plasma phenytoin levels suggesting that this allele may act as a predisposing factor for the occurrence of phenytoin intoxication among patients of tuberculous meningitis or tuberculoma with seizures and taking INH and phenytoin simultaneously. Murali *et al*[25] showed that C481T genotype of *NAT2* gene was responsible for phenytoin toxicity in epileptic patients.

Pharmacogenetic analysis in TBM patients having convulsion can help understand the interaction between INH and phenytoin. It explains that with simultaneous administration of INH and phenytoin, drug dose should be manipulated according to patients *NAT-2* genetic status. Those with rapid acetylator status, manipulation of drug dose can be ignored, but among slow acetylators, lower dose of phenytoin should be preferred without compromising the relief in seizures. Knowing the acetylator status by *NAT2* gene analysis in TBM patients prior to treatment may result in better improvement in patient's condition with minimum drug toxicity. As this was a pilot study due to limited time and resources, small sample size was a limitation. Further, genetic status of *CYP2C9* and *CYP2C19* was not evaluated. In this study phenytoin metabolites were not estimated as phenytoin toxicity was only due to phenytoin *per se* and not due to its metabolites[25].

In conclusion, rapid acetylator genotypes were comparatively predominant as compared to the slow acetylator genotypes among the patients with TBM having seizures. Slow acetylators showed significantly higher plasma phenytoin level resulting in toxicity as compared to rapid acetylators. Lethargy, dysarthria, disorientation, nystagmus and ataxia were found predominantly among phenytoin intoxicated patients. Patients with phenytoin toxicity had plasma phenytoin levels above the therapeutic level. T481T was found to be the most frequent genotype among the phenytoin intoxicated patients. Our findings suggest that T481T allele may act as a predisposing factor for the occurrence of phenytoin intoxication among patients of TBM or tuberculoma with seizures taking INH and phenytoin simultaneously.

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