The Professional Medical Journal
www.theprofesional.com

DOI: 10.29309/TPMJ/2019.26.01.2584

ORIGINAL PROF-0-2584

POST-OPERATIVE PATIENTS;

INFLUENCE OF POLYMORPHISMS IN THE DRUG TARGET GENE ON ANTI-EMETIC EFFICACY OF ONDANSETRON IN POST-OPERATIVE PATIENTS.

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Article received on:
31/01/2018
Accepted for publication:
15/09/2018
Received after proof reading:
04/01/2019

ABSTRACT… Objective: To explore the effect of genetic polymorphism -100_-102AAG deletion in the 5-hydroxytryptamine type 3B (5-HT3B) gene on the incidence of post-operative vomiting (POV). **Study Design**: A prospective, clinical trial. **Place and Duration of study:** 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:** This study included two hundred and sixty patients planned for elective laparoscopic cholecystectomy. 4 mg ondansetron was administered intravenously thirty minutes before the end of surgery. A total of 140 patients with the complaints of vomiting and 120 patients without vomiting were analyzed for -100_-102delAAG deletion polymorphism with the help of direct sequencing method. **Results:** A significant association was found between the incidence of vomiting and the -100_-102AAG insertion/deletion polymorphism of the 5-HT3B gene at 2 hours after surgery. **Conclusion:** The -100_-102AAG deletion variant of the 5-HT3B gene may affect POV and predict the responsiveness to ondansetron.

Keywords: 5-hydroxytryptamine type 3B (5-HT3B) receptor gene, Genotyping, Ondansetron, Polymorphism, Post-operative vomiting.

Article Citation: Farhat K, Shaukat A, Ali S, Waheed A, Ismail M. Post-operative patients; influence of polymorphisms in the drug target gene on anti-emetic efficacy of ondansetron in post-operative patients. Professional Med J 2019; 26(1):181-185. **DOI:** 10.29309/TPMJ/2019.26.01.2584

INTRODUCTION

Despite the fact that the anti-emetics are widely used, the incidence of post-operative vomiting (POV) remains very high and in the high risk groups the incidence rises up to 80%.[1] POV has a multifactorial genesis and is said to be provoked by stimulation of 5-hydroxytryptamine type 3 (5-HT3) receptors in gastrointestinal tract (GIT) or in the central nervous system (CNS). This predicts the role of the serotonin system in the genesis of emesis and the antagonists to this receptor have a role in its treatment.[2]

Among the many anti-emetics in current use the antagonists of 5-HT3 receptor (5-HT3 RAs) are said to be playing an effective role in treatment of POV.[3] The target site for this class of drugs is an ion channel that is ligand gated -5-HT3 receptor. In humans multiple subunits of this receptor from A, B, C, D to E have been identified. The 5-HT3 receptor antagonists bind to a 5-HT_{3A}, 5-HT_{3B} receptor complex and produce their effects. The 5-HT_{3B} subunit however appears to have a larger role in its functionality which in humans is encoded by the 5-HT3B gene.[4] Clinically relevant and functional genetic polymorphisms in this gene has been detected from various studies.[5,6,7,8] One such polymorphism which is of particular significance is the deletion variant of the 5-HT3B gene present in the promotor region. It has been observed that this genetic variations has been modulating susceptibility to vomiting. The possible contribution of genetic polymorphisms in 5-HT3B gene to vomiting has been examined in a small number of studies around the world. Up till now inconclusive and contradictory results have been reported with this polymorphism. Our region of the world has so far not provided any information in this regard. Keeping the researches done in cancer patients and other populations as base, we have hypothesized a possible impact of the -100_ -102AAG deletion of 5-HT3B gene in the promoter region on treatment outcomes in post-operative Pakistani patients undergoing

laparoscopic cholecystectomy under general anesthesia being given prophylactic ondansetron.

METHODS

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 study consisted of 260 adults both male and female undergoing elective laparoscopic cholecystectomy.[9] The patients having an age group between 18 to 65 years were included in the study. The current good clinical practices were followed while conducting this study.

Any patient having preoperative reflux diseases, any obstruction in GIT or any history of intake of anti-emetic drugs were excluded from the study. Consent was taken from all the subjects in writing. Each subject was evaluated with detailed medical history which included the history of smoking, motion sickness and any past experience of POV.

All the vitals of the patients were continuously monitored in the operation theatre. Thiopental in a dose of 4-5 mg/kg was used for induction of anesthesia, rocuronium in a dose of 0.6 mg/kg was used for endotracheal intubation and for anesthesia maintenance sevoflurane (1.5-2.0 vol%) and nalbuphine 0.1mgkg^{-1} was used. A bispectral index score (BIS) was kept between 50 and 60 using a monitor. All the patients were given 4 mg ondansetron intravenously half an hour before the procedure was to be ended. The total dose of nalbuphine consumption during anesthesia was noted down. In the first 02 hours after surgery all the patients were observed for symptoms of 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 designated a responders group if they remained without vomiting postoperatively. A 5 ml of blood sample was taken from all the patients included in the study.

The standard organic methods of DNA extraction were used to extract the genomic DNA from whole blood.[10] Genotyping the -100_-102AAG deletion variants was carried out by DNA direct sequencing method. For amplification of one 5-HT3B fragment, UCSC In-Silico PCR was used. For the purification of products Multi-Screen384-PCR Filter Plate (Millipore, Billerica, MA, USA) was used. Next the sequencing of the purified products were carried out. And finally mutation analyses were performed.[11]

Statistical Analysis

The data was analyzed using Statistical Package for the Social Sciences (SPSS) 21.0. 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 POV were compared by chi-squared test. A *p* value of less than 0.05 was considered significant.

RESULTS

The DNA extraction was carried out in 275 subjects but we could make out the genotypic profile of 260 subjects. Out of which 151 patients had Ins/Ins genotype, 81 patients had Ins/del and 28 patients had del/del genotype. The characteristics of patients and the clinical data is summarized in the Table-II. The characteristics and clinical data did not differ significantly in accordance to genotypes ($p>0.05$).

The incidence of POV after surgery was significantly lower in patients with the Insertion genotype than other genotypes (Ins/Ins *vs* Non-Ins/Ins, $p < 0.036$). The occurrence of POV was significantly higher in patients with deletion genotype than other genotypes (del *vs* non-del/del; $p< 0.048$) (Table-III).

SNP	Genotypes (n=260)		
100_-102AAG deletion	Ins/Ins n(%)	Ins/Del n(%)	Del/Del n(%)
	151(58%)	81(31%)	28(11%)

Table-I. Genotype frequencies of -100_-102AAG deletion variants in study subjects

Variables	Genotypes (n=260)		
	Ins/Ins (n = 151)	Ins/Del (n = 81)	Del/Del (n = 28)
Sex: M/F	53/98	32/49	9/19
Age (years)	42.74 ± 9.61	42.73 ± 8.10	41.92 ±8.69
History of Smoking	14	18	15
History of PONV	5	11	9
History of motion sickness	14	2	7
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-II. The characteristics and clinical parameters of the patients in accordance with -100_-102AAG deletion variants. Values are number or Mean ± SD.

	Genotypes		
	Ins/Ins	Ins/Del	Del/Del
Non Responders (n=140)	73	47	20
Responders (n=120)	78	34	8
Comparing Ins/Ins vs non-Ins/Ins			
	Ins/Ins	Non Ins/Ins (Ins/Del+ Del/Del)	*p*-values
Non-Responders	73	67	0.036*
Responders	78	42	
Comparing Del/Del vs non-Del/Del			
	Non Del/Del (Ins/Ins+ Ins/Del)	Del/Del	*p*-values
Non-Responders	120	20	0.048*
Responders	112	8	

Table-III. The effects of -100_-102AAG deletion variants of the 5-HT3B receptor gene polymorphism on the anti-emetic efficacy of ondansetron

DISCUSSION

Ondansetron a 5-HT3 receptor antagonist is a widely used drug for post-operative vomiting (POV) in our country due to its good efficacious profile. However not all the patients treated with it respond in the same way. And one of the main reasons for this inter-individual variability is said to be the genetic makeup. Previous studies in Caucasians have shown that the genetic variability of the target site for this drug and the clinical response are associated to each other. We in this study explored the e□ect of genetic polymorphism -100_-102AAG deletion in the 5-HT3B receptor gene on the incidence of POV.

There is a huge list of drugs that act as agonist and antagonist at 5-HT3 receptor. However the antagonist have more frequent clinical uses. They have been employed in treating chemotherapy-induced nausea and vomiting (CINV), in POV and in relieving vomiting during pregnancy.[12]

The anti-emetic response to 5-HT3 receptor antagonists and the gene encoding 5-HT3B site have been shown to be associated to each other. The polymorphism of the 5-HT3B receptor gene in Japanese patients having psychiatric problems was observed to be affecting the nausea induced by anti-depressant drug. It was observed that the patients having deletion AAG had more frequent nausea and a significant association was found between the phenotype and genotype.[13] Another study by Yamada also suggested an important role for HTR3B in major depression in women.[14]

In 2003 Tremblay and his colleagues documented a polymorphism rs3831455 in the promoter region of 5-HT3B gene. This polymorphism was said to be effecting the structure of mRNA, the translation and ultimately the protein binding. Thus enhanced the promoter activity. This pointed towards the existence of association between the genetic change and clinical response.

They conducted this study on cancer patients undergoing highly emetogenic chemotherapy. It was concluded that the patients having variant allele of this polymorphism had more incidence of nausea and vomiting than the other patients most probably due to the increased translation. Such patients required more doses of anti-emetics due to failure of response.[8] Rueffert came up with the conclusion suggesting the possible genetic role of HTR3B gene in the individual risk of developing POV.[5] So far very few studies have studied the impact of this polymorphism in post-operative patients. We did confirm this specific finding, We observed genetic impact of HTR3B variant in POV patients.

All the risk factors that can be labelled as predictors of POV were all considered and risk factors did not differ significantly in accordance to the genotypes. We enrolled patients undergoing similar surgical procedure and the anesthesia protocol was also kept standardized for all the subjects in an attempt to minimize the influence of anesthetic and surgical factors on our results. We had confirmed that the genotype distribution of -100_-102AAG deletion of the 5-HT3B receptor gene was within the Hardy-Weinberg equilibrium, pointing to the fact that these findings of ours were most likely correct. The functional aspects of this polymorphisms couldn't be made obvious as for that an in vitro study would be required. Though Frank et al could demonstrate a modification in the structure of mRNA due to this polymorphism.[15] Moreover Meineke et al could also come up with increased promotor activity of the gene due to this polymorphism. Thus the polymorphism be responsible for varied activity of receptor encoded by this gene.[16]

CONCLUSION

The POV is affected by -100_-102AAG deletion variant of the 5-HT3B gene and this genetic variability can predict the responsiveness to ondansetron.

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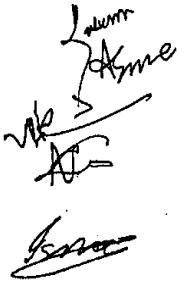
AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Kulsoom Farhat	Conception and design of the study part of PhD project.	
2	Asma Shaukat	Data collection at hospital.	
3	Shabana Ali	Statistical analysis and its verification.	
4	Akbar Waheed	Discussion of the results and contribution towards the final manuscript.	
5	Muhammad Ismail	Carried out the genetic analyis at IBGE	