Pharmacogenetics of antiemetics in Indonesian cancer patients

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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*

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ABSTRACT

Objective: Suboptimal treatment of chemotherapy-induced nausea and vomiting (CINV) and unsatisfactory response to antiemetic drugs cause impairment of cancer patient's daily functioning. This study was aimed to investigate the association of selected germline polymorphisms with ondansetron and metoclopramide response in Indonesian cancer patients treated with highly emetogenic chemotherapy.

Methods: We enrolled 202 chemotherapy naïve patients treated with cisplatin at a dosage ≥ 50 mg/m^2 as monotherapy or as combined chemotherapy. Ondansetron 8 mg and dexamethasone 8 mg intravenously were the standard antiemetic therapy for prevention of acute chemotherapy-induced nausea and vomiting. Metoclopramide 10 mg orally, 3 times per day as fixed prescription was given until 5 days after chemotherapy to prevent delayed chemotherapy-induced nausea and vomiting. Primary and secondary outcomes were the occurrence of chemotherapy-induced nausea and vomiting in acute and delayed phase. The following single nucleotide polymorphisms were determined in *ABCB1*: rs1045642, rs2032582, rs1128503; in *5-HT3B receptor*: rs45460698, rs4938058, rs7943062 and in *CYP2D6*: rs16947 (*CYP2D6*2*), rs3892097 (*CYP2D6*4*), rs1065852 (*CYP2D6*10*) using Taqman assays.

Results: During the acute phase, 21.8% and 30.2% patients experienced Grade 3 and 4 nausea and vomiting, respectively, whereas 38.6% patients experienced nausea and/or vomiting in the delayed phase. Carriers of CTG haplotype of the *ABCB1* gene experienced Grade 3 and 4 CINV more often than other haplotypes in the delayed phase ($P < 0.05$). No associations were found with the 5-HT3B receptor haplotypes and CYP2D6-predicted phenotypes.

Conclusions: Our study shows that in Indonesian cancer patients treated with highly cytostatic emetogenic, carriership of the CTG haplotype of the *ABCB1* gene is related to an increased risk of delayed CINV.

INTRODUCTION

Chemotherapy-induced nausea and vomiting (CINV) is the most common side effect of cancer patients treated with highly emetogenic chemotherapy[1] and has a significant effect on the patients' daily functioning and well-being.[2] Poor control of acute CINV, which occurs within 24 hours after chemotherapy, may be used as predictor of delayed CINV.[3] However, patients with delayed CINV, which persists from 24 to 120 hours after chemotherapy, experience more severe impact of daily functioning than patient with acute CINV.[4]

The introduction of 5-hydroxytriptamine 3 receptor antagonists (5-HT3RAs) significantly improved the control of CINV.[4] However, the use of 5-HT3RAs in combination with dexamethasone as antiemetic treatment in patients treated with highly emetogenic chemotherapy provides only 70-80% complete protection in the acute phase[2,6] and 60% complete protection in delayed emesis.[5]

Ondansetron is the first 5-HT3RA and the most widely used in Indonesia community hospitals. Standard antiemetic treatment for prevention of acute CINV in Indonesia is ondansetron in combination with dexamethasone. For prevention of delayed CINV, metoclopramide is given orally from 24 hours until 120 hours after chemotherapy. We realize that the combination of a 5-HT3RA, a neurokinin-1 antagonist and a corticosteroid is more effective and is therefore frequently given to cancer patients treated with high emetogenic chemotherapy.[6,7] This combination increases the complete protection of acute emesis, with 10-15% increased response in comparison with the combination of 5-HT3RA and a corticosteroid,[8,9] currently the neurokinin-1 antagonist, aprepitant is not available in Indonesia.

Next to the antiemetic treatment regimen, patient characteristics such as age, gender, history of motion sickness, history of alcohol drinking are known to influence antiemetic drug efficacy. In addition, in recent years it appeared that also genetic variation in genes encoding drug transporters, metabolic enzymes and drug targets may influence drug efficacy.[3] Indeed, variability in ondansetron transport, biotransformation and receptor affinity may cause variations in ondansetron's efficacy.[10] More specifically, ondansetron is transported into the blood-brain barrier by the drug transporter P-glycoprotein (P-gp) and is partially metabolized by, for example, cytochrome P450 2D6 (CYP2D6) and has moderate affinity on the 5-HT3 receptors.[10-12]

In a previous study, it has been reported that the gene *ABCB1* encoding P-gp has a role in the pharmacology of ondansetron. The ondansetron transepithelial transport decreased when an inhibiting agent was added into a MDR1 cell line. In other words, the passive diffusion rate of ondansetron was increased by P-gp.[13] This mechanism was found in both the gastrointestinal and blood-brain barrier.[11,12] In addition, a polymorphism in the

ABCB1 gene, 3435C>T, showed a significant association with the occurrence of acute CINV in cancer patients.[13] Regarding ondansetron metabolism, it was reported in a Caucasian population that the ultrarapid metabolizers (UM) of CYP2D6 experienced the most severe nausea and vomiting after chemotherapy treatment.[14] It has been shown that ondansetron is mainly metabolized by CYP1A2, CYP2D6 and CYP3A4.[15] Finally, other studies suggested that variation of 5-HT3B, 5-HT3C and 5-HTR3D receptors could be the predictors of 5-HT3RAs' efficacy in cancer patients.[16-18]

For metoclopramide, gene variations of protein transporter and drug metabolizing enzyme are suggested to influence efficacy and adverse drug reaction.[19,20] The passage of metoclopramide across the blood brain barrier is also influenced by the P-gp transporter,[19] whereas its metabolism is highly dependent on CYP2D6.[20,21]

In theory, not only the response to antiemetic drugs may be genetically determined but also the susceptibility to emetogenic drugs leading to interindividual differences of vomiting and nausea at baseline. However, as our knowledge, there are no studies relating genetic variants to severity of chemotherapy-induced emesis. The aim of this study was to investigate the association of *ABCB1*, *5-HT3B* receptor polymorphisms and CYP2D6-predicted phenotypes with ondansetron and metoclopramide antiemetic response of Indonesian cancer patients treated with highly emetogenic chemotherapy.

PATIENTS AND METHODS

Study population

The study population involved various cancer patients in the Oncology Department of Dr Sardjito Hospital, Yogyakarta, Indonesia, from January 2009 until April 2010, who were treated with cisplatin at a dosage ≥ 50 mg/m^2 as monotherapy or in combination chemotherapy regimens. Ondansetron 8 mg intravenously and dexamethasone 8 mg intravenously were standard antiemetic therapy for prevention of acute CINV. Metoclopramide, 10 mg orally, 3 times per day as fixed presciption, was given to the patients after cytostatic administration until 5 days after chemotherapy in order to prevent delayed CINV.

Patients were eligible for this study if they were ≥ 18 years old with a Karnofsky performance scale (KPS) of ≥ 50%. We used self-reported ethnicity. However, to make a more accurate assessment of ethnicity also the ethnicity of the parents and grandparents were verified. Exclusion criteria were: the presence of nausea or vomiting 24 hours before chemotherapy; the use of other antiemetics such as benzodiazepines or neuroleptics, radiotherapy within

24 hours before start of chemotherapy, the use of opioids within the last 2 weeks, the use of inducers of CYP3A4 or inhibitors of CYP2D6, patients with concomitant diseases that might cause nausea or vomiting (e.g. ulcerations or obstruction of the upper gastrointestinal system, aspartate aminotransferase/alanine aminotransferase > 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/min, brain metastases, artificial stoma or pregnancy.

This study has been approved by The Ethical Committee of the Medical Faculty of Universitas Gadjah Mada, Yogyakarta, Indonesia. All of the patients signed the consent form before enrollment.

Nausea and vomiting assessment

Every patient completed a daily record up to 5 days starting at initiation of cytotoxic drugs administration. The daily record contained the number of episodes of vomiting, the 0-100 scale of Nausea Visual Analog Scale (NVAS) and the antiemetic therapy that was consumed over 5 days. Patients were informed that an episode of vomiting that was separated at least 1 minute from the previous one counted as single episode.[22]

Study outcome definitions

The primary outcome was acute nausea and vomiting which was categorized based on the National Cancer Institute Common Toxicity Criteria v.3 (NCI CTC v.3).[23] We grouped the acute nausea and vomiting into Grade 1-2 and Grade 3-4 nausea vomiting. Patients were discharged from the hospital on day 1, a few hours after the cytostatic administration. Therefore, we could not categorize the secondary outcome based on the NCI CTC v.3. The secondary outcome was delayed nausea and vomiting scored dichotomic (yes or no). Patients without delayed emesis (no) were defined as patients without vomiting and/or had less than a 5 score on the NVAS scale, while patients with delayed emesis (yes) were patients with vomiting and/or scored ≥ 5 scale of NVAS.[24,25]

SNPs selection and genotyping assays

Three SNPs in the *5-HT3B* receptor gene: rs45460698 (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), rs1128503 (exon 12) and three SNPs of *CYP2D6*; rs16947 (CYP2D6*2), rs3892097 (CYP2D6*4), rs1065852 (CYP2D6*10) were selected from the National Center for Biotechnology Information SNP database. The selection of the SNPs

was based on the following 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 $r^2 \geq 0.7$) and/or indications for relevance based on previous publications.[18,26-29]

DNA was extracted from saliva samples. DNA was quantified using Nanodrop (Isogen, Maarssen, The Netherlands). Genotypes were established using commercially available pre-designed Taqman assays and analysed on ABI 7500 realtime PCR System from Applied Biosystems (Nieuwerkerk aan den IJssel, The Netherlands) according to manufactures' protocol of allelic discrimination. As a quality control at least 5% of samples were genotyped in duplicate and no inconsistencies were found. Overall genotyping success rate of the samples was more than 96%.

Statistical methods

The genotype frequencies were assessed for deviations from Hardy Weinberg equilibrium and they did not deviate from Hardy Weinberg equilibrium. The gPlink software was used to estimate the haplotype frequency and to set the individual haplotypes from raw genotype data. The estimation of haplotype frequencies/phases was ≥ 0.01 and phases consideration was ≥ 0.01.[30]

The predicted phenotypes of SNPs in CYP2D6 gene were defined as follows: CYP26*2 is an active allele, *10 is a decreased activity allele and *4 is a defective allele.[14,31,32] Therefore, the definition of extensive metabolizers (EMs) include *2/*2, *2/*10, the intermediate metabolizers (IMs) include *2/*4, *4/*10, *10/*10, and poor metabolizers (PMs) include *4/*4.

The χ^2 test was performed to test the association of patient characteristics and primary and secondary outcome. Moreover, the association of 5-HT3B receptor and ABCB1 haplotypes and CYP2D6-predicted phenotypes with primary and secondary outcome were analyzed by χ^2 test. These associations are considered to be the result of ondansentron as the antiemetic drug in the acute phase and metoclopramide as the antiemetic drug in delayed phase. A *P* value of < 0.05 was considered as significant association. This study is explorative and hypothesis generating, and therefore we decided not to correct for multiple testing.

RESULTS

A total of 202 patients were enrolled in this study. Table 3.1 presents the patient characteristics.

Table 3.1 Characteristics of cancer patients treated with antiemetics ($n = 202$)

Characteristic		
Age (mean ± SD)	48.6 ± 9.6	
Gender	*n*	%
Male	14	6.9
Female	188	93.1
Diagnosis		
Cervical cancer	121	59.9
Ovarian cancer	58	28.7
Lung cancer	3	1.6
Nasopharyngeal cancer	13	6.4
Vulva cancer	7	3.4
Stage of cancer		
Stage I and II	139	68.8
Stage III and IV	63	31.2
Cytostatic agent		
Cisplatin	81	40.1
Cisplatin and other agent	121	59.9
Cisplatin dose		
50-70 mg/m^2	183	90.6
75-100 mg/m^2	19	9.4
BMI		
Underweight (16-18.5 kg/m^2)	49	24.3
Normal (18.5-25kg/m^2)	117	57.9
Overweight and obese (> 25 kg/m^2)	36	17.8
Karnofsky Performance Status		
80-100%	182	90.1
50-70%	20	9.9
Comorbidity		
None	109	53.9
At least 1	93	46.1
History of motion sickness		
Yes	39	19.3
No	163	80.7
History of morning sickness during pregnancy		
Yes	45	22.3
No	134	66.3
NA	23	11.4
Patients' perception for having nausea and vomiting after chemotherapy		
Yes	79	39.1
No	123	60.9
Anxiety		
Yes	90	44.6
No	112	55.4

SD, standard deviation; BMI, body mass index; NA, not applicable because patients have not been pregnant yet

The most frequent diagnosis was cervical cancer (59.9%), mostly diagnosed as Stage 1 or 2 of cancer (68.8%). The majority of the patients (90.6%) were treated with an intermediate dose of cisplatin (50-70 mg/m^2) either as monotherapy or in combination therapy, the remaining patients (9.4%) were treated with cisplatin at a dosage of 75-100 mg/m^2.

The presence of nausea and vomiting during the acute and delayed phase is presented in Table 3.2.

In the acute phase, 21.8% patients experienced acute nausea and 30.2% patients experienced acute vomiting, whereas 38.6% patients experienced nausea and/or vomiting in the delayed phase. Figure 3.1 and Figure 3.2 present the means of vomiting episodes and NVAS score over 5 days. The peak of vomiting episodes and NVAS score was seen on day 2, with a gradual decline afterwards.

Table 3.3 depicts the association between patient characteristics and primary and secondary outcome measurements. No significant associations of patient characteristics and primary or secondary endpoint were found. However, the data suggest that Grade 3 and 4 acute CINV and delayed CINV are more frequent in younger patients with low performance and a history of motion sickness but the associations did not reach significance. The statistical analyses were performed in the female subjects to understand the association between gene variants, patients' characteristic and the primary/secondary outcome. However, we found no significant association in the analysis results (data not shown).

In Table 3.4 the association of gene haplotypes and phenotypes with primary and secondary endpoint are presented. A statistical significant association was found between the CTG haplotype in the *ABCB1* gene and the presence of nausea and vomiting in

Table 3.2 The occurrence of acute and delayed chemotherapy induced nausea and vomiting

	n	%
Acute nausea		
Grade 1 and 2	158	78.2
Grade 3 and 4	44	21.8
Acute vomiting		
Grade 1 and 2	141	69.8
Grade 3 and 4	61	30.2
Delayed CINV		
None	124	61.4
Yes	78	38.6

CINV, chemotherapy induced nausea vomiting.

the delayed phase. Carriers of the *ABCB1* CTG haplotype experienced more frequent Grade 3/4 CINV compared to the other haplotypes ($P < 0.05$). Multivariate analysis demonstrated that age and gender did not alter this result (data not shown).

In our population, no predicted phenotypes of CYP2D6, the UMs or PMs were found; the percentages of EMs and IMs were 59.9% and 32.7%, respectively.

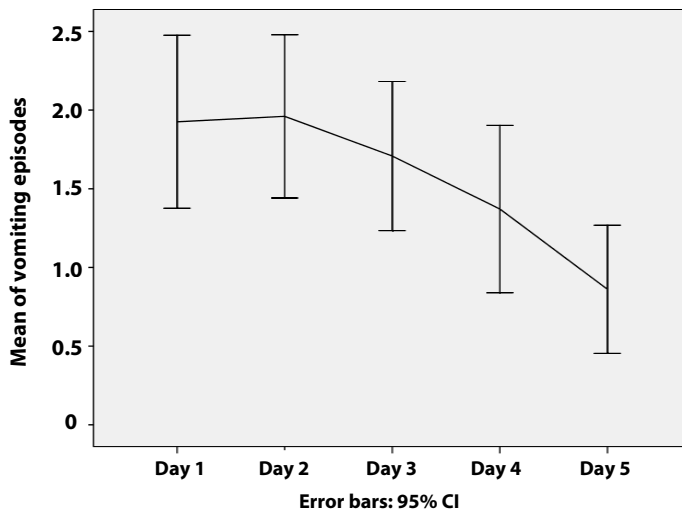

Figure 3.1 Mean (± SD) number of vomiting episodes over 5 days after initiation of chemotherapy.

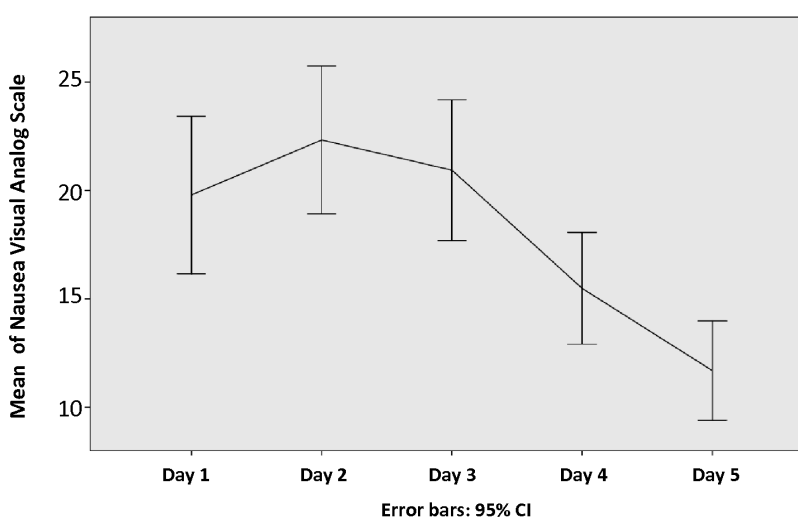

Figure 3.2 Mean (± SD) of Nausea Visual Analog Scale over 5 days after initiation of chemotherapy.

Table 3.3 Univariate analysis of patients characteristics and primary-secondary outcome

Patients characteristics	Acute nausea [*n* (%)		*P* value	Acute vomiting [*n* (%)]		*P* value	Delayed CINV		*P* value
	Grade 1 & 2 (*n* = 158)	Grade 3 & 4 (*n* = 44)		Grade 1 & 2 (*n* = 141)	Grade 3 & 4 (*n* = 61)		None (*n* = 78)	Yes (*n* = 124)	
Age, mean ± SD	48.4 ± 9.9	47.7± 8.8	0.67	48.6 ± 9.4	47.6 ± 10.0	0.51	48.8 ± 9.8	47.4 ± 9.4	0.29
Gender									
Male	12 (8.2)	1 (2.3)	0.20	10 (7.1)	3 (6.6)	0.75	115 (8.1)	74 (5.1)	0.55
Female	146 (91.8)	43 (97.7)		131 (92.9)	58 (93.4)		9 (91.9)	4 (94.9)	
Diagnosis									
Cervical cancer	95 (60.1)	26 (59.1)	0.86	88 (62.4)	33 (54.1)		79 (63.7)	42 (53.8)	0.31
Ovarian cancer	46 (29.1)	12 (27.3)		39 (27.7)	19 (31.1)	0.46	41 (25.0)	27 (34.6)	
Others	17 (10.8)	6 (13.6)		14 (9.9)	9 (14.8)		13 (11.3)	9 (11.5)	
Stage of cancer									
Stage I and II	106 (67.1)	33 (75.0)	0.32	94 (66.7)	45 (73.8)	0.32	86 (69.4)	53 (67.9)	0.83
Stage III and IV	52 (32.9)	11 (25.0)		47 (33.3)	16 (26.2)		38 (30.6)	25 (32.1)	
Cytostatic agent									
Cisplatin	59 (37.3)	22 (50.5)	0.13	56 (39.7)	25 (41.0)	0.87	53 (42.7)	28 (35.9)	0.33
Cisplatin + other agents	99 (62.7)	22 (50.0)		85 (60.3)	36 (59.0)		71 (57.3)	50 (64.1)	
Cisplatin dose									
< 50-70 mg/m^2	143 (90.5)	40 (90.9)	0.93	130 (92.2)	53 (86.9)	0.24	114 (91.9)	69 (88.5)	0.41
75-100 mg/m^2	15 (9.5)	4 (9.1)		11 (7.8)	8 (13.1)		10 (8.1)	9 (11.5)	
BMI									
Underweight (< 16 kg/m^2)	42 (26.6)	7 (15.9)	0.16	38 (27.0)	11 (18.0)	0.05	27 (21.8)	22 (28.2)	0.15
Normal (16-18.5 kg/m^2)	86 (54.4)	31 (70.5)		74 (52.5)	43 (70.5)		70 (56.5)	47 (50.3)	
Overweight and obese (> 18.5 kg/m^2)	30 (19.0)	6 (13.6)		29 (20.6)	7 (11.5)		27 (21.8)	89 (11.5)	

Karnofsky Performance Status									
80-100%	143 (90.5)	39 (88.6)	0.71	129 (91.5)	53 (86.9)	0.95	113 (91.1)	69 (88.5)	0.54
50-70%	15 (9.5)	5 (11.4)		12(8.5)	8 (13.1)		11 (8.9)	9 (11.5)	
Comorbidity									
None	83 (52.5)	26 (59.1)	0.44	74 (52.5)	35 (57.4)	0.52	70 (66.9)	39 (50.0)	0.37
At least 1	75 (47.5)	18 (40.9)		67 (47.5)	26 (42.6)		54 (33.1)	39 (50.0)	
Motion sickness history									
No	131 (82.9)	32 (72.7)	0.13	119 (84.4)	44 (72.1)	0.09	103 (83.1)	60 (76.9)	0.28
Yes	27 (17.1)	12 (27.3)		22 (15.6)	17 (27.9)		21 (16.9)	18 (23.1)	
Morning sickness history									
No	106 (67.1)	28 (63.6)	0.23	99 (70.2)	35 (57.4)	0.13	84 (67.7)	50 (64.1)	0.27
Yes	32 (20.3)	13 (29.5)		27 (19.1)	18 (29.5)		21 (19.4)	21 (26.9)	
NA	20 (12.7)	3 (6.8)		15 (10.6)	8 (13.1)		16 (12.9)	7 (9.0)	
Patients' perception for NV after chemotherapy	97 (61.4)	26 (59.1)	0.78	91 (64.5)	32 (52.5)	0.11	74 (59.7)	49 (62.8)	0.66
No	61 (38.6)	18 (40.9)		50 (35.5)	29 (47.5)		50 (40.3)	29 (37.2)	
Yes									
Anxiety									
No	84 (53.2)	28 (63.6)	0.21	77 (54.6)	35 (57.4)	0.72	68 (54.8)	44 (56.4)	0.83
Yes	74 (46.8)	16 (36.4)		64 (45.4)	26 (42.6)		56 (45.2)	34 (43.6)	

Table 3.4 Univariate analysis of gene haplotypes and primary-secondary outcome

Gene	Acute nausea [*n* (%)]		*P* value	Acute vomiting [*n* (%)]		*P* value	Delayed CINV [*n* (%)]		*P* value
	Grade 1 & 2	Grade 3 & 4		Grade 1 & 2	Grade 3 & 4		None	Yes	
ABCB1 gene	*n* = 150	*n* = 38		*n* = 136	*n* = 52		*n* = 119	*n* = 69	
CCG									
Other haplotypes	87 (58.0)	23 (60.5)		82 (60.3)	28 (53.8)		67 (56.3)	43 (62.3)	
Carrier of CCG haplotype	63 (42.0)	15 (39.5)	0.78	54 (39.7)	24 (46.2)	0.42	52 (43.7)	26 (37.3)	0.42
CTG									
Other haplotypes	76 (50.7)	22 (57.9)		68 (50.0)	30 (57.7)		70 (58.8)	28 (40.6)	
Carrier of CTG haplotype	74 (49.3)	16 (42.1)	0.43	68 (50.0)	22 (42.3)	0.35	49 (41.2)	41 (59.4)	0.02*
CTT									
Other haplotypes	138(92.0)	38 (100.0)	0.07	125 (91.9)	51 (98.1)	0.12	109 (91.6)	67 (97.1)	0.14
Carrier of CTT haplotype	12(8.0)	0 (0.0)		11 (8.1)	1 (1.9)		10 (8.4)	2 (2.9)	
TTT									0.90
Other haplotypes	60 (40.0)	12 (31.6)		53 (39.0)	19 (36.5)		46 (38.7)	26 (37.7)	
Carrier of TTT haplotype	90 (60.0)	26 (68.4)	0.34	83 (61.0)	33 (63.5)	0.76	73 (61.3)	43 (62.3)	
5HT3B gene	*n* = 150	*n* = 36		*n* = 131	*n* = 55				
AAGAG									
Other haplotypes	34 (22.7)	10 (27.8)		32 (24.4)	12 (21.8)				
Carrier of AAGAG haplotype	116 (77.3)	26 (72.2)	0.52	99 (75.6)	43 (78.2)	0.70			
AAGGG									
Other haplotypes	106 (70.7)	21 (58.3)		87 (66.4)	40 (72.7)				
Carrier of AAGGG haplotype	44 (29.3)	15 (41.7)	0.15	44 (33.6)	15 (27.3)	0.40			
AAGAA									
Other haplotypes	112 (74.7)	30 (83.3)		99 (75.6)	43 (78.2)				
Carrier of AAGAA haplotype	38 (25.3)	6 (16.7)	0.28	32 (24.4)	12 (21.8)	0.70			
Del AG									
Other haplotypes	107 (71.3)	26 (72.2)		96 (73.3)	37 (67.3)				
Carrier of delAG haplotype	43 (28.7)	10 (27.8)	0.92	35 (26.7)	18 (32.7)	0.41			
CYP2D6 predicted phenotype	*n* = 150	*n* = 37		*n* =133	*n* = 54		*n* = 117	*n* = 70	
EM	93 (62.0)	28 (75.7)		86 (64.7)	35 (64.8)		76 (65.0)	45 (64.3)	
IM	57 (38.0)	9 (24.3)	0.12	47 (35.3)	19 (35.2)	0.98	41 (35.0)	25 (35.7)	0.93

* significant value. EM, extensive metabolizers; IM, intermediate metabolizers; CINV, chemotherapy induced nausea vomiting.

DISCUSSION

Our study confirms that prevention of CINV is suboptimal, ondansetron and dexamethasone could prevent about 80% of patients from acute nausea and 70% of patients from acute vomiting. In the delayed phase, with metoclopramide, 60% of the patients experienced no nausea and/or vomiting. These percentages are lower than commonly seen with newer antiemetic drugs such as aprepitant or with the use of 5HT3RAs for prevention during the delayed phase but these are no standard therapies in Indonesia.

To date, the reasons of variability in antiemetic drug response are largely unknown. To some extent, patient characteristic such as age and gender may contribute to variable drug response. Although we did not find significant association between patient characteristic and primary or secondary outcome in this study, a non-significant trend analysis supported that young patients were more susceptible to experience higher grade of acute and delayed nausea and vomiting. A previous study in cancer patients showed that female gender and younger age were associated with higher risk of CINV.[9] A reason for not replicating these findings in our study is that our patients were mostly women, of relatively young age and with a narrow distribution of age, resulting in limited power to find associations with gender and age. Remarkably, patients-related risk factors such as age play no role in individualizing choice of antiemetic-treatment in patients treated with highly emetogenic chemotherapy.[33]

Variations in genes which are involved in the pharmacology of antiemetic drugs may explain interpatient variability in response to these drugs. Indeed, our study shows that carriership of the CTG haplotype in the *ABCB1* gene increases the risk of delayed CINV and may therefore modify the effect of metoclopramide. In contrast, our study shows that genetic variants in *ABCB1*, *5-HT3B receptor* and *CYP2D6* are not related to ondansetron efficacy in acute CINV.

Interestingly, while the CTG haplotype of *ABCB1* is related to delayed CINV it is not related to acute CINV. This could be explained by the mechanism of cisplatin-induced nausea and vomiting which is probably mostly mediated by the serotonin release in the gastrointestinal enterochromaffin cells, and not in the central nervous system.[34] Thus the haplotype of *ABCB1* which could theoretically increase the amount of ondansetron that crosses the blood-brain barrier did not show significant impact in the ondansetron response. However, in a previous pharmacogenetic study in Caucasian cancer patients it was shown that the TT genotype of 3435C>T of *ABCB1* experienced less severe of emesis, because it was supposed that higher concentrations of ondansetron were available in the central nervous system.[13]

The significant association between the carrier of CTG haplotype in *ABCB1* gene and delayed nausea vomiting indicates that metoclopramide efficacy is modified by the *ABCB1* gene variation. The proposed mechanism is that passage of metoclopramide across the blood-brain barrier is increased in absence of an active P-gp. Indeed, metoclopramide's site of action as an antiemetic is thought to be in the fourth ventricle, which is located behind the blood-brain barrier. The role of P-gp in metoclopramide transport in the central nervous system is consistent with the finding of and increased metoclopramide concentration in the central nervous system in patients with an inactive P-gp leading to extra pyramidal symptoms.[19]

In the current study, the percentage of patients who experienced acute nausea and vomiting seemed to be higher in carriers of the AAGAG haplotype in *5-HT3B* receptor gene, although it did not reach statistical significance. Patients carrying the deletion AG haplotype in 5-HT3B receptor experienced a lower grade of nausea and a higher grade of vomiting in the acute phase compared to the other haplotypes.

We performed a haplotype analysis because we could consider information about human evolutionary history and genetic variants by finding the LD.[35] Previous studies in Caucasian cancer patients used the genotype of 3435C>T of *ABCB1* gene and the -100_-102 AAG deletion variant of *5-HTR3B* gene and performed an association analysis rather than a haplotype analysis.[13,18] Therefore, we cannot compare our study findings with the previous studies in Caucasian cancer patients. Teh et al. reported that the allele frequencies in 3435C>T of *ABCB1* gene were different between Asians and Caucasians.

Among our patients, no predicted phenotypes of CYP2D6 PMs or UMs were identified and the frequency of EMs exceeded that of the IMs. Similar results were found in a previous study in healthy subjects of Malaysian Chinese origin, presenting that there were no PM and the frequency of EM in this population was also around 60%.[31] Indeed, in subjects of Asian origin the PM phenotype is very rare. The previous study of Kaiser et al. in Caucasian cancer patients showed that a different antiemetic response to ondansetron was found in both CYP2D6 UMs and PMs. The PMs and UMs showed the lowest and the highest score of nausea and vomiting in acute phase, respectively.[14] Since the incidence of predicted phenotypes of CYP2D6 PMs and UMs in subjects with Indonesian origin is very low, the role of CYP2D6 phenotype in explaining variability in ondansetron and metoclopramide efficacy in Asians seems to be limited if present at all.

While there are two reports suggesting that CYP2D6 has a significant role in metoclopramide metabolism,[20,21] we found no association between CYP2D6-predicted phenotype and metoclopramide efficacy. The EMs and IMs as the only predicted phenotypes found in our study may be the reasons for these results.

In conclusion, our study suggests that the carriers of CTG haplotype of *ABCB1* gene have increased risk of CINV during the delayed phase. However, variants in the genes encoding *ABCB1*, *CYP2D6* and *5-HT3B* receptor are not associated with antiemetic efficacy of ondansetron in Asian cancer patients during the acute phase. Further studies are needed to confirm the application of these results in clinical practice.

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REFERENCES

1. Hilarius DL, Kloeg PH, van der Wall E, van den Heuvel JJ, Gundy CM, Aaronson NK. Chemotherapy-induced nausea and vomiting in daily clinical practice: a community hospital-based study. Support Care Cancer 2011.
2. Lohr L. Chemotherapy-induced nausea and vomiting. Cancer J 2008; 14(2):85-93.
3. Schnell F. Chemotherapy-Induced Nausea and Vomiting: The Importance of Acute Antiemetic Control . The Oncologist 2003; 8(2):187-198.
4. Hesketh PJ. New treatment options for chemotherapy-induced nausea and vomiting. Support Care Cancer 2004; 12(8):550-554.
5. de Wit R, Herrstedt J, Rapoport B, Carides AD, Carides G, Elmer M et al. Addition of the oral NK1 antagonist aprepitant to standard antiemetics provides protection against nausea and vomiting during multiple cycles of cisplatin-based chemotherapy. J Clin Oncol 2003; 21(22):4105-4111.
6. Kris MG, Hesketh PJ, Somerfield MR, Feyer P, Clark-Snow R, Koeller JM et al. American Society of Clinical Oncology guideline for antiemetics in oncology: update 2006. J Clin Oncol 2006; 24(18):2932-2947.
7. Roila F, Herrstedt J, Aapro M, Gralla RJ, Einhorn LH, Ballatori E et al. Guideline update for MASCC and ESMO in the prevention of chemotherapy- and radiotherapy-induced nausea and vomiting: results of the Perugia consensus conference. Ann Oncol 2010; 21 Suppl 5:v232-v243.
8. de Jongh FE, van Veen RN, Veltman SJ, de Wit R, van der Burg ME, van den Bent MJ et al. Weekly high-dose cisplatin is a feasible treatment option: analysis on prognostic factors for toxicity in 400 patients. Br J Cancer 2003; 88(8):1199-1206.

9. Hesketh PJ, Aapro M, Street JC, Carides AD. Evaluation of risk factors predictive of nausea and vomiting with current standard-of-care antiemetic treatment: analysis of two phase III trials of aprepitant in patients receiving cisplatin-based chemotherapy. Support Care Cancer 2010; 18(9):1171-1177.

10. Ho KY, Gan TJ. Pharmacology, pharmacogenetics, and clinical efficacy of 5-hydroxytryptamine type 3 receptor antagonists for postoperative nausea and vomiting. Curr Opin Anaesthesiol 2006; 19(6):606-611.

11. Schinkel AH, Wagenaar E, Mol CA, van DL. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest 1996; 97(11):2517-2524.

12. Tamai I, Saheki A, Saitoh R, Sai Y, Yamada I, Tsuji A. Nonlinear intestinal absorption of 5-hydroxytryptamine receptor antagonist caused by absorptive and secretory transporters. J Pharmacol Exp Ther 1997; 283(1):108-115.

13. Babaoglu MO, Bayar B, Aynacioglu AS, Kerb R, Abali H, Celik I et al. Association of the ABCB1 3435C>T polymorphism with antiemetic efficacy of 5-hydroxytryptamine type 3 antagonists. Clin Pharmacol Ther 2005; 78(6):619-626.

14. Kaiser R, Sezer O, Papies A, Bauer S, Schelenz C, Tremblay PB et al. Patient-tailored antiemetic treatment with 5-hydroxytryptamine type 3 receptor antagonists according to cytochrome P-450 2D6 genotypes. J Clin Oncol 2002; 20(12):2805-2811.

15. Hsu ES. A review of granisetron, 5-hydroxytryptamine3 receptor antagonists, and other antiemetics. Am J Ther 2010; 17(5):476-486.

16. Fasching PA, Kollmannsberger B, Strissel PL, Niesler B, Engel J, Kreis H et al. Polymorphisms in the novel serotonin receptor subunit gene HTR3C show different risks for acute chemotherapy-induced vomiting after anthracycline chemotherapy. J Cancer Res Clin Oncol 2008; 134(10):1079-1086.

17. Hammer C, Fasching PA, Loehberg CR, Rauh C, Ekici AB, Jud SM et al. Polymorphism in HTR3D shows different risks for acute chemotherapy-induced vomiting after anthracycline chemotherapy. Pharmacogenomics 2010; 11(7):943-950.

18. 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(11):2147-2155.

19. Doran A, Obach RS, Smith BJ, Hosea NA, Becker S, Callegari E et al. The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: evaluation using the MDR1A/1B knockout mouse model. Drug Metab Dispos 2005; 33(1):165-174.

20. van der Padt A, van Schaik RH, Sonneveld P. Acute dystonic reaction to metoclopramide in patients carrying homozygous cytochrome P450 2D6 genetic polymorphisms. Neth J Med 2006; 64(5):160-162.

21. Desta Z, Wu GM, Morocho AM, Flockhart DA. The gastroprokinetic and antiemetic drug metoclopramide is a substrate and inhibitor of cytochrome P450 2D6. Drug Metab Dispos 2002; 30(3):336-343.

22. Double-blind, dose-finding study of four intravenous doses of dexamethasone in the prevention of cisplatin-induced acute emesis. Italian Group for Antiemetic Research. J Clin Oncol 1998; 16(9):2937-2942.

23. Hamadani M, Chaudhary L, Awan FT, Khan JK, Kojouri K, Ozer H et al. Management of platinum-based chemotherapy-induced acute nausea and vomiting: is there a superior serotonin receptor antagonist? J Oncol Pharm Pract 2007; 13(2):69-75.

24. Bloechl-Daum B, Deuson RR, Mavros P, Hansen M, Herrstedt J. Delayed nausea and vomiting continue to reduce patients' quality of life after highly and moderately emetogenic chemotherapy despite antiemetic treatment. J Clin Oncol 2006; 24(27):4472-4478.

25. Hassan BA, Yusoff ZB. Negative Impact of Chemotherapy on Breast Cancer Patients. Asian Pac J Cancer Prev 2010; 11(6):1523-1527.

26. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. Pharmacogenomics 2002; 3(2):229-243.

27. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J 2005; 5(1):6-13.

28. Teh LK, Ismail R, Yusoff R, Hussein A, Isa MN, Rahman AR. Heterogeneity of the CYP2D6 gene among Malays in Malaysia. J Clin Pharm Ther 2001; 26(3):205-211.

29. Teh LK, Lee WL, Amir J, Salleh MZ, Ismail R. Single step PCR for detection of allelic variation of MDR1 gene (P-glycoprotein) among three ethnic groups in Malaysia. J Clin Pharm Ther 2007; 32(3):313-319.

30. Anonymous. Whole genome association analysis toolset. gPlink [2009 [cited 2010 Aug. 22]; Available from: URL:http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml.

31. Ismail R, Teh LK, Amir J, Alwi Z, Lopez CG. Genetic polymorphism of CYP2D6 in Chinese subjects in Malaysia. J Clin Pharm Ther 2003; 28(4):279-284.

32. Ishiguro A, Kubota T, Ishikawa H, Iga T. Metabolic activity of dextromethorphan O-demethylation in healthy Japanese volunteers carrying duplicated CYP2D6 genes: duplicated allele of CYP2D6*10 does not increase CYP2D6 metabolic activity. Clin Chim Acta 2004; 344(1-2):201-204.

33. Jordan K, Kasper C, Schmoll HJ. Chemotherapy-induced nausea and vomiting: current and new standards in the antiemetic prophylaxis and treatment. Eur J Cancer 2005; 41(2):199-205.

34. Minami M, Endo T, Hirafuji M, Hamaue N, Liu Y, Hiroshige T et al. Pharmacological aspects of anticancer drug-induced emesis with emphasis on serotonin release and vagal nerve activity. Pharmacol Ther 2003; 99(2):149-165.

35. Zhao H, Pfeiffer R, Gail MH. Haplotype analysis in population genetics and association studies. Pharmacogenomics 2003; 4(2):171-178.