RESEARCH ARTICLE

Association of anti-emetic efficacy of Ondansetron with 18792A>G polymorphism in a drug target gene 5-HT3B in Pakistani population

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Abstract

Objective: To evaluate the association of anti-emetic efficacy of ondansetron with 18792A>G polymorphism in the target gene of 5-hydroxytryptamine type 3 subtype B.

Method: The prospective clinical study was conducted at Combined Military Hospital, Rawalpindi and the genetic analysis was carried out at Institute of Biomedical and Genetic Engineering, Islamabad from August 2012 to September 2013. The subjects enrolled were undergoing elective laparoscopic cholecystectomy under general anaesthesia. All the patients were given anti-emetic ondansetron (4mg) intravenously 30 minutes before the end of surgery. Within the first two hours after surgery the response to ondansetron was noted down. Patients with the complaints of vomiting and those who had no vomiting were analysed for 18792A>G polymorphism using polymerase chain reaction- restriction fragment length polymorphism method.

Results: Of the 350 patients, 183(52%) had complaints of vomiting and 167(48%) had no such complaints. Overall, 195(56%) patients had 18792AA genotype, 130(37%) had genotype AG, and 25(7%) had GG genotype. No significant association was found between the incidence of vomiting and the 18792A>G genotypes at 2 hours after surgery ($p>0.05$).

Conclusion: No association of anti-emetic efficacy of ondansetron with 18792A>G polymorphism in the target gene of 5-hydroxytryptamine type 3 subtype B was found.

Keywords: 18792A>G, Ondansetron, Polymorphism, Post-operative nausea vomiting. (JPMA 68: 733; 2018)

Introduction

Post-operative nausea and vomiting (PONV) is associated with general anaesthesia with a very high incidence of 80% especially in high-risk groups.[1] The stimulation of the 5-hydroxytryptamine type 3 (5-HT3) receptors in the gastrointestinal tract and central nervous system is said to be one leading factor in the genesis of emesis.[2] That is why the drugs that act as antagonists to these receptors help in treating emesis. The 5-HT3 receptor antagonists (5-HT3 RAs) have proved themselves to be very effective in preventing and treating PONV.[3] Ondansetron is a widely used drug of this class. Its site of action is a receptor which is an ion channel with multiple subunits (A, B, C, D andE). It is cation selective and produces excitation of nerves within the central and peripheral nervous systems.[4]

Ondansetron mainly exerts its effects through its action on the 5-HT3A and 5-HT3B subunits. Among these two subunits the major contributor to its functions is the 5-HT3B subunit. This subunit is encoded by a gene-5-HT3B located close together on human chromosome 11q23.1.[2]

This gene is, however, known to be altering the response to the drugs that act on this site. The underlying cause to this altered response has been attributed to the variations in the gene.[5] Many genetic variations in the 5-HT3B gene have been identified in different populations.[6,7] But not all the polymorphisms have been studied extensively. One such polymorphism is 18792A>G at the intron position of the 5-HT3B gene, on which much less studies have been carried out in contest to observing its effect on modulating the clinical response. The results that have been put forth are unconvincing and have not answered questions satisfactorily. Moreover, so far no such study has ever been conducted on post-operative patients. The little amount of work that has been carried out across the world is on cancer patients. Keeping the researches done in cancer patients and other populations as base, we hypothesised a possible association of the 18792A>G in the intron position of the 5-HT3B gene with the treatment outcomes in post-operative Pakistani patients undergoing laparoscopic cholecystectomy under general anaesthesia being given prophylactic ondansetron.

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Patients and Methods

The prospective clinical study was conducted at Combined Military Hospital, Rawalpindi and the genetic analysis was carried out at Institute of Biomedical and Genetic Engineering, Islamabad from Aug 2012 to Sep 2013. After getting approval from the ethical committee of the Institute patients providing written informed consent were enrolled. Patients of either gender aged 18-65 years with an American Society of Anaesthesiologists (ASA) grade I and II undergoing elective laparoscopic cholecystectomy were included. The patients were randomly selected through non-probability consecutive sampling belonging to different regions of Pakistan to provide representation from all areas.[8] The current good clinical practices were followed in the true spirits. Any patient who had a history of gastro-oesophageal reflux disease, any obstruction in the tract or any history of anti-emetic ingestion were excluded from the study.

A preclinical proforma was completed for each subject that included the detailed history and detailed physical examination. All the patients were given a standardised anaesthesia procedure. As the intravenous (IV) line was secured, a 5 ml blood sample was drawn from all the patients for future genetic testing. Thiopentone (4-5 mg/kg) was used for induction, rocuronium (0.6 mg/kg) for intubation and sevoflurane (1.5-2.0 vol %) for maintenance of anaesthesia. Ondansetron in a dose of 4mg was given IV to all the patients 30 minutes before the end of surgery.

Nausea and vomiting experienced by any subject was noted down in the first 2 hours after surgery in the recovery room. Here the subjects were allocated to two groups; those with complaints of nausea and vomiting were placed in the non-responders group, and those with no complaints of nausea and vomiting were placed in the responders group.

The deoxyribonucleic acid (DNA) was extracted using the standard organic methods.[9] The genomic DNA was amplified using forward: 5'-CCTTATGGTCCATCTGTG-3' and reverse 5'-GAGGCTGAGGCAGGAGAA-3' primers for the region harbouring the 18792A>G single nucleotide polymorphism (SNP). Polymerase chain reaction (PCR) was carried out in a final volume of 25 µl containing 10X PCR buffer without Mg2+, 25 mM MgCl2, 2 mMdNTPs, 5U Taq polymerase, 10 µM forward and reverse primers and 40 nanogram (ng) genomic DNA. Then the amplified PCR products of 18792A>G were digested with restriction enzyme (Ppu10I). The digested DNA products were then analysed by 2% agarose gel electrophoresis and visualised by ultraviolet light.[10]

To calculate the sample size, we relied on an earlier study.[11] SPSS 21.0 was used for analysing the data. 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. P<0.05 was considered significant.

Results

Of the 350 patients, 183(52%) had complaints of vomiting and 167(48%)had no such complaints. Overall, 195(56%) patients had 18792AA genotype, 130(37%) had genotype

Table-1: Genotype frequencies of 18792A>G variants in study subjects.

SNP	Genotypes (n=350)		
	AA n(%)	AG n(%)	GG n(%)
18792A>G	195 (55.7%)	130 (37.1%)	25 (7.1%)

Expected values: 191.1; 133.7, 23.1, Chi square= 0.270, p=0.6032.
SNP: Single-nucleotide polymorphism.

Table-2: The characteristics and clinical parameters of the patients in accordance with -18792A>G variants. Values are number or Mean ± SD.

Variables	Genotypes (n=350)			p value
	AA (n = 195)	AG (n = 130)	GG (n = 25)	
Gender: M/F	65/130	43/87	9/16	0.95
Age (years)	42.74 ± 9.61	42.73 ± 8.10	41.92 ±8.69	0.909
History of Smoking	18	16	3	0.657
History of PONV	16	12	5	0.163
History of motion sickness	17	11	3	0.845
Duration of Surgery	78.56 ± 11.49	77.81 ±12.30	77.84 ± 12	0.843

SD: Standard deviation
PONV: Post-operative nausea and vomiting.

Table-3: The effects of 18792A>G variants of the 5-HT3B receptor gene polymorphism on the anti-emetic efficacy of ondansetron.

	Genotypes		
	AA (n=195)	AG (n=130)	GG (n=25)
Non Responders (n=183)	97	76	10
Responders (n=167)	98	54	15
Comparing AA vs Non AA			
	AA	Non AA (AG+ GG)	p-values
Non-Responders (n=183)	97	86	0.2855^{NS}
Responders (n=167)	98	69	
Comparing GG vs Non GG			
	Non GG (AA+ AG)	GG	p-values
Non-Responders (n=183)	173	10	0.2018^{NS}
Responders (n=167)	152	15	

^{NS}- not significant.
5-HT3B: 5-hydroxytryptamine type 3 subtype B.

AG, and 25(7%) had GG genotype (Table-1).

There was no significant difference in patient characteristics and clinical data like age, gender, history of smoking, past history of PONV, history of motion sickness and duration of surgery, in accordance with the genotypes (Table-2).

There was no significant difference in the incidence of PONV among genotypes of 18792A>G during the first 2 hours after surgery (p>0.05) (Table-3).

Discussion

The efficacious profile of ondansetron as anti-emetic has placed this drug in the category of a widely used one in our clinical settings. It has been effectively used in treating chemotherapy-induced nausea and vomiting (CINV), PONV and relieving vomiting during pregnancy.[12] The response to the drug, however, differs from person to person. And among the many factors responsible for this discrepancy, one important factor is said to be the gene that encodes the target site of the drug. The variations of this gene and the ultimate outcome under their influence have been evaluated in fewer studies that have confirmed the role of polymorphisms in 5-HT3B gene in altered response to the anti-emetic treatments.[13-16]

We selected this polymorphism as there has been no work reported encompassing the frequency distribution or the effect of 18792A>G variability on anti-emetic response from our population. The association of 18792A>G genotypes with the incidence of PONV was evaluated in this study. Much less work has been carried out with this variant. One study carried out on Indonesians has shown that this genetic variant of 5-HT3B gene and the clinical response were not associated to each other.[11] Recently a study conducted on Chinese Han population could also not find any significant association between 18792A>G polymorphism and the incidence of CINV in patients of acute myeloid leukaemia.[10] We too couldn't observe any significant impact of HTR3B variant on the anti-emetic response in our post-operative patients. The findings of this study, however, need to be confirmed with a much larger sample size.

The expression of 5-HT3 A and B complex is affected by the genetic variations of these subunits. This predisposes the individuals to increased or decreased effects.[17] The genetic variations in the regulatory region of the gene alters the structure as well as the designated function of the protein.[18,19] Moreover, the variations in the coding regions of genes will have an effect on the transcription and signalling cascade.[20-22] We recommend further work to be done taking into account the functional aspects of this polymorphism through an invitro study. This will help in better understanding the discrepancies between the protein expression and activity.

We had confirmed that the genotypic distribution of 18792A>G was in accordance with Hardy-Weinberg equilibrium, as the observed and expected values were not significantly different, suggesting that our findings involving this receptor gene was likely robust.[23]

In a clinical study like ours, multiple factors could have affected the results.[24] The effects of multiple anaesthetic and surgical factors could only be minimised by recruiting patients in a way strictly following inclusion and exclusion criteria. And that we had ensured. All our patients were undergoing similar procedure may it be surgery or anaesthesia. We found no significant differences in the risk factors according to the genotypes.

Conclusion

The study has provided data regarding genotypic frequency of 18792A>G of 5-HT3B gene in our population, but this variant did not affect PONV and thus may not predict the responsiveness to ondansetron. This is the very first study to provide the genotypic frequency of 18792A>G of 5-HT3B gene in our population.

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Conflict of Interest: None.

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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. Browning KN, Travagli RA. Central Nervous System Control of Gastrointestinal Motility and Secretion and Modulation of Gastrointestinal Functions. Compr Physiol. 2014; 4: 1339-68.
4. Barnes NM, Hales TG, Lummis SC, Peters JA. The 5-HT3 receptor: the relationship between structure and function. Neuro pharmacol. 2009; 56: 273-84.
5. Choi EM, Lee MG, Lee SH, Choi KW and Choi SH. Association of ABCB1 polymorphisms with the efficacy of ondansetron for postoperative nausea and vomiting. Anaesth. 2010; 65: 996-1000.
6. Rueffert H, Thieme V, Wallenborn J, Lemnitz N, Bergmann A, Rudlof K, et al. Do Variations in the 5-HT 3A and 5-HT3B Serotonin Receptor Genes (HTR3A and HTR3B) Influence the Occurrence of Postoperative Vomiting? Anesth Analg. 2009; 109: 1442-7.
7. Janicki PK, Sugino S. Genetic factors associated with pharmacotherapy and background sensitivity to postoperative and chemotherapy-induced nausea and vomiting. Exp Brain Res. 2014; 232: 2613-25.

8. Farhat K, Waheed A, Hussain A, Ismail M, Mansoor Q, Pasha AK, et al. Influence of genetic variations in ABCB1 on the clinical efficacy of ondansetron-A pharmacogenetic analysis of Pakistani population. J Pak Med Assoc. 2015; 65: 963-66.
9. Sambrook J, Rusell DW. In: Sambrook J, Rusell DW eds. Molecular Cloning: Laboratory Manual, 3rd ed.New York, USA: Cold Spring Harbour Laboratory Press, 2001; pp 51-54
10. Cao HL, Wu ZY, Deng MH. Relationship between 5-hydroxytryptamine (serotonin) type 3 receptor and nausea and vomiting. Int J Clin Exp Pathol. 2016; 9: 11944-50.
11. 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: 34-8.
12. Smith HS, Cox LR, Smith EJ. 5-HT3 receptor antagonists for the treatment of nausea/vomiting. Ann Palliat Med. 2012; 1: 115-20.
13. Tremblay PB, Kaiser R, Sezer O, Rosler N, Schelenz C, Possinger K, et al. Variations in the 5-hydroxytryptamine type 3B receptor gene as predictors of the efficacy of antiemetic treatment in cancer patients. J Clin Oncol. 2003; 21: 2147-55.
14. Tanaka M, Kobayashi D, Murakami Y, Ozaki N, Suzuki T, Iwata N, et al. Genetic polymorphisms in the 5-hydroxytryptamine type 3B receptor gene and paroxetine-induced nausea. Int J Neuropsycho Pharmacol. 2008; 11: 261-7.
15. Ma XX, Chen QX, Wu SJ, Hu Y, Fang XM. Polymorphisms of the HTR3B gene are associated with post-surgery emesis in a Chinese Han population. J Clin Pharm Ther. 2013; 38: 150-55.
16. Hammer C, Cichon S, Muhleisen TW, Haenisch B, Degenhardt F, Mattheisen M. Replication of functional serotonin receptor type 3A and B variants in bipolar affective disorder: a European multicenter study. Trans Psychia. 2012; 2, e103.
17. Krzywkowski K. Do polymorphisms in the human 5-HT3 genes contribute to pathological phenotypes? BiochemSoc Trans. 2006, 34: 872-76.
18. Niesler B, Flohr T, Nothen MM, Fischer C, Rietschel M, Franzek E, et al. Association between the 5' UTR variant C178T of the serotonin receptorgene HTR3A and bipolar affective disorder. Pharmacogenet. 2001; 11: 471-75.
19. 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.
20. Thompson AJ, Sullivan NL, Lummis SC. Characterization of 5-HT3 receptor mutations identified in schizophrenic patients. J Mol Neurosci. 2006; 30: 273-81.
21. 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.
22. 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.
23. 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: 28-33.
24. Farhat K, Iqbal J, Waheed A, Mansoor Q, Ismail M, Pasha AK, et al. 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: 486-90.