

ORIGINAL ARTICLES

THE IMPACT OF ABCB1 (C3435T) POLYMORPHISM ON THE EFFICACY OF ONDANSETRON FOR POST-OPERATIVE NAUSEA AND VOMITING IN PAKISTANI POPULATION

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ABSTRACT

Objective: To investigate the association of adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1) polymorphism C3435T with anti-emetic efficacy in patients treated with ondansetron for preventing post-operative nausea and vomiting (PONV).

Study Design: A prospective, clinical trial.

Place and Duration of Study: Clinical data collection and blood sampling carried out at Combined Military Hospital, Rawalpindi and genetic analysis carried out at Institute of Biomedical and Genetic Engineering, Islamabad from Aug 2012 to Sep 2013.

Material and Methods: Ondansetron was administered in a dose of 4 mg intravenously 30 minutes before the end of surgery. Peripheral blood was withdrawn from 491 patients who had planned laparoscopic cholecystectomy under general anesthesia. A total of 249 patients with complaints of nausea and vomiting and 242 patients without nausea and vomiting were analyzed for C3435T polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: The results showed the patients with CC and CT genotype of ABCB1 C3435T had significantly higher incidence of nausea and vomiting ($p < 0.05$) while patients with TT genotype had significantly lower incidence of post-operative nausea and vomiting during the first two hours after surgery ($p < 0.05$).

Conclusion: It is concluded that polymorphism of ABCB1 may be a good guide for predicting responsiveness of ondansetron and there is a role of genetics in the management of PONV.

Keywords: Adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1), Polymorphism, post-operative nausea and vomiting, P-glycoprotein (P-gp).

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INTRODUCTION

Post-operative nausea and vomiting (PONV) is a frequently occurring problem in patients after surgery. It occurs in an estimated 35% of all patients and in as much as 70% of high-risk patients¹. It is a significant problem in laparoscopic surgery². A great deal of money, time and effort is being spent every year dealing with prevention and treatment of PONV after laparoscopic surgery procedures which cause extra burden on poor economies of countries such as Pakistan³. Ondansetron is the most

widely used selective 5-hydroxytryptamine receptor antagonist (5-HT₃RA) to prevent PONV, however all patients do not respond to the drug in the same way. The P-glycoprotein (P-gp) is an efflux pump driven by ATP which is encoded by ABCB1. ABCB1 is highly polymorphic gene. One SNP has been widely studied in healthy populations as well as different clinical conditions and that is the mutation at position 3435 in exon 26 (C3435T). This being the only silent polymorphism which has been found to influence the expression of P-gp in diverse human tissues as well as in diverse races. The marked variability in P-gp activity is in turn reflected in terms of effects of the drugs on inter individual basis⁹.

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A complex of genetic and acquired factors are said to be the basis of this variability⁴. It has been observed that even when patients receive anti-emetics during surgery, there are 26% patients who still require additional treatment in the post-operative period, and 40% of patients are those who even require additional treatment for this complaint following discharge⁵. So far the reasons for this variability in antiemetic drug response are largely unknown. Besides many reasons accounting for this variability in antiemetic drug response, recently the role of genetics is highlighted in this regard⁶.

This drug is recognized and transported by P-glycoprotein (P-gp) a drug transporter encoded by adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1) in the blood brain barrier. This in turn determines the concentration of drug in central nervous system. However this ABCB1 is highly polymorphic; the polymorphism of which may affect availability of drug in the central nervous system affecting efficacy and treatment outcomes⁷. We hypothesized that the polymorphism C3435T in this transporter gene may be playing a role in inter-individual variation affecting the response to pharmacotherapy of PONV.

MATERIAL AND METHODS

The study consisted of 491 adults both male and female undergoing elective laparoscopic cholecystectomy. The patients aged between 18 and 65 years with an American Society of Anesthesiologists (ASA) physical status of I or II were included in the study. The current good clinical practices were followed while conducting this study. The protocol of the study was approved by Ethical Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College Rawalpindi Pakistan. The clinical data collection and sampling was done at operation theatre, Combined Military Hospital Rawalpindi. The analytical procedures were carried out at Institute of Biomedical and Genetic Engineering (IBGE), Islamabad. An informed consent was taken from

all the patients in writing and each subject was evaluated with detailed medical history.

The anesthesia protocols were standardized for all the patients included in this study. Thiopental in a dose of 4-5 mg/kg was used for induction of anesthesia and to facilitate endotracheal intubation Rocuronium was given in a dose of 0.6 mg/kg. The patient's lungs were ventilated with 50% oxygen in air. Sevoflurane 1.5–2.0 volume% was used for maintenance of anesthesia. During the procedure a bispectral index score (BIS) monitor was used continuously and depth of anaesthesia was maintained appropriately between 50 and 60. All the patients were given 4 mg (0.1 mg/kg) Ondansetron intravenously thirty minutes before the end of surgery. The total dose of Nalbuphine consumption during anesthesia was noted down. On arrival in the post-anesthesia care unit the pain score were recorded. All the patients were observed for symptoms of nausea and vomiting after surgery which was recorded in the first 2 hours and at 2-24 hours. Patients having any complaints of nausea or vomiting were allocated to non-responders group. These patients were considered to have failed therapy and were given rescue anti-emetic. Patients were allocated to the responders group if they did not complain of any nausea or vomiting postoperatively. Out of 500 patients enrolled initially in this study, 9 patients were later excluded from this study. 4 patients did not complete the post-operative questionnaire, 3 patients did not provide the complete bio data and 2 patients were administered antiemetic other than ondansetron. A 5 ml of blood sample was taken from all the patients included in the study.

The genomic DNA from whole blood was isolated using the standard organic methods⁸. The genotyping for C3435T was made by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The genomic DNA was amplified using sense: 5'-TGC AGG CTA TAG GTT CCA GG – 3' and anti-sense: R5'-TTT AGT TTG ACT CAC CTT CCC G–3' primers for the region harboring the C3435T. The PCR

was carried out in a final volume of 20 μ l containing 1X PCR buffer without Mg^{2+} , 1 mM Mg Cl_2 , 1.5 mM dNTPs, 1U TaqDNA polymerase, 10 μ M forward and reverse primers and 40 ng genomic DNA. The PCR products were subjected to digestion with Mbo1 restriction enzyme. After digestion the homozygous individuals for major allele C had three fragments of 172 base pair (bp), 60bp, 16 bp. The heterozygous containing both the major and minor allele T and C yielded four fragments of 248bp, 172bp, 60bp, 16bp. The minor allele T homozygous individuals produced a single

calculated for quantitative variables like age, weight, height, duration of anesthesia, doses of opioid. Frequency and percentages were calculated for qualitative variables like gender, ASA grade, history of smoking, PONV and motion sickness and genotypes. Chi-Square test was used for comparing categorical variables such as genotypes and alleles. For comparing continuous variables student's t-test and one-way ANOVA were used. Chi-square test was used for allelic and genotypic analyses for the SNP. ANOVA was used for the analysis of the three genotypes of the SNP. One-way ANOVA test

Table-1: The characteristics and clinical parameters of the patients in accordance with the ABCB1 C3435T presented as number or mean (SD).

Variables	Genotype			p-value
	CC (n=35)	CT (n=290)	TT (n=166)	
Sex: M/F	14/21	126/164	84/82	0.136 NS
Age (years)	44.06 \pm 9.22	42.64 \pm 8.34	41.89 \pm 8.84	0.356 NS
BMI (kg/m ²)	23.2 \pm 1.01	23.0 \pm 1.06	22.9 \pm 1.12	0.291 NS
History of Smoking (M/F)	4/0	30/2	22/3	0.209 NS
History of PONV	3	21	13	0.811 NS
History of motion sickness	4	23	14	0.79 NS
Duration of Surgery	80.20 \pm 11.32	76.84 \pm 11.23	76.43 \pm 10.92	0.186 NS
Nalbuphine doses in operating room (mg/kg)	6.67 \pm 0.41	6.63 \pm 0.40	6.67 \pm 0.38	0.646 NS

NS $p > 0.05$

Table-2: The effects of C3435T ABCB1 polymorphism on the anti-emetic efficacy of ondansetron presented as number.

Genotype	First 02 hours		2-24 hours	
	Responders	Non responders	Responders	Nonresponders
CC	4(11.4%)	31 (88.6%)	24 (68.6%)	11(31.4%)
CT	114(39.3%)	176 (60.7%)	250 (86.2%)	40(13.8%)
TT	124(74.7%)	42 (25.3%)	154 (92.8%)	12(7.2%)
p-value			p-value	
CC + CT vs TT	< 0.001			0.008
CC vs CT + TT	< 0.001			0.001

fragment of 248 bp.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 16.0 was used for analysis of data. Descriptive statistics were used to describe the data. Mean and standard deviation (SD) were

used for in-group comparison. Genotype frequencies for this SNP were assessed for deviation from the Hardy-Weinberg equilibrium using the Chi-square test. Frequency differences in genotype, demographic data, and incidence of PONV were compared by chi-square test. A p value of less than 0.05 was considered significant.

RESULTS

The figure-1 shows CC, CT and TT genotypes being classified into different band sizes after digest by Mbo I. The characteristics and clinical data did not differ significantly according to the genotypes which is summarized in table-1. The frequencies of ABCB1 C3435T were as follows; 7.1% for CC, 59.1% for CT and 33.8% for TT. The genotype frequencies of this SNP was in Hardy-Weinberg equilibrium ($p>0.05$).

Among 3435C >T variants, the incidence of PONV during the first 2 hours as well as between 2 to 24 hours after surgery was significantly lower in patients with the 3435TT genotype than other 3435 genotypes (TT vs Non-TT, $p<0.05$) (table-2).

The occurrence of PONV was significantly higher in patients with CC genotype at 2 hours. There were more non-responders as compared to responders with CC genotype at this time

significant difference has been found between the response to ondansetron and all the three genotypes between 2 hours and 24 hours after surgery ($p<0.05$). The complaint of the patients with nausea and vomiting had decreased in this time period and it occurred only in 13% of patients.

DISCUSSION

There are inconclusive and contradictory results regarding this SNP in various populations depicting varied expression and function of P-gp. It has been associated with increased¹⁰, at times decreased and even with no effect on plasma concentrations of P-gp substrates^{11,12}. That is why we selected C3435T polymorphism of ABCB1 and attempted to find out its impact on the efficacy of ondansetron in preventing emesis in Pakistani population.

Recently the polymorphism of the ABCB1 has been found to have its impact on pharmacology of ondansetron. The drug is

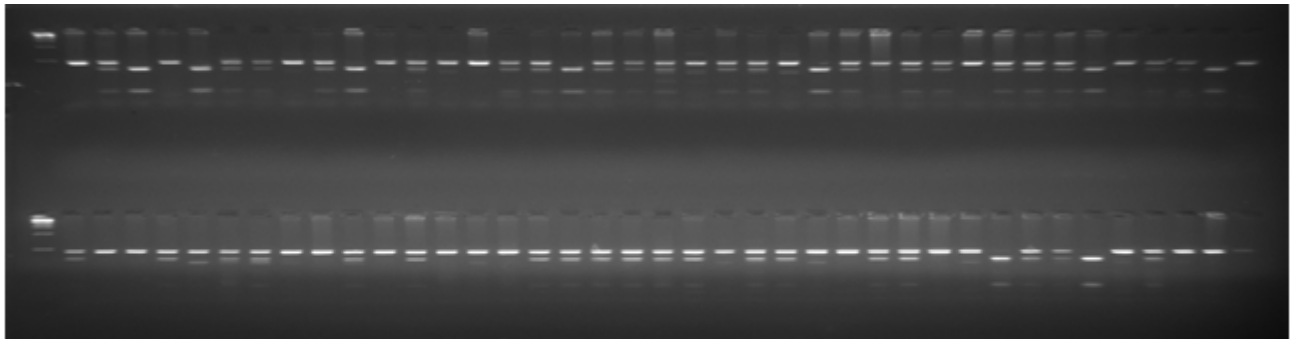


Figure-1: Electrophoresis pattern for ABCB1 C3435T alleles analyzed by PCR-RFLP.

($p<0.05$). Among the individuals with CT genotype, the incidence of PONV was significantly higher during the first 2 hours after surgery ($p<0.05$). There were more non-responders as compared to responders with this genotype. Among the individuals with TT genotype, the incidence of PONV during the first 2 hours after surgery was significantly lower ($p<0.05$). There were more responders as compared to non-responders among the individuals with TT genotype. At 2-24 hours there were more responders as compared to non-responders in all the genotypes. A statistically

transported into the brain by the drug transporter P-gp and when an agent that inhibited this transport was added into multi drug resistant 1 (MDR1) cell line a reduction in the transport was observed. Moreover in cancer patients, nausea and vomiting induced by chemotherapy was significantly associated with C3435T polymorphism¹³. Choi and his colleagues have also demonstrated the impact of C3435T polymorphism on the anti-emetic efficacy in preventing post-operative nausea and vomiting⁶.

We have observed that individuals with TT genotype at 3435 responded well to the drug at 2

hours and between 2 to 24 hours in post-operative period. We have found that the efficacy of ondansetron was significantly associated with C3435T polymorphism in ABCB1 during the two time periods. A study conducted on cancer patients concluded that individuals with TT genotype of C3435T of ABCB1 experienced less severe emesis and it was assumed that ondansetron must be available in higher concentrations in the central nervous system¹³.

In our study we observed that the frequencies of CC and CT genotypes were elevated in non-responders at 2 hours and between 2 to 24 hours after surgery, showing that C allele at 3435 position was significantly associated with worse or poor response to ondansetron in post-operative patients. This indicates that the carriers with 3435C allele might have more chances of resistance to the drug. The same has been supported from three different studies that 3435CC genotype has a higher probability of treatment failure as seen from complete remission in AML patients¹⁴, as with the drug-resistant epilepsy¹⁵, and longer steroid treatment in pediatric heart transplant patients¹⁶.

An interesting finding with this study was that the different genotype groups depicted significant differences in the incidence of PONV between 2 to 24 hours after surgery. The incidence of PONV was in 13 % patients only. This finding has never been reported earlier.

CONCLUSION

We can finally conclude that in this population the response of individuals to ondansetron seems to be affected by ABCB1 gene C3435T polymorphism. Individuals with TT genotype had the least complaints of nausea and vomiting after prophylactic treatment with ondansetron while the individuals with CC and CT genotype had the maximum complaints. The genotypes of ABCB1 can be considered as predictor in guiding responsiveness to ondansetron for PONV in this region of the world. Hence, genotyping of ABCB1 gene polymorphism (C3435T) including the CC, CT

and TT genotypes may prove helpful in designing individualized therapy based on genetic makeup. Our results indicate that antiemetic treatment may be improved by identification of non-responders on a pharmacogenetic basis.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by author.

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