ORIGINAL ARTICLE

Association of Anti-Emetic Efficacy of Ondansetron with G2677T Polymorphism in a Drug Transporter Gene ABCB1 in Pakistani Population

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ABSTRACT

Objective: To determine the association of ABCB1polymorphism G2677T with anti-emetic efficacy in patients treated with ondansetron for preventing postoperative nausea and vomiting.

Study Design: A clinical trial.

Place and Duration of Study: Combined Military Hospital, Rawalpindi and Institute of Biomedical and Genetic Engineering, Islamabad, from 2012 to 2013.

Methodology: Four mg ondansetron was administered intravenously 30 minutes before the end of surgery. A total of 246 patients with the complaints of nausea and vomiting and 244 patients without nausea and vomiting were analyzed for G2677T polymorphism using PCR-RFLP method. Results were described as frequency percentages and chi-square test with significance at $p < 0.05$.

Results: The patients with TT genotype had significantly lower incidence of postoperative nausea and vomiting during the first 2 hours ($p < 0.001$) and between 2 - 24 hours after surgery as compared to other genotypes ($p < 0.001$). The patients with GG genotypes had significantly higher incidence of this complaint ($p=0.014$).

Conclusion: Polymorphism of ABCB1 has an association with responsiveness for ondansetron. There is a role for genetics in the management of PONV.

Key Words: *ABCB1. Polymorphism. G2677T. Ondansetron. Postoperative nausea and vomiting.*

INTRODUCTION

Postoperative Nausea and Vomiting (PONV) is a disturbing and a frequently occurring complication after surgery with an incidence upto 80% in high risk patients.[1] It has consequences far more reaching than hitherto appreciated.[2] Although the exact mechanism of PONV is unclear, it is known that the 5-Hydroxytryptamine Type-3 (5-HT3) receptor in the chemoreceptor trigger zone is involved in the occurrence of PONV.[3]

Ondansetron, a serotonin receptor antagonist is the widely used agent for PONV due to its efficacy and safety profile. However, in spite of its use, 35% of the patients still complain of PONV. So far, the reasons for this variability in antiemetic response are largely unknown. Besides, many risk factors attributing to this nausea and vomiting, it is now realized that there may be a role of genetics responsible for this variability.[4] Ondansetron is recognized by adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1) drug transporter in the blood brain barrier which in turn determines the concentration of drug in central nervous system. However, ABCB1 is highly polymorphic; the polymorphism of which may affect the expression and function of P-gp and thus can influence the drug disposition in the central nervous system affecting efficacy and treatment outcomes.[5,6]

The authors hypothesized that there may be a role of ABCB1 polymorphism in determining the response of postoperative patients to ondansetron and the polymorphism in this transporter gene may be playing a role in inter-individual variation.

The objective of this study was to determine the association of ABCB1polymorphism G2677T with anti-emetic efficacy in patients treated with ondansetron for preventing postoperative nausea and vomiting.

METHODOLOGY

The study was conducted in accordance with the current Good Clinical Practices (GCP). 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

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Operation Theatre, Combined Military Hospitals, Rawalpindi. The analytical procedures were carried out at Institute of Biomedical and Genetic Engineering (IBGE), Islamabad, from 2012 to 2013. All the patients included in the study provided a written informed consent. It comprised of 500 adults both males and females randomly selected patients belonging to different regions of Pakistan to provide representation from all areas. Patients aged between 18 and 65 years with an American Society of Anesthesiologists (ASA) physical status of I or II scheduled to undergo elective laparoscopic cholecystectomy were enrolled in the study. Patients with a history of any known hyper-sensitivity to 5-HT3 receptor antagonist, hepatic or renal disease or who had used anti-emetics within the 24 hours before the study were excluded. Each subject was evaluated with detailed medical history which included the history of smoking, motion sickness and any past experience of PONV.

Vital signs of the patient including electrocardiography, noninvasive blood pressure and pulse oximetry were monitored continuously once the patient was received in the operation theatre. Induction was carried out with 4 - 5 mg/kg Thiopental, and endotracheal intubation was carried out with 0.6 mg/kg Rocuronium. 1.5 - 2.0 vol% Sevoflurane was used for maintenance of anaesthesia. 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, 30 minutes before the end of surgery. The total dose of Nalbufen consumption during anaesthesia was noted down. On arrival in the post-anaesthesia care unit, the pain score were recorded. In the postoperative period, all the patients were observed for symptoms of nausea and vomiting. PONV in the first 2 hours and at 2 - 24 hours were recorded. 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. A 5-ml of blood sample was taken from all the patients included in the study. Out of 500 patients enrolled initially in this study, 10 patients were later excluded from this study. Four patients did not complete the postoperative question-naire, 03 patients did not provide the complete bio-data and 03 patients were administered antiemetic other than ondansetron.

The standard organic methods of DNA extraction were used to extract the genomic DNA from whole blood.[7] The genotyping for G2677T was made by 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 G2677T SNP. The PCR was carried out in a final volume of 20 µl containing 10 x PCR buffer without Mg_2+, 25 mM $MgCl_2$, 2 mM dNTPs, 5U Taq polymerase, 10 µM forward and reverse primers and 40 nanogram (ng) genomic DNA. The PCR products were subjected to digestion with *Ban1* restriction enzyme. After digestion the homozygous individuals for major allele had single fragment of 224 bp. The heterozygous containing both the major and minor allele yielded three fragments of 224 bp, 198 bp and 26 bp. The minor allele homozygous individuals produced 198 bp and 26 bp fragments. The 26 bp fragment was not visible on agarose gel. Figure 1 shows the electrophoresis patterns for ABCB1 alleles analyzed by PCR-RFLP.

The data was analyzed using Statistical Package for the Social Sciences (SPSS) version 16.0. The frequencies of this single nucleotide polymorphism were assessed for deviation from Hardy-Weinberg equilibrium using Fisher's exact test. Frequency differences in genotype and incidence of PONV were compared by chi-square test. A p-value of less than 0.05 was considered significant.

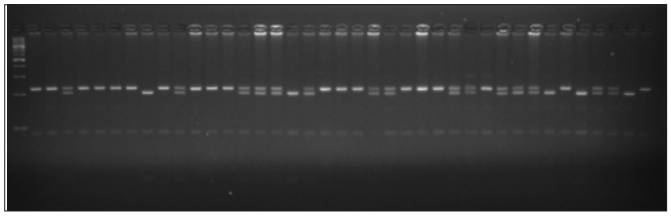

Figure 1: Electrophoresis patterns for ABCB1 alleles analyzed by PCR-RFLP.

RESULTS

Figure 1 shows the genotype results for ABCB1 G2677T polymorphisms. The homozygous wild type GG, heterozygous GT and homozygous variant TT was classified into different band sizes after digest by the specific restriction enzyme (*Ban 1*). Seventy three patients had GG genotype, 232 patients had GT and 185 patients had TT genotype. The frequencies of ABCB1 2677 genotypes are given in Table I. The allele and genotype frequencies of this SNP was in Hardy-Weinberg equilibrium (p=0.99). The characteristics of patients and the clinical data is summarized in the Table II. There were no significant differences in the characteristics and clinical data according to genotypes ($p > 0.05$).

Among G2677T variants, the incidence of PONV during the first 2 hours after surgery was significantly lower in patients with the 2677TT genotype than other 2677 genotypes (TT vs. GG + GT, $p < 0.001$). The occurrence of PONV was significantly higher in patients with GG genotype at 2 hours than other 2677genotypes (GG vs GT + TT; $p < 0.001$) (Figure 2). The incidence of PONV during the 2 - 24 hours after surgery was significantly

lower in patients with the 2677TT genotype than other 2677 genotypes (TT vs GG + GT; p = 0.014). However, the occurrence of PONV was not significantly higher in patients with GG genotype than other 2677 genotypes at 2 - 24 hours (GG vs GT + TT; p = 0.816) (Table III).

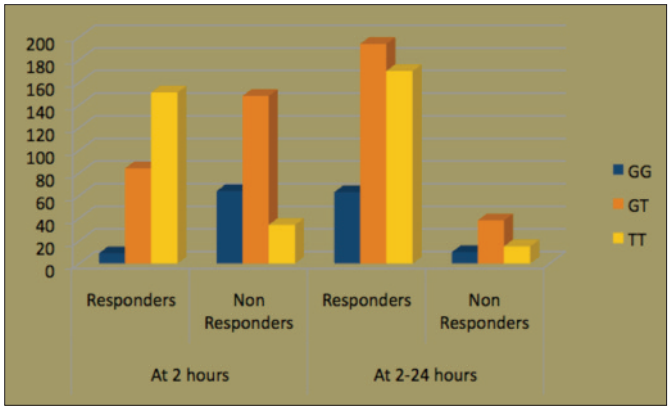

Figure 2: Association between G2677T genotypes and the response to ondansetron at 2 hours and between 2-24 hours.

Table I: Allele and genotype frequencies of ABCB1 G2677T in study subjects.

SNP	Alleles (n=980)		Genotypes (n=490)		
G2677T	G	T	GG	GT	TT
n (%)	382 (39%)	598 (61%)	73 (14.9%)	232 (47.3%)	185 (37.8%)

Table II: The characteristics and clinical parameters of the patients in accordance with ABCB1 G2677T. Values are number or Mean ± SD.

Variables	Genotype		
	GG (n = 73)	GT (n = 232)	TT (n = 185)
Sex: M/F	27 / 46	104 / 128	89 / 96
Age (years)	42.74 ± 9.61	42.73 ± 8.10	41.92 ±8.69
History of smoking	9	26	27
History of PONV	5	19	14
History of motion sickness	4	25	12
Duration of surgery	77.56 ± 10.49	76.81 ±11.30	76.84 ± 11
Nalbuphine doses in operating room (mg/kg)	7.10 ± 0.28	6.80 ± 0.54	6.53 ± 0.76

Table III: The effects of G2677T ABCB1 polymorphism on the anti-emetic efficacy of ondansetron between 2-24 hours.

	At 2-24 hours n=490		p-values
	Responders (n=427)	Non-Responders (n=63)	
Genotypes			
GG	63 (86.3)	10 (13.7%)	
GT	194 (83.6%)	38 (16.4%)	
TT	170 (91.9%)	15 (8.1%)	
Comparing TT vs. Non-TT			
TT	170 (39.8%)	15 (23.8%)	0.014*
Non-TT (GG+GT)	257 (60.1%)	48 (76.1%)	
Comparing GG vs. Non-GG			
GG	63 (14.7%)	10 (15.8%)	0.816^{NS}
Non-GG(GT+TT)	364 (85.2%)	53 (84.1%)	

*$p < 0.05$; $^{NS}p > 0.05$

The frequencies of G and T alleles differed significantly between responders and non-responders. The occurrence of PONV was significantly lower in patients with T-allele at 2 hours. There were more non-responders as compared to responders with G-allele at this time (p < 0.001). However, no statistically significant difference has been found between the response to ondansetron and both the alleles between 2 hours and 24 hours after surgery (p=0.065).

DISCUSSION

P-glycoprotein (P-gp) encoded by ABCB1 is considered to be an important efflux transporter in blood brain barrier that limits the accumulation of various drugs including the anti-emetics.[8] This gene is highly polymorphic; over 50 Single Nucleotide Polymorphisms (SNPs) in ABCB1 have been documented.[9] One SNP is of particular significance. It is a mutation in exon 21 at position 2677 (G2677T) which has been extensively studied and has been reported to be the most common polymorphism among various populations,[5,6,10,11] depicting high inter-ethnic variability.[12-15] So far, very few studies have studied the impact of ABCB1 polymorphism in postoperative patients. Our region of the world has so far not provided any information in this regard. Keeping the research done in cancer patients and other populations as base, the authors hypothesized a possible impact of the G2677T polymorphism on treatment outcomes in postoperative patients. Thus, this study focused to determine the associations between G2677T and treatment outcomes in a large number of Pakistani patients undergoing laparoscopic cholecystectomy under general anaesthesia being given prophylactic ondansetron.

Up till now inconclusive and contradictory results have been reported with this polymorphism. It has been associated with increased, at times decreased and even with no effect on plasma concentrations of P-gp substrates.[14,16-18] The authors observed that TT genotype at 2677 was associated with a better drug response at 2 hours and at 2 - 24 hours in postoperative period. Choi and his colleagues demonstrated the same but only at 2 hours.[4] It is most possible that patients with 2677TT genotype have a more availability of ondansetron in the central nervous system and thus a better response to ondansetron pointing to reduced activity of ABCB1 transporter. Therefore, it is reasonable to hypothesize that genetic variance of this transporter could influence the efficacy of ondansetron. Likewise in one of the studies it has been reported that there was lower expression of P-gp in individuals with the TT genotype than those who had GG genotype,[19] thus pointing to lower resistance to drugs being transported across the transporter. Tanabe and his colleagues have also reported that the individuals homozygous for 2677T had the lowest level of expression of ABCB1 within

placental tissue.[20] In this study, the patients who were most responsive to ondansetron were those who had TT genotype, therefore, the authors suggest that the TT genotype is the chief factor determining the better response to ondansetron. It was also observed that the patients with GG genotype had more complaints of PONV, thus pointing to the fact that it may be a predictor of poor response to this substrate of P-gp. It was found that the response to ondansetron for PONV did differ significantly according to genotypes between 2 hours and 24 hours after surgery (GG + GT vs. TT p=0.014). However, the incidence of PONV in that time period was in 13% of patients.

Previous studies have supported the influence of genetic variability on the efficacy of anti-emetics. The effectiveness of ondansetron in preventing nausea and vomiting has been linked to genetic polymorphism.[21-23] Babaoglu and his colleagues found an association of ABCB1 polymorphism with the anti-emetic efficacy of serotonin receptor antagonists in patients undergoing chemotherapy treatment.[21] If one can predict the response of patient to ondansetron, without the fear of facing distress due to unresponsiveness, it may be possible to target treatment appropriately. However, much work needs to be done to evaluate the effect of genetic variability in the ABCB1 gene on the expression of P-gp in the blood-brain barrier. In this study, the allele frequency of G2677T polymorphism differed significantly between responders and non-responders for G and T alleles, showing that G-allele at 2677 position was significantly associated with worse response in post-operative patients. This shows that the carriers of 2677G allele might have a more risk of drug resistance. Increased P-gp expression has been reported with the G-allele and was said to be responsible for increased efflux of P-gp substrates. In a study on patients of chronic myeloid leukemia receiving imatinib therapy, Dulucq and colleagues reported that the G-allele at 2677 position was linked with worse response.[24]

It is believed that PONV is a multifactorial problem. Numerous risk factors have been described but only few seem to be unequivocally proven. The gender, the patient's history of PONV or motion sickness, non-smoking status, volatile anaesthetics, nitrous oxide, and opioids have shown to be independent predictors for PONV in various centers.[25] This multifactorial genesis of PONV is currently regarded as a major cause of possible treatment failure.[23] There are several risk factors affecting PONV. We had observed no significant differences in these risk factors in accordance with the genotypes. The other factors that affect PONV like the anaesthetic agent and type of surgery were also controlled in this study so as to minimally affect the results.

This study has served as one of the many efforts towards evaluating the impact of ABCB1genetic variability on the anti-emetic efficacy in postoperative Pakistani patients. This polymorphism has never been studied in such a large group of patients so far. It has also served as a step ahead in the implementation of the concept of efficacious use of ondansetron by pointing out a possible role of ABCB1 polymorphism G2677T for treatment outcomes. However, in order to implement genetic testing in routine clinical practice, further validation is required. It is suggested that the possible role of other polymorphisms of all the proteins that are contributing a role in the transport of drugs be also evaluated in order to combat the problem of drug resistance.

CONCLUSION

The response to ondansetron for PONV was significantly influenced by ABCB1 gene G2677T polymorphism in Pakistani population. ABCB1 genotypes may be a clinical predictor of responsiveness for ondansetron for PONV in this region of the world. Hence, genotyping of ABCB1 gene polymorphism (G2677T) including the TT, TG and GG genotypes might be helpful in planning the individualized therapy based on the genetic makeup and the anti-emetic therapy can be upgraded by identifying non-responders.

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