Impact of 46698G>A Polymorphism in the 5-Hydroxytryptamine Type 3b Receptor Gene on the Anti-Emetic Efficacy of Ondansetron

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ABSTRACT

OBJECTIVE: To evaluate the possible effect of a single nucleotide polymorphism- 46698G>A, of 5-hydroxytryptamine type 3B (5-HT3B) receptor gene on anti-emetic efficacy of ondansetron in preventing post-operative nausea and vomiting (PONV).
STUDY DESIGN: A prospective, clinical trial.
Place and Duration: Clinical data collection and blood sampling was carried out at Combined Military Hospital, Rawalpindi. Genetic analysis was carried out at Institute of Biomedical and Genetic Engineering, Islamabad from 01 Aug 2012 to 22 Sep 2013.
METHODS: A single nucleotide polymorphism of 5-HT3B receptor gene, 46698G>A in 3' near gene position was selected and genotyped in 368 Pakistani post-operative patients. These patients had undergone elective laparoscopic cholecystectomy and were given anti-emetic ondansetron 0.1 mg/kg half an hour before the end of surgery. 46698G>A was screened using Taqman assays. The relationship between genetic polymorphisms and clinical outcomes of ondansetron treatment was investigated.
RESULTS: No significant association was found between the incidence of nausea and vomiting and the 46698G>A polymorphism of the 5-HT3B gene at 2 hours after surgery.
CONCLUSION: The 46698G>A variant of the 5-HT3B gene may affect PONV and predict the responsiveness to ondansetron.
KEYWORDS: Laparoscopic Cholecystectomy, Nausea, Vomiting, Ondansetron, Polymorphism, 5-Hydroxytryptamine Type 3B (5-HT3B) Receptor Gene, Genotyping.

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INTRODUCTION

Post-operative nausea and vomiting (PONV) is a problematic side effect of general anesthesia[1]. The vomiting is provoked by the release of serotonin and subsequently the action of this serotinin on the 5-hydroxytryptamine type 3 (5-HT3) receptors present in the center and in the periphery. The drugs that block these receptors are found to have a significant role in controlling the vomiting, may this control be in the form of prevention or treatment [2]. Ondansetron is the most widely used drug of this class having its role in PONV, chemotherapy induced nausea and vomiting and pregnancy induced nausea and vomiting[3]. Ligand gated ion channel having a number of subunits, is the site where it acts. The repeatedly observations have lead to the conclusion that it's the 5-HT3B subunit that appears to be contributing largely to its functions. 5-HT3B gene encodes this subunit. This gene lie in a 90-kb region on chromosome 11q23.[1] A continuously growing list of polymorphisms are being identified and reported for this gene[2]. We selected the 46698G>A polymorphism as this polymorphism has been identified and reported in a very few populations. And the frequency distribution of its genotypes have been carried out in just a few number of studies [4-6] the effect of 46698G>A variability on anti-emetic response have never been studied in Pakistani patients. This area of research being unfocussed has provoked us to carry out a prospective study taking into account 5-HT3B gene variant in the post-operative settings. Therefore this study was aimed to evaluate the effect of this polymorphism in 5-HT3B gene on PONV in our population and to see if any significance is established or not. If yes then it would definitely be a potential determinant of altered drug response. This study was conducted to evaluate the possible effect of a single nucleotide polymorphism- 46698G>A, of 5-hydroxytryptamine type 3B (5-HT3B) receptor gene on anti-emetic efficacy of ondansetron in preventing post-operative nausea and vomiting (PONV).

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METHODOLOGY

This prospective clinical trial was carried out at Combined Military Hospital, Rawalpindi from 1st Aug 2012 to 22nd Sep 2013. After getting the approval of ethical committee and excluding the patients with any history of gastrointestinal

problem or ingestion of anti-emetics, a total of 368 subjects undergoing elective laparoscopic cholecystectomy were included in this study. A written informed consent from each of the subject was a must. All the demographic parameters were asked and noted down in a pre-clinical proforma. Anesthesia was kept standardized in contest to induction, intubation and maintenance of anesthesia for all the subjects, the study drug ondansetron was administered 30 minutes before the end of the surgery. In the first 02 hours of post-operative period all the patients were observed for symptoms of nausea and vomiting. Patients with the complaints of 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 vomiting postoperatively. A 5 ml of blood sample was taken from all the patients included in the study. Genetic analysis was carried out at Institute of Biomedical and Genetic Engineering, Islamabad The standard organic methods of DNA extraction were used to extract the genomic DNA from whole blood[7]. The genotypes of genotypes of 46698G>A were determined using Taqman assays and analyzed on ABI 7500 real-time PCR System from Applied Biosystems according to the manufacturer's protocol[4].

Data analysis: Statistical Package for the Social Sciences (SPSS) version 21.0 was used for analyzing the data. The genotypic frequencies were assessed through Fisher's exact test for deviation from Hardy–Weinberg equilibrium. The genotypic frequencies and the incidence of PONV were compared by chi-square test. A *p* value of less than 0.05 was considered significant.

RESULTS

In the total sample of 368, the frequency of GG genotypes was 70% (n=258), the frequency of GA genotypes was 27% (n=99) and the frequency of AA genotype was 3% (n=11) (Table I). Our population was in Hardy Weinberg equilibrium as chi-square was 0.1598 with a *p* value equal to 0.6892. There were no significant differences in patient characteristics and clinical data (age, gender, history of smoking, past history of PONV, history of motion sickness and duration of surgery) in accordance with the genotypes. There were no significant differences in the incidence of PONV among genotypes of 46698G>A during the first 2 hours after surgery (*p* >0.05) (Table II).

Table-I: Genotype frequencies of 46698G>A variants in study subjects (N=368)

SNP	Genotypes (n=368)		
46698G>A	**GG n(%)**	**GA n(%)**	**AA n(%)**
	258 (70%)	99 (27%)	11 (3%)

Chi square= 0.1598, *p*=0.6892

Table-II: The effects of 46698G>A variants of the 5-HT3B receptor gene polymorphism on the anti-emetic efficacy of ondansetron (N=368)

	Genotypes		
	GG	**GA**	**AA**
Non Responders (n=198)	132	59	7
Responders (n=170)	126	40	4

Comparing GG vs non-GG			
	GG	**Non GG (GA+ AA)**	***p*-values**
Non-Responders (n=198)	132	66	0.1195^{NS}
Responders (n=170)	126	44	
Comparing AA vs non-AA			
	Non AA (GG+ GA)	**AA**	***p*-values**
Non-Responders(n=198)	191	7	0.5066^{NS}
Responders(n=170)	166	4	

Among 46698G>A variants, the incidence of PONV during the first 2 hours after surgery didn't differ significantly among patients with the 46698GG genotype and 46698 non GG genotypes (GG *vs* Non-GG; *p*=0.1195). The occurrence of PONV also differed insignificantly in patients with AA genotype and 46698 non-AA genotypes (AA *vs* Non-AA; *p*=0.5066) (Table II).

DISCUSSION

Post operative nausea and vomiting has been effectively prevented and treated with the 5-HT3 receptor antagonists[8]. However the response of the patients to these drugs varies [9]. Some respond with therapeutic failure and some show toxicity. The therapeutic failure has underlying complex mechanisms, and one among is said to be the genetic makeup. We selected 46698G>A polymorphism of the 5-HT3B subunit gene to be investigated. Since neither the frequency distribution nor the effect of 46698G>A variability on anti-emetic response have ever been being studied in Pakistani patients so we carried out this study. We hypothesized that any functional variant in the gene encoding the 5-HT3 receptor will potentially influence the response to the HT3 receptor antagonists. To the best of our knowledge, this is the first study to report the genotype frequencies of 46698G>A in the 5-HT3B gene in Pakistani post-operative patients. The present study couldn't reveal a significant association between the 46698G>A polymorphism and the efficacy of ondansetron as the 46698G>A genotypes were not related to the clinical response to ondansetron. Much less work has been carried out with this variant. One such study carried out on Indonesians have shown that this genetic variant of 5-HT3B gene and the clinical response were not associated to each other[10]. We too

couldn't observe any significant impact of 5-HT3B variant on the anti-emetic response in our post-operative patients. The findings of this study however needs to be confirmed by taking into account a much larger sample size. The polymorphism of the 5-HT3B gene affects the expression of this target site which in turn translates into increased or decreased effects[11]. Usually a polymorphism in the regulatory region of the gene brings about changes in the structure and function of the protein[12,13]. Moreover the polymorphism in the coding regions of genes affects the transcription and signaling cascad[14-16]. An invitro study clarifying the discrepancies between the protein expression and activity can answer the questions pertaining to the functional aspects of this polymorphism. The genotypic distribution of this variant 46698G>A was in in accordance with Hardy-Weinberg equilibrium, as there was insignificant differences between the observed and expected values, suggesting that our findings involving this receptor gene was likely to be correct[17]. This study has recruited patients undergoing similar anesthesia and surgical procedures, it was a deliberate effort to minimize the effects of different anesthetic and surgical factors on the results[18]. Moreover there were no significant differences in the risk factors according to the genotypes. Our study has provided the data regarding the genotypic frequency of 46698G>A of 5-HT3B gene in our population. The information gained through this study will help in drafting guidelines for newer researchers in the field of pharmacogenetics. We will add not only to the knowledge of etiology of PONV which is considered to be multifactorial, but we may be able to explore the genetic basis for the inter-individual variations to the study drug. This recognition of the relation between genotype and drug response is highly expected to affect the medical practice.

CONCLUSION

The 46698G>A variant of the 5-HT3B gene may affect PONV and predict the responsiveness to ondansetron.

CONTRIBUTION OF AUTHORS

Waheed A: conceived idea, design methodology
Pasha AK: Data Collection
Farhat K: Manuscript writing, carried out the genetic and statistical analysis.

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REFERENCES

1 Gan TJ, Diemunsch P, Habib AS, Kovac A, Kranke P, Meyer TA et al. Consensus Guidelines for the Management of Postoperative Nausea and Vomiting. Anesth Analg. 2014; 118:85–113.

2 Farhat K, Ismail M, Ali S, Pasha AK. Resistance to Ondansetron: Role of Pharmacogenetics in Post-Operative Nausea and Vomiting. Egypt J Hum Med Genet. 2013; 14: 331-36.

3 Smith HS, Cox LR, Smith EJ. 5-HT3 receptor antagonists for the treatment of nausea/vomiting. Ann Palliat Med. 2012; 1 (2): 115–20.

4 Perwitasari DA, Wessels JA, van der Straaten RJ. Association of ABCB1, 5-HT3B receptor and CYP2D6 genetic polymorphisms with ondansetron and metoclopramide antiemetic response in Indonesian cancer patients treated with highly emetogenic chemotherapy. Jpn J Clin Oncol. 2011; 41:1168-76.

5 Yang J. Association and interaction analyses of 5 HT3 receptor and serotonin transporter genes with alcohol, cocaine, and nicotine dependence using the SAGE data. Hum Genet. 2014; 133: 905–18.

6. Currow DC, Quinn S, Ekstrom M. Can variability in the effect of opioids on refractory breathlessness be explained by genetic factors? BMJ Open 2015; 5:e006818. doi: 10.1136/bmjopen-2014-006818

7 Sambrook J, Rusell DW. Molecular Cloning: Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA. 2001; 151-54.

8 Browning KN, Travagli RA. Central Nervous System Control of Gastrointestinal Motility and Secretion and Modulation of Gastrointestinal Functions. Compr Physiol. 2014; 4(4): 1339–68.

9 Zoto T, Kilickap S, Yasar U, Celik I, Bozkurt A, Babaoglu MO. Improved Anti-Emetic Efficacy of 5-HT3 Receptor Antagonists in Cancer Patients with Genetic Polymorphisms of ABCB1 (MDR1) Drug Transporter. Basic Clin Pharmacol Toxicol, 2015; 116: 354–60.

10 Perwitasari DA, van der Straaten RJ, Mustofa M. Differences in 5-hydroxytryptamine-3B haplotype frequencies between Asians and Caucasians. Int J Biol Markers. 2012; 27 (1):34–38.

11 Krzywkowski K. Do polymorphisms in the human 5-HT3 genes contribute to pathological phenotypes? Biochem Soc Trans. 2006; 34: 872-76.

12 Niesler B, Flohr T, Nothen MM, Fischer C, Rietschel M, Franzek E, et al. Association between the 5' UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. Pharmacogenetics. 2001; 11: 471-75.

13 Meineke C, Tzvetkov MV, Bokelmann K, Oetjen E, Hirsch-Ernst K, Kaiser R et al. Functional characterization of a -100_-102delAAG deletion-insertion polymorphism in the promoter region of the HTR3B gene. Pharmacogenet Genomics. 2008; 18: 219-30.

14 Thompson AJ, Sullivan NL, Lummis SC. Characterization of 5-HT3 receptor mutations identified in schizophrenic patients. J Mol Neurosci. 2006; 30: 273-81.

15 Krzywkowski K, Jensen AA, Connolly CN, Brauner-Osborne H. Naturally occurring variations in the human 5-HT3A gene profoundly impact 5-HT3 receptor function and expression. Pharmacogenet Genomics. 2007; 17: 255-66.

16 Krzywkowski K, Davies PA, Feinberg-Zadek PL, Brauner-Osborne H, Jensen AA. High frequency HTR3B variant associated with major depression dramatically augments the signaling of the human 5-HT3AB receptor. Proc Natl Acad Sci. 2008; 105: 722-27.

17 Nazir N, Waheed A, Farhat K, Ismail M, Mansoor Q, Qais N. Prevalence of CYP2D6*4 genotype and its association with tamoxifen induced hot flashes in Pakistani female breast cancer patients. J Postgrad Med Inst. 2015; 29 (1): 28-33.

18 Farhat K, Iqbal J, Waheed A, Mansoor Q, Ismail M, Pasha AK. Association of anti-emetic efficacy of Ondansetron with G2677T polymorphism in a drug transporter gene ABCB1 in Pakistani population. J Coll Phys & Surg Pak. 2015; 25 (7): 486-90.