ORIGINAL ARTICLE

Prevalence of Cytidine Deaminase and Ribonucleotide Reductase Polymorphism in Thai Healthy Volunteers

Duanggamon Muengsaen, MSc[1], Thikhumporn Areesinpitak, MSc[1], Kosin Wirasorn, MD[2], Wichittra Tassaneeyakul, PhD[1], Suda Vannaprasaht, MD[1]

[1] Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[2] Oncology Unit, Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Background: Gemcitabine is a chemotherapy used in many types of solid tumor treatment. It is metabolized to inactive forms by the cytidine deaminase (CDA) enzyme. Gemcitabine inhibits ribonucleotide reductase (RRs) activity, decreasing the nucleotide substrate for DNA replication. Recent data showed that *CDA* and *Ribonucleotide reductase subunit 1 (RRM1)* genotypes are associated with gemcitabine efficacy and adverse effects.

Objective: This study investigated the prevalence of *CDA* and *RRM1* polymorphisms in the Thai population and the correlation between *CDA* polymorphisms and CDA activity.

Materials and Methods: One hundred and forty healthy Northeastern Thai volunteers were enrolled. CDA enzyme activity was evaluated by measuring residual enzyme activity in plasma. The principle of the assay was based on the conversion of cytidine to uridine, which releases ammonium (NH_3). The released NH_3 was then measured by spectrophotometry. Total proteins in plasma were assayed using the standard Bradford method. *CDA* and *RMM1* genotypes were analyzed using a real-time PCR with specific TaqMan® probes.

Results: The prevalence of *CDA*1/*1* and *CDA*1/*2* in the Thai healthy volunteers was 80.72% and 19.28%, respectively. *CDA*3* allele mutant was not found in the present study. The prevalence of *CDA+435C>T* consisted of *CDA+435C/C* 70.54%, *CDA+435C/T* 26.36% and *CDA+435T/T* 3.1%. There was no statistical difference in the CDA activity of *CDA*2* and *CDA+435C>T* allele mutants. However, males presented significantly higher CDA activity than females (p=0.027). Moreover, the result showed that the activity was significantly lower in older volunteers (over 50 years old) than in younger volunteers (p=0.047).

The prevalence of *RRM1-37C>A* genotypes were *RRM1-37C/C* 48.84%, *RRM1-37C/A* 41.86% and *RRM1-37A/A* 9.03%. Finally, the prevalence of *RRM1-524T>C* gene genotype was *RRM1-524T/T* 49.61%, *RRM1-524T/C* 37.98%, and *RRM1-524C/C* 12.4%.

The prevalence of *CDA*2* and *CDA+435T* allele frequency was statistically significantly lower than in Caucasian populations (p<0.001). The prevalence of *RRM1-524C* allele frequency was lower than in Caucasian populations (p<0.003).

Conclusion: CDA activity was not related to *CDA* genotypes. However, CDA activity was related to age and gender. Allele frequencies of *CDA* and *RRM1* allele mutants in Thais that were different from Caucasian populations may affect the efficacy and toxicity of gemcitabine in Thais.

Keywords: Gemcitabine; *CDA*; *RRM1*; CDA activity; Polymorphism

Received 7 February 2024 | Revised 23 May 2024| Accepted 30 May 2024

J Med Assoc Thai 2024;107(Suppl. 1):S55-62

Website: http://www.jmatonline.com

Gemcitabine (2', 2'-difluorodeoxycytidine, dFdC) is a potent chemotherapy drug for treating pancreatic cancer, non-small cell lung cancer (NSCLC), ovarian cancer, breast cancer, and cholangiocarcinoma patients[1]. DNA synthesis of cancer cells is inhibited by replacing cytidine base (C) in DNA replication with gemcitabine active metabolite (dFdCTP), causing chain elongation suppression and inducing chain termination[2]. Moreover, another metabolite of gemcitabine (dFdCDP) also inhibits ribonucleotide reductases (RRs) activity, RRM1, RRM2, and RRM28, bringing about a decrease in nucleotide substrate for the replication process (Figure 1)[3]. Terminating DNA synthesis leads to the induction of malignant cell apoptosis. Previous studies revealed a significant association between overexpression of RRM1 in lung cancer or pancreatic cancer cell lines and gemcitabine resistance[4]. By contrast, a meta-analysis study demonstrated a rising gemcitabine response rate associated with low RRM1 expression. In addition,

Correspondence to:

Vannaprasaht S.

Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.

Phone: +66-43-363148

Email: sudvan@kku.ac.th

How to cite this article:

Muengsaen D, Areesinpitak T, Wirasorn K, Tassaneeyakul W, Vannaprasaht S. Prevalence of Cytidine Deaminase and Ribonucleotide Reductase Genotype Polymorphism in Thai Healthy Volunteers. J Med Assoc Thai 2024;107(Suppl.1):S55-62.

DOI: 10.35755/jmedassocthai.2024.S01.S55-S62

the progress-free survival (PFS) of NSCLC patients with low expression of RRM1 treated with gemcitabine was longer than that of the high expression group[5]. In another pathway, gemcitabine is metabolized to inactive form (2', 2'-difluorodeoxyuridine; dFdU) by cytidine deaminase (CDA) enzyme. This inactive metabolite played an important role in regulating the transportation, accumulation, and cytotoxicity of gemcitabine (Figure 1)[6-8]. Therefore, CDA activity is a crucial enzyme in the pharmacokinetics of gemcitabine. A previous study reported a significant association between low CDA activity and adverse effects in patients treated with gemcitabine[9]. Moreover, patients with low CDA activity had more improved clinical outcomes and disease progression than patients with high CDA activity[10]. It was found that the response rate was significantly higher in the low-activity patients than in the high-activity group. Similarly, low-activity patients had a longer time to progress and overall survival than others[11].

Several studies reported that polymorphisms of the *CDA* gene affected CDA activity, such as *CDA*2*, *CDA*3*, and *CDA+435C>T*[13]. The mutant allele of *CDA*2* (79A>C; K27Q) (rs2072671) correlated with an increase in CDA activity. NSCLC patients carrying the *CDA*2* allele had significantly higher CDA activity than wild-type patients[14]. Moreover, wild-type patients had longer time to progression and overall survival than *CDA*2* homozygous mutant patients. In contrast to *CDA*2* mutation, CDA activity was decreased in *CDA*3* (208G>A; Ala70Thr) (rs60369023) carriers. Thus, the elimination of gemcitabine was reduced in the mutation group. Gemcitabine clearance (CL) of the patients with *CDA*3/*3* was lower than the other groups (*CDA*1/*1, *1/*3*), leading to an increase in gemcitabine concentration and drug toxicity[15-18]. Another *CDA* polymorphism reported in correlation with the activity was *CDA+435C>T* (Thr145Thr; rs1048977). There was high *CDA* gene expression in individuals who carried the *CDA +435T/T* genotype, tending to increase the activity[19].

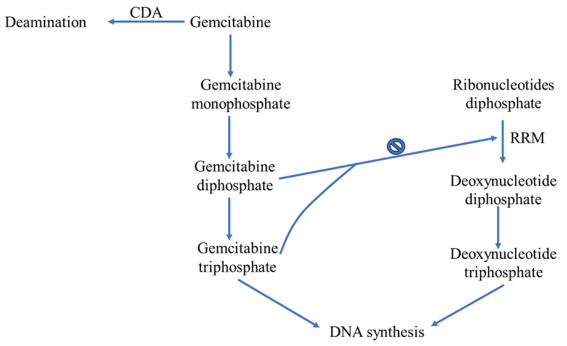

Figure 1. Metabolism and mechanism of action of gemcitabine[12].

However, a recent study showed that patients with the *CDA +435T* allele respond better to gemcitabine treatment but experience increased adverse activity[20].

In addition, several studies reported a significant correlation between *RRM1* polymorphisms, especially RRM1 at promoter; -37C>A (rs12806698) and -524T>C (rs11030918), and gemcitabine response. Previous studies revealed that RRM1 expression in patients with *RRM1-37C/C* and *RRM1-524T/T* genotypes was lower than in patients with other genotype groups. Moreover, the patients with these two genotypes had longer OS and DFS than others (p=0.032)[21]. In NSCLC patients, the individuals with *RRM1-37C/A* and *RRM1-524T/C* showed significantly higher gemcitabine response rates than other genotypes[22].

However, no prevalence study of *CDA* and *RRM1* polymorphisms in the Thai population has been conducted. Therefore, the present study aimed to investigate the prevalence of *CDA* and *RRM1* polymorphisms in the Thai population and the correlation between *CDA* polymorphisms and CDA activity.

Materials and Methods

Blood sampling

One hundred and forty healthy Northeastern Thai volunteers were enrolled in this study at the Central Blood Bank, Srinagarind Hospital. Seventy-four were men. Three microliters of blood were sampled from each volunteer into potassium ethylene diamine tetra-acetic acid (EDTA)--containing vacutainer tubes. The blood was centrifuged to obtain plasma and the buffy coat. The plasma was used to evaluate CDA activity, while the buffy coat was extracted to collect genomic DNA. This study was achieved based on the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines approved by the Ethics Committee for Human Research, Khon Kaen University (HE571402).

Bradford assay

Bradford assay is an assay to measure a quantity of protein. The assay principle is the color alteration of Coomassie Brilliant Blue dye when its acidic solution binds to the protein[23]. The concentration of BSA solution was prepared at 40, 30, 20, 10, 5, and 2.5 µg/ml as a standard protein solution. In a 96-well plate, 50 µl of 5,000-fold diluted plasma sample and the standard solution was added with 200 µl Bradford solution. The mixture was incubated at room temperature for 10 minutes. Then, the color shift was detected by spectrophotometry at a wavelength of 620 nm. The number of proteins was calculated from a color intensity.

CDA activity assay

The CDA activity assay of this study was performed following the previous study[23]. Ammonium solution was

used as a standard control that was prepared by mixing 10 µl of solution C (4 M ammonium chloride) in 10 ml of solution A (0.07 M KH_2PO_4 + 0.07 M Na_2HPO_4, pH 7.0). Its concentration was 40 CDA activity Units (U). Then, it was diluted to 20, 10, 5, 2.5, 1.25, and 0 U by solution A. One hundred microliters of the standard solution and the plasma sample were added with 400 µl solution F (2mM cytidine in solution A) and incubated at 37°C for 16 hours. Meanwhile, 100 µl plasma and solution blank were incubated with 400 µl solution A. All reaction was stopped by precipitating proteins with 200 µl solution B (sodium tungstate 0.35 M) and solution G (sulfuric acid 1 N) and centrifuged at 4,700 rpm, 25°C, 5 min before transferring 450 µl supernatant of plasma sample to a new tube. All mixtures added one point five-milliliter solution D (phenol solution) and two-milliliter solution E (hypochlorite solution). Then, they were incubated at 37°C for 30 minutes. Two hundred and seventy microliter mixtures were pipetted into a 96-well plate for measuring absorbance at 620 nm by spectrophotometry.

CDA activity calculation

The CDA activity was calculated by NH_3 concentration (U) divided by total proteins in plasma (mg)[24]. Ammonium concentration was calculated from the linear equation of the calibration curve plotted from the OD value of the ammonium standard solution. Similarly, the total protein volume in the plasma was calculated from the linear equation of the calibration curve from the Bradford technique.

DNA extraction and genotyping

The buffy coat from the blood was extracted by QIAamp® DNA Blood Mini Kits according to the manufacturer's instructions. Then, five single nucleotide polymorphisms (SNPs) were determined, such as *CDA*2*, *CDA*3*, *CDA 435C>T*, *RRM1 -37C>A*, and -524T>C.

*CDA*2* (79A>C, Lys27Gln; rs2072671), *CDA*3* (208G>A, Ala70Thr; rs6039023) and *CDA+435 C>T* (Thr145Thr; rs1048977) were detected by following TaqMan allelic discrimination assay by using the Light-Cycler 480 technology (Roche Diagnostics, Meylan, France) with specific TaqMan probe according to the manufacturer's instructions.

On the other hand, *RRM1*-37C>A (rs12806698) and *RRM1*-524T>C (rs11030918) were performed by PCR-restriction fragment length polymorphism (RFLP) technique. As in the previous study, two pairs of specific primers were designed using a published sequence (Gen Bank accession number AF107045, nucleotide 1-1362)[21]. PCR was carried out in a 25 µl reaction containing 2 µl of 10X reaction buffer, 2.5 µl of $MgCl_2$, 2.5 µl of each dNTP (2.5 mM), 1 µl of each primer (10 pmol/µl), 0.25 µl of1 U Taq DNA polymerase and 2 µl of template DNA. PCR conditions included 95°C and 5 min for DNA denaturation. The annealing step was set at 95°C for 30 sec, 70°C for 30 sec, and 72°C for 30 sec, in total 35 cycles, followed by 72°C for 7 min and 4°C for holding. The PCR product of *RRM1*-37C>A and *RRM1*-524T>C were identified by *Bbs*I and *Apo*I restriction enzyme digestion, respectively. The result of restriction was confirmed via 3% agarose gel electrophoresis.

Statistical analysis

The allele frequencies of *CDA* and *RRM1* polymorphisms were analyzed with Hardy-Weinberg equilibrium. The prevalence of five polymorphisms was compared between the Thai population and other ethnicities by Pearson Chi-square test. The association between *CDA* polymorphisms and CDA activity was determined by an independent sample t-test. All analyses used SPSS program version 17.0 (SPSS Inc., Chicago, USA).

Results

CDA polymorphisms

The prevalence of *CDA* polymorphisms in Thai healthy volunteers was summarized in Table 1. The distribution of all *CDA* SNPs was in accordance with Hardy–Weinberg equilibrium ($p>0.05$). The *CDA*2* allele frequencies were 90.36% for *CDA*1* and 9.64% for *CDA*2*. Moreover, the frequencies of *CDA+435C>T* were 81.43% for the C allele and 18.57% for *T*. However, no *CDA*3* mutant allele was found in the Thai population. The prevalence of *CDA*2* SNP in Thai was not different from that of the Chinese and Indian populations. On the other hand, there was a significant difference between Japanese, Korean, Caucasian, and African populations ($p<0.05$) (Table 2). Moreover, the *CDA+435C>T* allele frequency result demonstrated no statistically significant difference between Thai and other Asian populations. However, the allele frequency of Thais was different from that of Caucasians, which was significant (Table 3).

Table 1. Prevalence of CDA genotype in Thai healthy volunteers

Genotype	Number	Frequency (%)
*CDA*2*		
**1/*1*	113	80.72
**1/*2*	27	19.28
**2/*2*	0	0
Total number	140	100
CDA+435C>T		
C/C	93	66.43
C/T	42	30.0
T/T	5	3.57
Total number	140	100

Table 2. *CDA*2* allele frequency comparison between Thai and other ethnic populations

Ethnic groups	N	n	*CDA*2* Allele frequency (%)		p-value	References
			*1	*2		
Thai	140	280	253 (90.4)	27 (9.6)		The present study
Asian						
Chinese	102	204	179 (87.7)	25 (12.3)	0.359	(Xu et al., 2011)[25]
Japanese	206	412	328 (79.6)	84 (20.4)	<0.001	(Sugiyama et al., 2009)[15]
Korean	200	400	339 (84.8)	61 (15.2)	0.032	(Sugiyama et al., 2009)[15]
Indian	17	34	30 (88.2)	4 (11.8)	0.759	(Soo et al., 2009)[26]
Caucasians						
American	150	300	202 (67.3)	98 (32.7)	<0.001	(Sugiyama et al., 2009)[15]
European	95	190	122 (64.0)	68 (36.0)	<0.001	(Fukunaga et al., 2004)[27]
African	85	170	163 (96.0)	7 (4.0)	0.032	(Fukunaga et al., 2004)[27]

Table 3. *CDA+435C>T* allele frequency comparison between Thai and other ethnic populations

Ethnic groups	N	n	*CDA+435* Allele frequency (%)		p-value	References
			C	*T*		
Thai	140	280	228 (81.4)	52 (18.6)		The present study
Asian						
Chinese	163	326	241 (73.9)	85 (26.1)	0.028	(Hu et al., 2021)[20]
Korean	78	156	116 (74.4)	40 (25.6)	0.083	(Yoon et al., 2015)[28]
Caucasians	100	200	131 (65.5)	69 (34.5)	<0.001	(Parmar et al., 2010)[19]

Correlation with CDA activity

The median CDA activity in Thai was 1,293.6 mU/mg proteins (IQR 984.8 to 1,553.7 mU/mg). Males presented significantly higher CDA activity than females (1,393.1 mU/mg; IQR 1,050.2 to 1,657.2 mU/mg in males and 1,178.6 mU/mg; IQR 884.8 to 1,470.9 mU/mg in females) (p=0.027). Moreover, the result showed the activity was significantly lower in older volunteers (more than 50 years; 1,050.2 mU/mg; IQR 844.4 to 1,382.4 mU/mg) than younger (1,317.4 mU/mg; IQR 1,023.0 to 1,560.6 mU/mg) (p=0.047). For genetic factors, the *CDA*1/*1* group had a median CDA activity of 1,316.7 mU/mg (IQR 985.0 to 1,622.0 mU/mg) (Figure 2). The CDA activity in individuals carrying heterozygous mutant genotype (*CDA*1/*2*) was 1,146.0 mU/mg (IQR 905.7 to 1,487.5 mU/mg). The activity in wild-type carriers tended to be higher than in the mutant group. However, there was no statistical difference in the median of CDA activity between these two groups (p=0.302). Furthermore, the activity in the *CDA+435 CC* carrier (1,189.90 mU/mg; IQR 926.8 to 1,548.4 mU/mg) was lower than in the *CDA+435 CT* or *TT* group (1,346.60 mU/mg; IQR 1,141.1 to 1,618.1 mU/mg). Nonetheless, no significant correlation with *CDA+435C>T* was observed (p=0.214) (Figure 3).

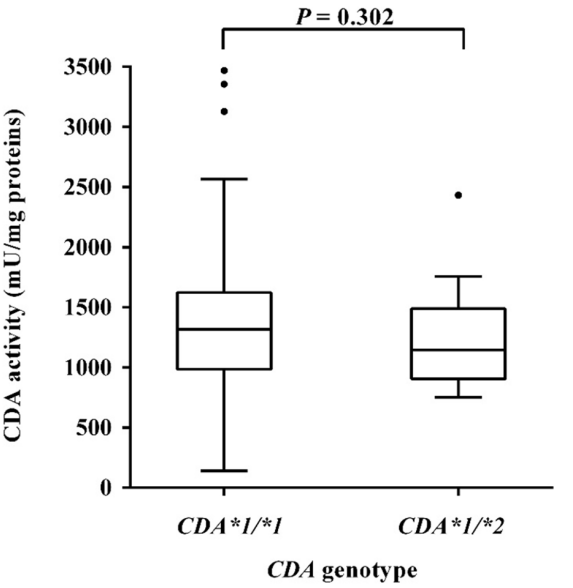

Figure 2. Correlation between *CDA*2* genotype and CDA activity.

RRM1 polymorphisms

The frequencies of *RRM1* polymorphisms assessed in Thai healthy volunteers were shown in Table 4. The distribution of *RRM1* genotype frequencies was following Hardy-Weinberg equilibrium (p>0.05). Estimated *RRM1-37C>A* allele frequencies were 73.6% for *C* and 26.4% for *A* (Table 5). There was no difference in these frequencies between Thai and other Asian or Caucasian groups such as Chinese, Korean, and American (p>0.05) (Table 5).

However, the frequencies in the Thai population differed from the African-American population (p=0.011). In addition, the allele frequencies of *RRM1-524T>C* were 73.57% for *C* and 26.43% for *A* (Table 6). These allele frequencies were not different among Thai, Chinese, and African-American populations (p>0.05), but they were significantly different from Korean (p=0.026) and American groups (p=0.003) (Table 6). Both *RRM1* genotypes showed strong linkage disequilibrium (D'=0.924, r^2=0.8236).

Discussion

Genetic polymorphisms of *CDA* and *RRM1* were factors that reported the correlation with the efficacy and toxicity of gemcitabine. The present study was the first to determine the prevalence of these SNPs in the Thai population. A previous study revealed that the prevalence of the *CDA*2* mutation allele in Asians was 12.3% for Chinese, 20.4% for Japanese, and 15.2% for Korean, respectively[15,26,30].

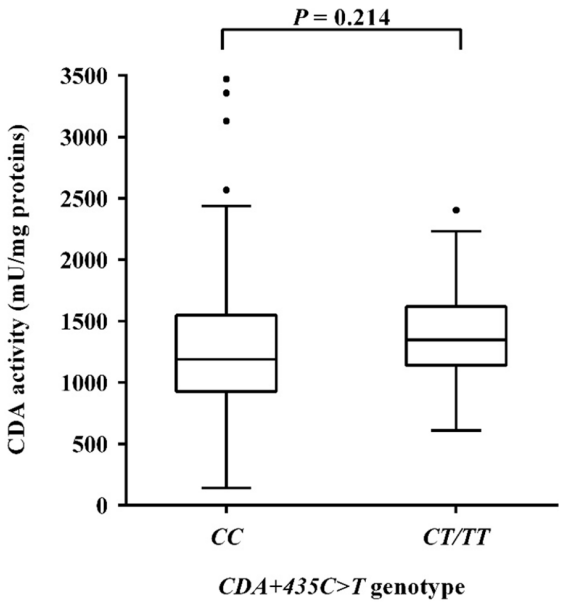

Figure 3. Correlation between *CDA+435C>T* genotype and CDA activity.

It showed similar mutant frequencies between the Asian and Thai populations except for the Japanese populations (9.6% vs. 20.4%, p<0.001). On the other hand, the *CDA*2* prevalence in the present study was significantly different from Caucasian populations such as Americans (32.7%) and Europeans (36%)[15,27]. For *CDA*3* gene mutation, it was an extremely rare SNP. It was found in only 0.98% of Chinese and 8.3% of Japanese, while no *CDA*3* mutant allele was detected in Caucasians or in Thai[30-33]. On the contrary, the *CDA+435 C>T* was the common mutant allele in several races, including Asian and Caucasian. The allele frequency of the *CDA+435T* mutant allele in the Asian population was found to be approximately 20 % (26.1% in Chinese, 22.7% in Singapore, and 25.6% in Korean)[20,26,28]. Thirty-five percent of the *CDA+435 T* allele was reported in Caucasians (23.7% in Italy and 44% in France)[9,19,34]. However, *CDA+435T* allele frequency in Thais significantly differed from Caucasians (18.6% vs. 34.5%, p<0.001) but not different from other Asian populations. The previous study showed *CDA*2* mutation associated with ineffective chemotherapy in non-small cell lung cancer patients. The odd ratio (OR) of ineffective chemotherapy increased for *CDA*1/*2* (OR 2.818; 95% CI 1.031, 7.705; p=0.043) and *CDA*2/*2* (OR 9.864; 95% CI 1.232, 78.966; p=0.031) when compared to wild-type[11,35]. Tibaldi C et al. reported that *CDA+435C/C* and *CDA+435C/T* had more prolonged

Table 4. Prevalence of *RRM1* genotype in Thai healthy volunteers

Genotype	Number	Frequency (%)
RRM1-37C>A		
CC	77	55.0
CA	52	37.14
AA	11	7.86
Total number	140	100
RRM1-524T>C		
T/T	77	55.0
C/T	50	35.71
C/C	13	9.29
Total number	140	100

Table 5. *RRM1-37C>A* allele frequency comparison between Thai and other ethnic populations

Ethnic groups	N	n	*RRM1-37* Allele frequency (%)		p-value	References
			C	A		
Thai	140	280	206 (73.6)	74 (26.4)		This study
Asian						
Chinese	213	426	319 (74.9)	107 (25.1)	0.696	(Feng et al., 2009)(29)
Korean	129	258	188 (72.9)	70 (27.1)	0.854	(Bepler et al., 2005)(21)
Caucasians						
American	855	1,710	1,260 (73.7)	450 (26.3)	0.968	(Bepler et al., 2005)(21)
African-American	45	90	78 (86.7)	12 (13.3)	0.011	(Bepler et al., 2005)(21)

Table 6. *RRM1-524T>C* allele frequency comparison between Thai and other ethnic populations

Ethnic groups	N	n	*RRM1-524* Allele frequency (%)		p-value	References
			T	C		
Thai	140	280	204 (72.9)	76 (27.1)		The present study
Asian						
Chinese	213	426	288 (67.6)	138 (32.4)	0.137	(Feng et al., 2009)[29]
Korean	129	258	165 (64.0)	93 (36.0)	0.026	(Bepler et al., 2005)[21]
Caucasians						
American	855	1,710	1,092 (63.9)	618 (36.1)	0.003	(Bepler et al., 2005)[21]
African-American	45	90	65 (72.2)	25 (27.8)	0.906	(Bepler et al., 2005)[21]

overall survival than *CDA+435T/T* (p=0.025). Therefore, the populations with a high prevalence of the *CDA*2* and *CDA+435T* mutant allele may decrease the effectiveness of gemcitabine-based chemotherapy. However, recent data showed a controversial correlation between *CDA* polymorphism and CDA activity[11].

CDA enzyme plays a crucial role in the metabolism of gemcitabine to inactive metabolite. Its activity involves the efficacy and toxicity of gemcitabine treatment[9]. There were two common methods to determine CDA activity. The first CDA activity could be evaluated by the metabolic ratio of gemcitabine with its metabolite. This method used chromatography with mass spectrometry to determine gemcitabine and metabolite, which were unavailable in our faculty. The other method, as in the present study, CDA activity expression could be determined by measuring the ammonia release during cytidine conversion into uridine by CDA after serum incubation. Ammonium concentration is assayed by spectrophotometry. CDA activity was expressed in the international unit. The present study was the first to determine the relationship between *CDA* polymorphisms and CDA activity in a healthy Thai population. Carpi FM et al. reported a significant association between *CDA*2* polymorphism and activity. *CDA* wild type had lower CDA activity than *CDA*1/*2* and *CDA*2/*2* (0.044±0.023 vs. 0.055±0.023 vs. 0.054±0.023 mU/mg; p=0.009). In contrast, the other two previous studies found no significant relationship between CDA activity and *CDA*2*[16,36]. The studies of Carpi et al. (2013) and Cohen et al. (2018) also revealed that *CDA+435 C>T* mutation did not significantly correlate with CDA activity[13, 36]. We found no significant association between the activity and *CDA*2* and *CDA*435 C>T* polymorphisms. The present study has no *CDA*2/*2* and a very low *CDA+435 T/T* genotype in our population when compared to the Caucasian population. Therefore, the present study cannot show the correlation between *CDA* genotypes and CDA activity. Moreover, recent reports showed that CDA activity correlated with age, gender, neutrophil counts, nutrition status, physical activity, and circadian rhythm[36,37]. Serum CDA activities were reported higher in breast cancer patients than in healthy volunteers (11.19 vs. 7.70 U/mg prot, p<0.0001)[37]. Moreover, CDA activity was lower in breast cancer patients treated with radiotherapy and chemotherapy than in healthy volunteers. Therefore, multiple environmental factors affected CDA activity. However, the *CDA* genotypes reported correlated with the outcome of gemcitabine treatment. Tibaldi C et al. demonstrated the correlation between *CDA*2* SNP and survival time of NSCLC patients treated with gemcitabine. Time to progression (TtP) of patients who carried *CDA*1/*1* and *CDA*1/*2* genotypes have longer TtP than patients carrying the *CDA*2/*2* genotype[11]. A previous study reported statistically significant more severe hematological toxicity occurred in patients who were treated with gemcitabine with either the *CDA*1/*1* and *CDA*1/*2* genotype when compared with *CDA*2/*2*. Therefore, *CDA* genotypes may be used to predict gemcitabine treatment outcomes[38].

Gemcitabine diphosphate and gemcitabine triphosphate bind to the RRM1 active site, inhibiting RRM1 activity. Therefore, low RRM1 activity affected deoxyribonucleotide diphosphate for DNA synthesis poorly. A previous study reported that overexpression of RRM1 in lung cancer or pancreatic cancer cell lines was associated with gemcitabine resistance[4]. Recently, studies reported that *RRM1* polymorphisms were correlated with gemcitabine response. The polymorphisms of *RRM1* at promoter -37C>A (rs12806698) and -524T>C (rs11030918) were reported as related to gemcitabine outcomes. The prevalence of the *RRM1-37* C>A mutant allele was 25.1% in Chinese, 21.3% in Korean, and 26.3% in American[21,29]. It was not significantly different from the Thai population in this study (26.4%). The frequency of *RRM1-524C* mutant allele was 32.4%, 36.1%, and 36.1% in Chinese, Korean, and American, respectively[21,29]. In contrast, only 27.1% of *RRM1-524C* in the Thai population was reported. Therefore, *RRM1-524C* allele frequency in Thai differed significantly from that in Korean and American. The significant difference in the prevalence of *CDA* and *RRM1* mutant alleles may affect the clinical outcome and toxicity of gemcitabine treatment in

different populations. Further investigation of the correlation between *CDA* and *RRM1* polymorphism in Thai patients who were treated with gemcitabine and clinical outcome and toxicity would be valuable.

Conclusion

The prevalence of *CDA*2* and *CDA+435C>T* mutant alleles in healthy Thai volunteers differed from that in the Caucasian population. Age and gender are non-genetic factors that affect CDA activity. The prevalence of *RRM1-37C/C* and *RRM1-524T/T* genotypes in Thai healthy volunteers differed from that in Korean and Caucasian populations.

What is already known on this topic?

The prevalence of *CDA* and *RRM1* genotypes was different between ethnic groups.

What this study adds?

This study is the first to show the prevalence of *CDA* and *RRM1* genotypes in the Thai population. Thais' allele frequencies were statistically significantly different from those of Caucasians. CDA activity was not related to the *CDA* genotype in the Thai population. Moreover, CDA activity correlated with age and gender.

Acknowledgements

The present study was supported by Khon Kaen University (600009). The authors thank the Department of Internal Medicine, Faculty of Medicine, Khon Kaen University, for supporting the publication.

Potential conflict of interest

The authors declare no conflict of interest.

References

1. Heinemann V, Xu YZ, Chubb S, Sen A, Hertel LW, Grindey GB, et al. Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2',2'-difluorodeoxycytidine. Mol Pharmacol 1990;38:567-72.
2. Gandhi V, Legha J, Chen F, Hertel LW, Plunkett W. Excision of 2',2'-difluorodeoxycytidine (gemcitabine) monophosphate residues from DNA. Cancer Res 1996;56:4453-9.
3. Heinemann V, Xu YZ, Chubb S, Sen A, Hertel LW, Grindey GB, et al. Cellular elimination of 2',2'-difluorodeoxycytidine 5'-triphosphate: a mechanism of self-potentiation. Cancer Res 1992;52:533-9.
4. Jordheim LP, Seve P, Tredan O, Dumontet C. The ribonucleotide reductase large subunit (RRM1) as a predictive factor in patients with cancer. Lancet Oncol 2011;12:693-702.
5. Gong W, Zhang X, Wu J, Chen L, Li L, Sun J, et al. RRM1 expression and clinical outcome of gemcitabine-containing chemotherapy for advanced non-small-cell lung cancer: a meta-analysis. Lung Cancer 2012;75:374-80.
6. Hodge LS, Taub ME, Tracy TS. The deaminated metabolite of gemcitabine, 2',2'-difluorodeoxyuridine, modulates the rate of gemcitabine transport and intracellular phosphorylation via deoxycytidine kinase. Drug Metab Dispos 2011;39:2013-6.
7. Rudin D, Li L, Niu N, Kalari KR, Gilbert JA, Ames MM, et al. Gemcitabine Cytotoxicity: Interaction of Efflux and Deamination. J Drug Metab Toxicol 2011;2:1-10.
8. Veltkamp SA, Pluim D, Van Eijndhoven MA, Bolijn MJ, Ong FH, Govindarajan R, et al. New insights into the pharmacology and cytotoxicity of gemcitabine and 2',2'-difluorodeoxyuridine. Mol Cancer Ther 2008;7:2415-25.
9. Ciccolini J, Dahan L, Andre N, Evrard A, Duluc M, Blesius A, et al. Cytidine deaminase residual activity in serum is a predictive marker of early severe toxicities in adults after gemcitabine-based chemotherapies. J Clin Oncol 2010;28:160-5.
10. Abbaspour A, Dehghani M, Setayesh M, Tavakkoli M, Rostamipour HA, Ghorbani M, et al. Cytidine deaminase enzyme activity is a predictive biomarker in gemcitabine-treated cancer patients. Cancer Chemotherapy and Pharmacology 2023;92:475-83.
11. Tibaldi C, Giovannetti E, Tiseo M, Leon LG, D'Incecco A, Loosekoot N, et al. Correlation of cytidine deaminase polymorphisms and activity with clinical outcome in gemcitabine-/platinum-treated advanced non-small-cell lung cancer patients. Ann Oncol 2012;23:670-7.
12. Alvarellos ML, Lamba J, Sangkuhl K, Thorn CF, Wang L, Klein DJ, et al. PharmGKB summary: gemcitabine pathway. Pharmacogenet Genomics 2014;24:564-74.
13. Carpi FM, Vincenzetti S, Ubaldi J, Pucciarelli S, Polzonetti V, Micozzi D, et al. CDA gene polymorphisms and enzyme activity: genotype–phenotype relationship in an Italian–Caucasian population. Pharmacogenomics 2013;14:769-81.
14. Giovannetti E, Laan AC, Vasile E, Tibaldi C, Nannizzi S, Ricciardi S, et al. Correlation between cytidine deaminase genotype and gemcitabine deamination in blood samples. Nucleosides Nucleotides Nucleic Acids 2008;27:720-5.
15. Sugiyama E, Kaniwa N, Kim SR, Hasegawa R, Saito Y, Ueno H, et al. Population Pharmacokinetics of Gemcitabine and Its Metabolite in Japanese Cancer Patients. Clin Pharmacokinet 2010;49:549-58.
16. Sugiyama E, Kaniwa N, Kim SR, Kikura-Hanajiri R, Hasegawa R, Maekawa K, et al. Pharmacokinetics of gemcitabine in Japanese cancer patients: the impact of a cytidine deaminase polymorphism. J Clin Oncol 2007;25:32-42.
17. Ueno H, Kaniwa N, Okusaka T, Ikeda M, Morizane C, Kondo S, et al. Homozygous CDA*3 is a major cause

of life-threatening toxicities in gemcitabine-treated Japanese cancer patients. Br J Cancer 2009;100:870-3.
18. Yonemori K, Ueno H, Okusaka T, Yamamoto N, Ikeda M, Saijo N, et al. Severe drug toxicity associated with a single-nucleotide polymorphism of the cytidine deaminase gene in a Japanese cancer patient treated with gemcitabine plus cisplatin. Clin Cancer Res 2005;11:2620-4.
19. Parmar S, Seeringer A, Denich D, Gärtner F, Pitterle K, Syrovets T, et al. Variability in transport and biotransformation of cytarabine is associated with its toxicity in peripheral blood mononuclear cells. Pharmacogenomics 2011;12:503-14.
20. Hu L, Mao X, Gao C, Xu Y, Li C, Wang T, et al. Cytidine deaminase 435C>T polymorphism relates to gemcitabine-platinum efficacy and hematological toxicity in Chinese non-small-cell lung cancer patients. Neoplasma 2021;68:638-44.
21. Bepler G, Zheng Z, Gautam A, Sharma S, Cantor A, Sharma A, et al. Ribonucleotide reductase M1 gene promoter activity, polymorphisms, population frequencies, and clinical relevance. Lung Cancer 2005;47:183-92.
22. Kim SO, Jeong JY, Kim MR, Cho HJ, Ju JY, Kwon YS, et al. Efficacy of gemcitabine in patients with non-small cell lung cancer according to promoter polymorphisms of the ribonucleotide reductase M1 gene. Clin Cancer Res 2008;14:3083-8.
23. Peters GJ, Honeywell RJ, Maulandi M, Giovannetti E, Losekoot N, Etienne-Grimaldi MC, et al. Selection of the best blood compartment to measure cytidine deaminase activity to stratify for optimal gemcitabine or cytarabine treatment. Nucleosides Nucleotides Nucleic Acids 2014;33:403-12.
24. Mercier C, Raynal C, Dahan L, Ortiz A, Evrard A, Dupuis C, et al. Toxic death case in a patient undergoing gemcitabine-based chemotherapy in relation with cytidine deaminase downregulation. Pharmacogenet Genomics 2007;17:841-4.
25. Xu B, Jiang Z, Kim SB, Yu S, Feng J, Malzyner A, et al. Biweekly gemcitabine-paclitaxel, gemcitabine-carboplatin, or gemcitabine-cisplatin as first-line treatment in metastatic breast cancer after anthracycline failure: a phase II randomized selection trial. Breast Cancer 2011;18:203-12.
26. Soo RA, Wang LZ, Ng SS, Chong PY, Yong WP, Lee SC, et al. Distribution of gemcitabine pathway genotypes in ethnic Asians and their association with outcome in non-small cell lung cancer patients. Lung Cancer 2009;63:121-7.
27. Fukunaga AK, Marsh S, Murry DJ, Hurley TD, McLeod HL. Identification and analysis of single-nucleotide polymorphisms in the gemcitabine pharmacologic pathway. Pharmacogenomics J 2004;4:307-14.
28. Yoon KA, Woo SM, Hong EK, Jung MK, Park WS, Bae K, et al. Cytidine Deaminase as a Molecular Predictor of Gemcitabine Response in Patients with Biliary Tract Cancer. Oncology 2015;89:345-50.
29. Feng JF, Wu JZ, Hu SN, Gao CM, Shi MQ, Lu ZH, et al. Polymorphisms of the ribonucleotide reductase M1 gene and sensitivity to platin-based chemotherapy in non-small cell lung cancer. Lung Cancer 2009;66:344-9.
30. Xu J, Zhou Y, Zhang J, Chen Y, Zhuang R, Liu T, et al. High incidence of severe neutropenia after gemcitabine-based chemotherapy in Chinese cancer patients with CDA 79A>C mutation. Clin Chim Acta 2012;413:1284-7.
31. Erculj N, Kovac V, Hmeljak J, Franko A, Dodic-Fikfak M, Dolzan V. The influence of gemcitabine pathway polymorphisms on treatment outcome in patients with malignant mesothelioma. Pharmacogenet Genomics 2012;22:58-68.
32. Joerger M, Burgers JA, Baas P, Doodeman VD, Smits PH, Jansen RS, et al. Gene polymorphisms, pharmacokinetics, and hematological toxicity in advanced non-small-cell lung cancer patients receiving cisplatin/gemcitabine. Cancer Chemother Pharmacol 2012;69:25-33.
33. Kasuya K, Tsuchida A, Nagakawa Y, Suzuki Y, Suzuki M, Aoki T, et al. Prediction of a side effect and efficacy of adjuvant chemotherapy with gemcitabine for post operative patient of pancreatic cancer by a genetic polymorphism analysis. Hepatogastroenterology 2012;59:1609-13.
34. Ludovini V, Floriani I, Pistola L, Minotti V, Meacci M, Chiari R, et al. Association of Cytidine Deaminase and Xeroderma Pigmentosum Group D Polymorphisms with Response, Toxicity, and Survival in Cisplatin/Gemcitabine-Treated Advanced Non-small Cell Lung Cancer Patients. Journal of Thoracic Oncology 2011;6:2018-26.
35. Li J, Xu D, Huang J, Wang YN, Ma XP, Lin ZY, et al. Associations of cytosine deaminase gene polymorphisms with effectiveness of gemcitabine/cisplatin chemotherapy in patients of Xinjiang Uyghur and Han nationality with non-small cell lung cancer. Int J Biol Markers 2019;34:389-97.
36. Cohen R, Preta LH, Joste V, Curis E, Huillard O, Jouinot A, et al. Determinants of the interindividual variability in serum cytidine deaminase activity of patients with solid tumours. Br J Clin Pharmacol 2019;85:1227-38.
37. Buhagiar-Labarchede G, Onclercq-Delic R, Vacher S, Berger F, Bieche I, Stoppa-Lyonnet D, et al. Cytidine deaminase activity increases in the blood of breast cancer patients. Sci Rep 2022;12:14062.
38. Farrell JJ, Bae K, Wong J, Guha C, Dicker AP, Elsaleh H. Cytidine deaminase single-nucleotide polymorphism is predictive of toxicity from gemcitabine in patients with pancreatic cancer: RTOG 9704. Pharmacogenomics J 2012;12:395-403.