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Ondansetron serum concentration and polymorphisms of *CYP2D6, ABCB1* and *5-HT3B* receptor genes in the treatment of chemoterapy induced nausea and vomiting

DA Perwitasari[1], Mustofa[2]
[1]Faculty of Pharmacy, University of Ahmad Dahlan, Yogyakarta, [2]Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta

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ABSTRACT

This study was aimed to understand differences of ondansetron serum concentration in each antiemetic responses, polymorphisms of *5HT3B* receptor, *CYP2D6* and *ABCB1* genes in Indonesian cancer patients treated with high emetogenic cytostatics. We recruited cancer patients in Dr Sardjito Hospital treated *w*ith cisplatin ($\geq$ 50 mg/m ^{2}) as monotherapy or combination therapy. Patients were treated with ondansetron 8 mg intravenously and dexamethasone 8 mg intravenously and metoclopramide (10 mg orally) after cytostatic administration until 5 days after chemotherapy. We cathegorized the nausea and vomiting grade according to the National Cancer Institute Common Toxicity Criteria v.3. We also determined some SNPs of *ABCB1, 5HT3B* and *CYP2D6* genes using realtime PCR. We recruited 191 cancer patients in this study with the average of ondansetron serum concentration reached 33.48 ng/ml (SD: 18.54). According to the patients' response to the antiemetic, during the acute phase, 21.8% patients experienced acute nausea and 30.2% patients experienced acute vomiting. Only the haplotype of CTG-CTG of *ABCB1* which have significant association with ondansetron serum concentration. EM patients of *CYP2D6* and patients with haplotype of delAG of *5HT3B* had lower ondansetron serum concentration. However, IM patients of *CYP2D6* showed higher ondansetron serum concentration and lower grade of nausea and vomiting. Variations of *ABCB1, CYP2D6* and *5HT3B* may be used as pharmacogenetic marker in predicting antiemetic response in cancer patients receiving highly emetogenic cytostatic.

ABSTRAK

Penelitian ini bertujuan untuk mengetahui perbedaan konsentrasi ondansetron serum dalam setiap respon antiemetik, polimorfisme gen reseptor *5HT3B* , *CYP2D6* dan *ABCB1* pada pasien kanker yang mendapatkan sitostatika emetogenik tinggi di Indonesia. Subjek dalam penelitian ini adalah pasien kanker di RSUP Dr Sarjito Hospital yang mendapatkan terapi sisplatin ($\geq$ 50 mg/m ^{2}) baik sebagai terapi tunggal maupun kombinasi. Subjek mendapatkan terapi ondansetron 8 mg, iv dan deksametason 8 mg, dan metoklopramid setelah pemberian sitostatika sampai hari kelima kemoterapi. Kategori mual muntah berdasarkan *National Cancer Institute Common Toxicity Criteria v.3*. Beberapa SNPs pada

Corresponding author: Lukman.apt@gmail.com

gen *ABCB1, 5HT3B* dan *CYP2D6* diidentifikasi dengan metode *realtime PCR.* Sejumlah 191 pasien berpartisipasi dalam penelitian ini dengan rerata konsentrasi ondansetron dalam serum adalah 33.48 ng/ml (SD:18.54). Pasien yang mengalami fase akut mual adalah 21.8% dan yang mengalami fase akut muntah adalah 30.2%. Haplotype CTG-CTG dari *ABCB1* mempunyai hubungan yang signifikan dengan kadar indansetron dalam serum. Pasien dengan fenotipe EM dari *CYP2D6* dan pasien dengan haplotipe delA dari *5HT3B* mempunyai kadar ondansetron rendah dalam serum. Pasien dengan fenotipe IM dari *CYP2D6* mempunyai konsentrasi ondansetron tinggi dan tingkat mual dan muntah yang rendah. Variasi gen *ABCB1, CYP2D6* dan *5HT3B* dapat digunakan sebagai penanda farmakogenetik untuk mengetahui respon antiemetik pada pasien kanker yang menerima sitostatik emetogenik tinggi.

Keywords : pharmacogenetics – antiemetic – cancer – polymorphism - Indonesia

INTRODUCTION

Nausea and vomiting is the most distressfull side effect of chemotherapy in cancer patients.[1,2] These symptoms may affect patient's quality of life and furthermore cause the decrease of patients' adherence in following the chemotherapy treatment.[3] In cancer patients treated by high and moderate emetogenic cytostatic as chemotherapy agent, the antiemetic which should be given to the patients are combination of 5-hydroxytriptamine 3 receptor antagonist (HT3RA), dexamethasone and neurokinin 1 receptor antagonist (NK1 antagonist).[4,5] However, around 30% of patients receiving high emetogenic cytostatic and moderate emetogenic cytostatic still did not have good response to this combination treatment.[4]

One of the causal of response variabilities of the antiemetic is the polymorphisms of *5-HT3B* receptor, *CYP2D6* and *ABCB1* genes. Even some previous studies did not show the consistency about the significancy of statistical results, however the pattern of the genotypes and the patients' response were similar.[6-10] One of the previous study supported that the polimorphism of *CYP2D6* resulted the significant difference of tropisetron serum concentration in poor metabolizer, extensive metabolizer and ultra-rapid metabolizer.[8]

This study is aimed to evaluate the association between ondansetron serum concentration and the antiemetic responses, polymorphisms of *5HT3B* receptor, *CYP2D6* and *ABCB1* genes in Indonesian cancer patients treated with high emetogenic cytostatics.

MATERIALS AND METHODS

Patients

We recruited cancer patients in Dr Sardjito General Hospital, Yogyakarta who were treated with cisplatin ($\geq$ 50 mg/m^2) as monotherapy or combination therapy. The standard antiemetic were ondansetron 8 mg intravenously and dexamethasone 8 mg intravenously. Patients were treated with metoclopramide (10 mg orally) after cytostatic administration until 5 days after chemotherapy. The inclusion criteria were patients with age $\geq$ 18 years old and they have a Karnofsky Performance Scale (KPS) of $\geq$ 50%. The patients were excluded if nausea or vomiting were present 24 hours before chemotherapy; the use of other antiemetics such as benzodiazepines or neuroleptics, radiotherapy within 24 hours before start of chemotherapy, use of opioids

within the last 2 weeks, use of inducers of CYP3A4 or inhibitors of CYP2D6, patients with concomitant diseases that might cause nausea or vomiting, patients with AST-ALT > 2,5 x ULN for patients without liver metastases > 5 x ULN for patients with liver metastases, renal dysfunction defined by creatinine clearance < 60 ml/minute, brain metastases and patients with artificial stoma or pregnancy.[8,9] We did the informed consent to all of the patients before the recruitment. This study has been approved by The Medical and Health Research Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Measurement of nausea and vomiting

We evaluated the nausea using 0-100 of Nausea Visual Analog Scale (NVAS) for 5 days after chemotherapy. The vomiting was assesed by quantifying the number of vomiting that was separated at least 1 minute betweeen each of vomiting episode.[9]

Study outcome definitions

We cathegorized the nausea and vomiting according to the previous study. The nausea and vomiting were grouped into grade 1-2 and grade 3-4 nausea vomiting of the National Cancer Institute Common Toxicity Criteria v.3 (NCI CTC v.3).[9,11]

Ondansetron serum concentration assay

The blood was taken from vena mediana cubiti 1.5 hour after intravenously injected to the patients. The ondansetron peak serum concentration reaches 30 ng/ml on the administration of 8 mg ondansetron intravenously.[12] Ondansetron serum concentration was measured using High Performance Liquid Chromatoghraphy as described in the previous method.[13,14]

Genotyping assay

We used patients' saliva as the samples. DNA concetration was measured using Nanodrop and the genotype assays were established using pre-designed Taqman assays and analysed on ABI 7500 realtime PCR System from Applied Biosystems. We also calculated the Hardy-Weinberg equilibrium and all of the assay were on the Hardy Weinberg equilibrium.

SNPs selection

We used three SNPs in the *5-HT3B* receptor gene: rs3831455 (deletion AAG in 5'-UTR position), rs4938058 (intron) , and rs7943062 (3' near gene); three SNPs in the *ABCB1* gene: rs1045642 (exon 26), rs2032582 (exon 22), rs 1128503 (exon 12) and three SNPs of *CYP2D6*; rs16947 (CYP2D6*2), rs3892097 (CYP2D6*4), rs1065852 (CYP2D6*10). Those particular SNPs were selected from the National Center for Biotechnology Information SNP database using these criteria: a minor allele frequency of >0.2, a validated SNP according to the NCBI database, and preferably a perfect Linkage Disequilibrium (LD) with other SNPs (for 5-HTR3B receptor gene: D'- =1 and r-square ≥0.7) and/or indications for relevance based on previous publications.[6,8-10]

Statistical analysis

The student-t test was performed to understand the differences between ondansetron serum concentration. We performed haplotype frequency estimation and individual haplotype from genotype data using gPlink. The metabolizer's phenotypes of SNPs of *CYP2D6* were defined according to the previous study, as CYP2D6*2, *4 and *10.[9]

RESULTS

We recruited 191 cancer patients in this study. The mean age of patients was 48.23 ± 9.7 years and most of them were female (93.1%). Around 58 % patients were diagnosed as cervical cancer , around 67 % of them were in the early stage and around 90% patients were treated with cisplatin (50-70 mg/m^2) either as monotherapy or in combination. We found that the average of ondansetron serum concentration reached 33.48 ± 18.54 ng/mL. This infomation is in line with the literature which reported that after 1.5 treatment of ondansetron intravenously, the peak ondansetron concentration will reach 30 ng/mL.[12] According to the patients' response to the antiemetic, during the acute phase, 21.8% patients experienced acute nausea and 30.2% patients experienced acute vomiting.

TABLE 1. Characteristics of cancer patients treated with antiemetics (n = 191)

Characteristic	Value	%
Ondansetron serum concentration (ng/mL)		
Mean ± SD	33.48 ± 18.54	
Minimal concentration	0.18	
Maximal concentration	117.36	
Age	48.2 ± 9.7	
Sex		
Male	13	6.8
Female	178	93.2
Diagnosis		
Cervical cancer	112	58.6
Ovarian cancer	57	29.8
Others	22	11.6
Stage of cancer		
Stage I and II	128	67.0
Stage III and IV	63	33.0
Cytostatic agent		
Cisplatin	76	39.8
Cisplatin and other agent	115	60.2
Cisplatin dose		
50-70 mg/m^2	173	90.6
75-100 mg/m^2	18	9.4

TABLE 2 shows the differences between *CYP2D6* phenotypes, nausea and vomiting grades and ondansetron serum concentration. There were no significant asssociation among them. However, we can see that EM patients, patients with higher grade of nausea and patients with lower grade of vomiting had lower ondansetron serum concentration.

TABLE 2. Differeces between ondansetron serum concentration and *CYP2D6* phenotypes, nausea and vomiting grade

Cathegory	Ondansetron serum concentration		p value
	n	Mean ± SD	
CYP2D6 phenotype			
EM	165	33.30 ± 18.96	0.862
IM	23	34.02 ± 14.83	
Nausea grade			
0 and 1	150	33.62 ± 18.14	0.850
2 and 3	41	32.99 ± 20.18	
Vomiting grade			
0 and 1	135	32.26 ± 17.94	0.158
2 and 3	56	36.43 ± 19.77	

FIGURE 1 shows the ondansetron serum concentration in the vomiting grades and *CYP2D6* phenotypes. In EM patients, serum concentration of ondansetron are higher in subjects with grade 2 and 3 vomiting than subjects with grade 0 and 1 vomiting. In contrast, among the subjects with IM phenotype, serum concentration of ondansetron are higher in subjects with low grade of vomiting than subjects with high grade of vomiting.

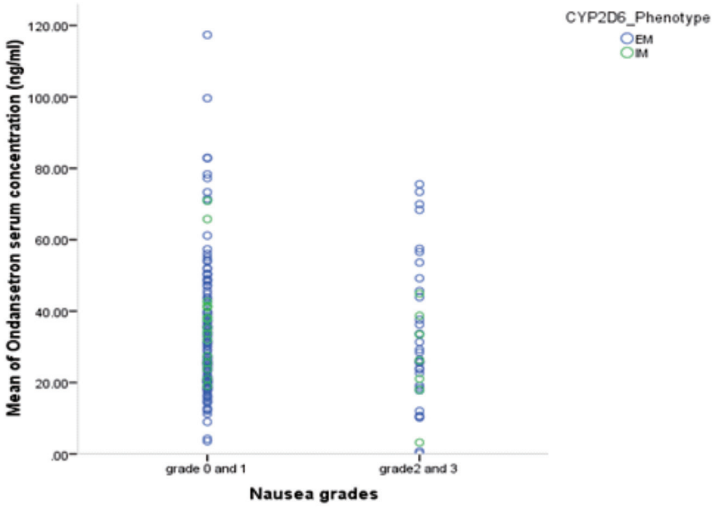

FIGURE 1. Serum concentration of ondansetron in CYP2D6 phenotypes and vomiting grades

TABLE 3 lists the differences between ondansetron serum concentration and haplotype of *5HT3B* and *ABCB1* genes. The significant difference of ondansetron concentration is shown in the variant of *ABCB1* CTG haplotype. Subjects with CTG-

CTG haplotype had lower ondansetron concentration than subjects with other haplotype. The subjects with delAG-delAG variant of 5*HT3B* had lower concentration of ondansetron than subjects with other haplotype.

TABLE 3. Differences between ondansetron serum concentration and haplotypes of *5HT3B* and *ABCB1* genes

Cathegory	Ondansetron serum concentration		p value
	n	Mean ± SD	
ABCB1 CCG			
Carriers of other haplotype	160	33.05 ± 18.54	0.624
CCG-CCG	19	35.24 ± 18.11	
ABCB 1 CTG			
Carriers of other haplotype	168	33.86 ± 18.75	0.-015
CTG-CTG	11	24.42 ± 10.26	
ABCB 1 TTT			
Carriers of other haplotype	160	32.89 ± 18.16	0.405
TTT-TTT	19	36.63 ± 21.33	
5-HT3BR AAGAG			
Wildtype	44	35.01 ± 23.67	0.674
Carriers of AAGAG	134	33.65 ± 16.65	
5-HT3BR AAGAG			
Carriers of other haplotype	119	33.59 ± 18.94	0.684
AAGAG-AAGAG	59	34.80 ± 17.91	
5-HT3BR AAGGG			
Carriers of other haplotype	172	33.99 ± 18.77	0.973
AAGGG-AAGGG	6	33.73 ± 12.43	
5-HT3BR AAGAA			
Carriers of other haplotype	176	34.17 ± 18.57	0.225
AAGAA-AAGAA	2	18.10 ± 12.88	
5-HT3BR delAG			
Carriers of other haplotype	172	34.01 ± 18.43	0.934
delAG--delAG	6	33.37 ± 24.16	

DISCUSSION

Our present study was aimed to understand the differences between the polymorphisms of *5HT3B, CYP2D6* and *ABCB1* genes and ondansetron serum concentration. Our preliminary data cannot show significant differences between the variables. However, we can present some pharmacogenetic markers which could affect the antiemetic response. Around 30% patients did not show good response to the ondansetron. The previous studies with almost similar regimen without NK1 antagonist also showed that the complete response of 5HT3RA reached around 70%.[2,15] In the future, Indonesia Health Institute should consider the use of NK1 antagonist in the guideline of prevention of chemotherapy-induced nausea vomiting in cancer patients treated with highly or moderately cytostatic agents.

As seen in the patients' characteristics, cervical cancer was the most diagnosed cancer in this study. This fact is in accordance with the statistic data on 2014 which was stated that the cervical cancer was the 2nd most frequent cancer in female and the 2nd most frequent cancer as well in the age range of 15-44 years old.[16] Cisplatin was mostly used in combination which may have the increase of CINV severity. This situation may prolong the existence of CINV in to the delayed phase.[17] Thus, the use of antiemetics should be closely monitored to increase patients' compliance in the treatment of chemotherapy.

In this study, we did not find patients with ultra rapid metabolizers phenotype of CYP2D6. However, we found that EM patients had lower serum concentration of ondansetron than IM patients. Over the IM patients, patients who experienced lower grade of nausea and vomiting showed higher serum concentration of ondansetron. This result is supported by previous study using tropisetron which showed that patients with higher number of active alleles experienced more nausea and vomiting and showed the lower serum concentration of tropisetron.[8] The other previous study with polymorphisms of OCT1 also supported this finding. Patients with higher number of active allele experienced higher grade of nausea vomiting with the lower serum concentration of ondansetron and tropisetron.[14]

According to the other two genes, only one haplotype of ABCB1 which shows significant differences between ondansetron serum concentration and the haplotype of CTG-CTG. However, related with the 5Ht3BRA gene, there were 6 patients with the haplotype of delAG-delAG had lower serum concentration of ondansetron. This finding is supported by previous study which stated that patients with the del AAG experienced higher score of nausea and vomiting.[10]

Our study has limitation about the number of patients. Because the number of variant in our study is low, thus we need further study with larger sample size to reach the significant result of statistical analysis. In our study, even though the most of statistical result is not significant, but we can show the difference patterns which are supported by some previous study.

CONCLUSION

Variations of ABCB1, CYP2D6 and 5HT3BRA may be used as pharmacogenetic marker in predicting antiemetic response in cancer patients receiving highly emetogenic cytostatic.

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