CYP2D6 Genotyping for Personalized Therapy of Tamoxifen in Indonesian Women with ER+ Breast Cancer

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Research Article

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Abstract

Purpose

Tamoxifen, common adjuvant therapy prescribed in estrogen receptor positive (ER+) breast cancer, is metabolized by CYP2D6 enzyme into endoxifen. The phenotypes of *CYP2D6*, a highly polymorphic gene, vary from ultrarapid (UM), normal (NM), intermediate (IM), and poor metabolizers (PM). Studies showed that reduced *CYP2D6* activity in IMs and PMs resulted in lower endoxifen level, thereby reducing therapy efficacy. This study aims to observe the distribution of *CYP2D6* profiles and their corresponding endoxifen levels in Indonesian ER+ breast cancer patients.

Methods

151 patients who have received tamoxifen therapy for ≥8 weeks were recruited prospectively. DNA and blood samples were collected with buccal swab and finger-prick methods, respectively. Genotyping was performed using the qPCR method while metabolites measurement was performed using HPLC-tandem MS. Patients with IM/PM *CYP2D6* profile were advised to increase their tamoxifen dose or switch to aromatase inhibitor, while patients with UM or NM *CYP2D6* profile remained on 20 mg daily dose. Tamoxifen metabolites levels of those given 40 mg/day of tamoxifen were measured eight weeks post dose adjustment.

Results

We found that 40.7% of patients recruited were IM. *CYP2D6*10* was the most abundant allele (28.8%) and **10/*36* was the most frequently observed diplotype (23.6%). Endoxifen levels between the NM-PM, NM-IM, and IM-PM were statistically significant, and dose increase of tamoxifen successfully increased endoxifen levels in IMs to a similar level with NMs at baseline.

Conclusion

Indonesian women have a relatively high proportion of IMs. The correlation between *CYP2D6* genotype and phenotype was shown in the significant difference in endoxifen levels among NMs, IMs, and PMs. Dose adjustment of tamoxifen to 40 mg daily positively increased endoxifen levels in IMs to a similar level as NMs. Implementing pharmacogenomics testing of *CYP2D6* on ER+ breast cancer women taking tamoxifen can potentially increase the likelihood of achieving better treatment efficacy.

Trial Registration

The trial was retrospectively registered at ClinicalTrials.gov on 18 March 2020 with identifier NCT04312347 (accessible at: https://clinicaltrials.gov/ct2/show/NCT04312347).

Introduction

Estrogen receptor (ER) expression is the primary indicator of potential responses to hormonal therapy, and approximately 70% of human breast cancers are hormone-dependent and ER+ (1). About 39.6% of breast cancer cases found in Indonesia are hormone-receptor positive (2). Tamoxifen is the current standard of care for ER+ breast cancer adjuvant therapy (3). Approximately 170,000 tamoxifen prescriptions were filed in 2015 (4), it is the most widely used antihormonal therapy in Indonesia to treat ER+ breast cancer, making up 59.7% of the total hormonal breast cancer therapy (5).

Tamoxifen is a prodrug that needs to be metabolized to its active form, endoxifen. *CYP2D6* gene that encodes CYP2D6 enzyme has more than 100 variants: some causing reduced activity, and others causing complete loss of function. These genetic polymorphisms can affect the function of CYP2D6 enzyme (6). Endoxifen is formed predominantly by CYP2D6 from N-desmethyltamoxifen, the most abundant metabolite (7). Serum endoxifen levels below 5.97 ng/mL has been linked to a 30% higher chance of having recurrence of breast cancer (8, 9). Additionally, individual variability of *CYP2D6* contributed 53% towards the ratio of N-desmethyltamoxifen and endoxifen, while combined other CYPs genetic factors (*CYP2C9, CYP2C19, CYP3A5*) and non-genetic factors (age, BMI) contributed to only 2.8% (10). In summary, genetic factors play an essential role in determining the clinical outcomes of tamoxifen therapy.

The spectrum of the *CYP2D6* enzymatic activity translates to different metabolizer profiles that are grouped into normal, ultrarapid, extensive, intermediate, and poor metabolizers (NM, UM, EM, IM, and PM, respectively), depending on how many reducing and/or loss of function alleles an individual carries. Asians and Africans were known to have up to 50% reduced activity alleles (11). IMs in Malays, Chinese, and Indians occur in 35%, 45.4%, and 15%, respectively (12–14). Meanwhile, Caucasians were commonly NMs (15). Such phenotypic proportions have not been observed yet in Indonesian population.

The Clinical Pharmacogenetics Implementation Consortium (CPIC) advices those with *CYP2D6* IM and PM phenotype profiles can increase tamoxifen dose to 40 mg daily, while patients who are clinically ineligible for tamoxifen dose increase according to clinical guidelines can be switched to aromatase inhibitor (16). Our study demonstrated the effectiveness of adjusting tamoxifen dosage as the first line of action for patients who are clinically eligible while still monitoring the possible side effects for those taking an increased one, serving as the first step in implementing pharmacogenomics in breast cancer adjuvant therapy by optimizing tamoxifen use to achieve better clinical outcomes and maximize cost-effectiveness. We aimed to observe the distribution of *CYP2D6* allele, haplotype, diplotype, phenotype frequencies, and their correlation with endoxifen levels in ER+ breast cancer patients in Indonesia.

Materials And Methods

Study Design and Ethics Approval

This is a single-arm, open-labelled, prospective intervention study. Patients were recruited from SJH Initiative, MRCCC Siloam Hospital Jakarta, Indonesia, from October 2019 to April 2021. Ethical approval

was granted by MRCCC Siloam Hospitals Semanggi Ethics Review Committee (Jakarta, Indonesia) under Reference Number 001/EA/KEPKK/RSMRCCC/V/2019. Patients' informed consent was obtained before they were enrolled in the study.

Study Participants

The inclusion criteria of this study were as follows: (1) was diagnosed with ER+ breast cancer and (2) had consumed tamoxifen for at least eight weeks. Patients who fulfilled the inclusion criteria were offered to participate in the study. The flow of recruitment steps is shown in Fig. 1. Ethnicities reported in this study were self-reported. Participants who identified with two or more ethnicities were categorized as mixed races.

DNA Extraction

Buccal swab sample was obtained for *CYP2D6* genotyping using ORAcollect•DNA OCR-100 (DNA Genotek) swab. Genomic DNA (gDNA) was extracted from buccal swab samples using Monarch Genomic DNA Purification Kit (NEB #T3010) following the manufacturer's instructions. Concentration of gDNA extracts was quantified using a BioDrop spectrophotometer. Acceptance criteria to further process the DNA extract for genotyping, include total DNA yield ≥ 500 ng, A260/280 ratio ≥ 1.75, and A260/230 ratio ≥ 1.75.

CYP2D6 **Genotyping**

CYP2D6 genotyping was performed using Nala PGx Core™, a Lab-Developed Test genotyping panel consisting of four pharmacogenes: *CYP2D6, CYP2C19, CYP2C9* and *SLCO1B1* (17). *CYP2D6* variants that were genotyped in this test included rs35742686, rs59421388, rs3892097, rs5030656, rs72549352, rs5030655, rs28371725, rs16947, rs1065852, rs267608319, rs769258, rs5030865, rs1135840, total copy number of intron 2 and a detection for the presence of exon 9 conversion. Genomic DNA extracts were diluted to 2ng/uL and added as template for Nala PGx Core™ qPCR runs on Bio-Rad CFX96 Touch™ Real-Time PCR Detection System. *CYP2D6* haplotypes, diplotypes, and phenotypes were inferred by Nala Clinical Decision Support™, a class A medical device (Health Sciences Authority, Singapore) compatible with Nala PGx Core™ qPCR output.

Measurement of Tamoxifen Metabolites

Peripheral whole blood was used as sample with Volumetric Absorptive Microsampling (VAMS) for collection technique. VAMS extraction was performed in methanol using a sonication-assisted extraction method for 25 minutes after 2 hours of VAMS drying. Separation was carried out using Acquity UPLC BEH C_{18} column (2.1 x 100 mm; 1.7 µm), with a flow rate of 0.2 mL/minute and the mobile phase gradient of formic acid 0.1% combined with formic acid 0.1% in acetonitrile for 5 minutes. The UPLC-MS/MS Waters Xevo TQD Triple Quadrupole with MassLynx Software controller (Waters, Milford, USA) was employed in metabolites measurement. Mass detection was carried out utilizing Triple Quadrupole with Multiple Reaction Monitoring (MRM) analysis modes and an electrospray ionization source using positive mode.

The method was developed in the Bioavailability and Bioequivalence Laboratory of Universitas Indonesia and validated according to FDA and EMA guidelines (18). The MRM values were set at m/z 372.28>72.22 for TAM; 374.29>58.22 for END; 388.29>72.19 for 4-HT; 358.22>58.09 for NDT; and 260.20>116.20 for propranolol as the internal standard.

Patient Follow Up

Patients with IM or PM *CYP2D6* profile who were clinically ineligible for tamoxifen dose increase were switched to aromatase inhibitor and were not monitored further for side effects and metabolite levels changes. Management of these patients was based on treating oncologists' clinical judgement, in accordance to the available clinical guidelines (3, 19, 20). In IM or PM, patients without contraindications to tamoxifen were recommended to adjust their dose to 40 mg/day, in comparison, UMs and NMs remained on 20 mg/day recommended dose. Patients who received 40 mg/day of tamoxifen had their tamoxifen metabolites levels measured eight weeks post adjustment and were monitored for possible side effects using the validated FACT-ES questionnaire (21).

Data Analysis

Data and statistical analysis were performed using Microsoft® Excel® for Microsoft 365 and R version 4.0.3. Deviation from Hardy-Weinberg equilibrium was performed on the haplotype frequencies using the chi-square statistical test, where Bonferroni correction was applied to determine the *p*-value threshold for significant deviation. Analysis of Variance (ANOVA) test was used to see if metabolite levels distribution at baseline were statistically different across all metabolites, followed by a paired T-test between each pair of metabolites when significance was found. Distribution of metabolite levels before and after dose adjustment was compared using a T-test, the same test was used to compare the distribution of metabolite levels in IMs post-dose adjustment against NMs (baseline). Concerning symptoms related to endocrine therapy post-dose adjustment on IMs were compared against NMs. Chi-square test was performed per symptom to check for the difference between the two groups.

Results

Demographics of Study Participants

Table 1 shows that the majority of participants were 50 years old and below (78.2%), followed by those aged 51-59 years old (17.9%) and a small proportion with age ≥60 years (4.0%). Most participants consisted of Chinese (33.8%) and Javanese (25.2%) descents. Participants with multiethnic and multiracial descents were also observed (16.6%), followed by small numbers of other Indonesian ethnicities. Among these participants, 47.3% underwent lumpectomy, while 44.0% underwent mastectomy. Aside from surgical intervention, 66.7% of these participants underwent post-operative adjuvant radiotherapy and 50% underwent adjuvant chemotherapy. Most patients were in the early stage of cancer at the time of recruitment: stage I (27.2%), stage IIa (23.8%), and stage IIb (13.9%). Participants in later stages of breast cancer were also observed in smaller proportions. About half of the study

participants (50.3%) were enrolled within 12 months after initial diagnosis of breast cancer. The other participants were enrolled within 13-24 (15.2%), 25-36 (13.3%), and 37-48 (9.3%) months after initial diagnosis while 10.6% were diagnosed more than four years ago. According to the available biopsy data, 44.4% of the participants had moderately differentiated tumors, while 27.8% and 11.9% had poorly and moderately differentiated tumors, respectively.

Table 1
Study respondents demographics

Age	n	%
<40	23	15.33%
40-49	88	58.67%
50-59	33	22.00%
>59	6	4.00%
Menopausal status**		
Premenopausal	54	36.00%
Post-menopausal	96	64.00%
Menarche		
7-11 years old	24	16.00%
12-13 years old	83	55.33%
>13 years old	37	24.67%
NA*	6	4.00%
Race		
Ambon	2	1.32%
Batak	8	5.30%
Betawi	5	3.31%
Chinese	51	33.77%
Javanese	38	25.17%
Minangkabau	5	3.31%
Palembang	2	1.32%
Sunda	9	5.96%
Mixed races	25	16.56%
NA*	6	3.97%
Past Breast Cancer Treatment		
Lumpectomy	7	4.67%

*NA: data not available; **this study includes both pre- and post-menopausal women who were taking tamoxifen by the time of study recruitment

Age	n	%
Lumpectomy, chemoterapy	2	1.33%
Lumpectomy, radiotherapy	34	22.67%
Lumpectomy, chemotherapy, radiotherapy	23	15.33%
Mastectomy	18	12.00%
Mastectomy, chemoterapy	16	10.67%
Mastectomy, radiotherapy	5	3.33%
Mastectomy, radiotherapy, chemoterapy	25	16.67%
Mastectomy, lumpectomy, radiotherapy, chemotherapy	2	1.33%
Radiotherapy	9	6.00%
Chemotherapy	2	1.33%
Radiotherapy, chemotherapy	5	3.33%
NA*	2	1.33%
Stage		
ST 0	0	0%
ST I	34	22.67%
ST IIA	48	32.00%
ST IIB	17	11.33%
ST IIIA	9	6.00%
ST IIIB	11	7.33%
ST IIIC	2	1.33%
ST IV	12	8.00%
NA*	17	11.33%
Time Recruited from Diagnosis (Months)		
1-12	76	50.33%
13-24	23	15.23%
25-36	20	13.25%

*NA: data not available; **this study includes both pre- and post-menopausal women who were taking tamoxifen by the time of study recruitment

Age	n	%
37-48	14	9.27%
>48	16	10.60%
NA*	1	0.66%
Tumor Grade		
Well differentiated / Grade 1	18	11.92%
Moderately differentiated / Grade 2	67	44.37%
Poorly differentiated / Grade 3	42	27.81%
NA*	23	15.33%
*NA: data not available; **this study includes both pre- and post-menopausal women who were taking tamoxifen by the time of study recruitment		

CYP2D6 **Haplotype Distribution**

All haplotypes observed were in Hardy-Weinberg equilibrium (*p*-value > 0.005). *CYP2D6*10* was found to be the most abundant haplotype in the population (28.8%, n=83/288), followed by *CYP2D6*36* (25.3%, n=73/288). Compared to PharmGKB database of the East Asian population, **10* was lower, but **36* was much higher in this study compared to the frequency reported by the database, 0.012 (Fig. 2). The reference haplotype *CYP2D6*1* was observed with frequency of 23.3% (n=67/288), and other haplotypes were also observed with frequencies as follows: **2* (12.8%, n=37/288), **41* (4.5%, n=13/288), **5* (2.1%, n=6/288), **3* (1.4%, n=4/288), **39* (0.7%, n=2/288), **4A* (0.7%, n=2/288), and **14* (0.3%, n=1/288).

CYP2D6 **Diplotype Distribution**

Our study demonstrated **10/*36* (23.6%, n=34/144) as the most abundant diplotype, followed by **1/*36* (13.2%, n=19/144) (Table 2). Other diplotypes that were observed in this study with frequencies between 0.1-0.05 were as follows: **2/*10* (9.7%, n=14/144), **1/*1* (9%, n=13/144), **2/*36* (8.3%, n=12/144), **1/*10* (7.6%, n=11/144), and **10/*10* (6.5%, n=9/144). Other diplotypes observed had frequencies lower than 5% (Table 2).

Table 2
CYP2D6 diplotype frequencies observed

Diplotype	Phenotype	Counts (N total = 144)	Frequency
*10/*36	Intermediate Metabolizer	34	23.6%
*1/*36	Normal Metabolizer	19	13.2%
*2/*10	Normal Metabolizer	14	9.7%
*1/*1	Normal Metabolizer	13	9.0%
*2/*36	Normal Metabolizer	12	8.3%
*1/*10	Normal Metabolizer	11	7.6%
*10/*10	Normal Metabolizer	9	6.5%
Others^		41	22.2%
^Other diplotypes were observed with frequency less than 0.05, these diplotypes were *1/*2, *36/*41, *1/*41, *10/*41, *1/*5, *2/*2, *3/*36, *5/*10, *5/*41, *1/*3, *1/*4A, *14/*36, *2/*3, *2/*39, *2/*41, *36/*39, and *4A/*10			

CYP2D6 **Phenotypes Distribution**

Our findings (Fig. 4) show that among the 150 patients genotyped, 40.7% (n=61/150) were IMs. It is significantly higher than the current known global prevalence of IMs which is between 0.4-11% (22). The frequency of NMs observed in this study was 54.0% (n=81/150). PMs were also observed in the population at 1.3% (n=2/150) (Fig. 3). UMs were not observed among the participants in this study. Distribution of the *CYP2D6* phenotypes among major ethnicities in this study's participants showed a higher proportion of IMs in Chinese (56.86%, n=29/51) compared to other ethnicities such as Javanese (23.7%, n=9/38). PM was observed in the Javanese group with 2.6% frequency (n=1). Ethnicities with participant count less than ten were grouped as others due to inefficient number of samples to conclude allele frequencies (Supplementary Table 2). Mixed races group showed 37.5% proportion of IM (n=6/16).

Tamoxifen Metabolite Concentration

Endoxifen levels among the three metabolizers were significantly different (*p*-value = 0.00307, Table 3). The rest of the metabolites did not show any statistically significant distribution among phenotypes (*p*-value = 0.96, 0.46, 0.44 for tamoxifen, 4-hydroxytamoxifen, and N-desmethyltamoxifen, respectively). T-test performed on endoxifen levels for each phenotype pair displayed significant difference among all phenotype pairs (*p*-value = $6.3x10^{-5}$, $9.1x10^{-5}$, and $4.7x10^{-3}$ for NM-PM, NM-IM, and IM-PM, respectively), demonstrating distinction of endoxifen levels across different phenotypes (Fig. 5). After grouping the endoxifen levels into five quintiles, it was observed that the highest number of IMs fall into the lowest quintile. In contrast, the highest number of NMs fall into the highest quintile (Supplementary Table 1).

Table 3
Summary of metabolite levels in relation to *CYP2D6* metabolizer profiles

***CYP2D6* Phenotype**		**Peripheral Whole Blood Concentration (ng/mL)**			
		Tamoxifen	**Endoxifen**	**4OH-tam**	**ND-tam**
Normal Metabolizer (N = 81)	SD	35.21	6.62	1.46	56.83
	Median	77.46	11.98	3.07	240.59
	Range	31.22 - 170.82	3.55 - 34.77	1.5 - 7.66	80.63 - 321.88
Intermediate Metabolizer (N = 61)	SD	37.20	4.35	1.67	58.01
	Median	81.72	8.33	3.27	241.55
	Range	14.22 - 210.39	3.17 - 22.97	1.5 - 9.31	77.61 - 337.29
Poor Metabolizer (N = 2)	SD	33.93	0.83	0.26	90.44
	Median	91.49	4.52	3.24	276.45
	Range	67.49 - 115.48	3.94 - 5.11	3.06 - 3.43	212.5 - 340.41
p-value (ANOVA)		0.964	0.00307*	0.461	0.443

*Statistically significant *p*-value was observed among phenotype groups for endoxifen level difference

Follow Up Action After PGx Testing

Among 66 IM/PM participants who were recommended to modify their medication based on their *CYP2D6* phenotype (Fig. 6), 18 patients (27.3%, n=18/66) were switched to aromatase inhibitors based on clinical guidelines or certain medical procedure such as post ovarian function suppression endocrine therapy. Thirty-eight patients (57.6%, n=38/66) were recommended by their physicians to adjust their tamoxifen dosage from 20 mg daily to 40 mg daily, while the remaining participants who did not follow the genotype-guided recommendation either passed away or experienced recurrence, thus ceasing adjuvant therapy temporarily (15.2%, n=10/66).

Metabolite Levels Post Dose Adjustment

Twenty-six patients who took 40 mg of tamoxifen daily for two months registered increase metabolite levels. After dose adjustment, the metabolites levels increased across all tamoxifen metabolites (Fig. 7). Metabolite levels before and after dose adjustment had p-value < 0.05 across all metabolites. The metabolite levels in IMs (n=26) post dose adjustment were compared against NMs (n=81) as the baseline, showing significant difference between the two groups (p-value < 0.05) for all metabolites except endoxifen (p-value = 0.4135). The distribution of endoxifen levels in IMs post dose adjustment (7.68-23.36 ng/mL) were similar to the endoxifen levels in NMs (3.55 - 34.77 ng/mL) at baseline (Fig. 8).

Side Effects Post Dose Adjustment

The most commonly reported side effects related to endocrine therapy in IMs were weight gain and mood swings (65.8%, n=17/26). Other common symptoms related to hormonal changes were also observed in participants who received 40 mg of tamoxifen daily such as hot flashes (50%, n=13/26), cold sweats (19.2%, n=5/26), night sweats (26.9%, n=7/26), vaginal discharge (42.3%, n=11/26), vaginal itching or irritation (15.4%, n=4/26), vaginal bleeding or spotting (23.1%, n=6/26), vaginal dryness (11.5%, n=3/26), pain or discomfort during intercourse (3.9%, n=1/26), lost interest in sex (15.4%, n=4/26), breast sensitivity or tenderness (53.9%, n=14/26), and irritability (61.5%, n=16/26). Other symptoms that might be related to endocrine therapy were also observed, such as lightheaded/dizziness (34.6%, n=9/26), vomiting (3.9%, n=1/26), headaches (53.9%, n=14/26), bloating (46.2%, n=12/26), and pain in joints (50%, n=13/26). No post-dose adjustment participants reported diarrhea.

Mood swings were also observed to be the most common side effect in patients who remained on 20 mg of tamoxifen daily (74.2%, n=23/31). Other common symptoms related to hormonal changes were also observed in NM participants such has hot flashes (35.5%, n=11/31), cold sweats (12.9%, n=4/31), night sweats (29.0%, n=9/31), vaginal discharge (38.7%, n=12/31), vaginal itching or irritation (22.6%, n=7/31), vaginal bleeding or spotting (16.1%, n=5/31), vaginal dryness (32.3%, n=10/31), pain or discomfort during intercourse (51.6%, n=16/31), lost interest in sex (64.5%, n=20/31), breast sensitivity or tenderness (41.9%, n=13/31), and irritability (58.1%, n=18/31). Other symptoms that might be related to endocrine therapy were also observed, such as lightheaded/dizziness (35.5%, n=11/31), vomiting (6.5%, n=2/31), diarrhea (3.2%, n=1/31), headaches (29.0%, n=9/31), bloating (38.7%, n=12/31), and pain in joints 67.7%, n=21/31).

Chi-square test showed statistically significant differences in the symptoms pain or discomfort during intercourse and lost interest in sex between the two groups who received 40 mg and 20 mg of tamoxifen daily. The other symptoms did not have significant difference among the two groups, indicating that dose escalation up to 40 mg daily did not increase potential toxicity or side effects (Table 4). Serious side effects including thrombosis, endometriosis, and endometrial cancer, were not observed.

Table 4
Number and percentage of patient responses related to adverse events in FACT-ES post eight weeks after dose adjustment.

Symptoms	NM participants who received 20 mg of tamoxifen daily (N = 31)		IM participants who received 40 mg of tamoxifen daily (N = 22)		*p*-value
	Patients reported side effect (n)	Patients reported side effect (%)	Patients reported side effect (n)	Patients reported side effect (%)	
Hot Flashes	11	35.48%	13	50.00%	0.269361
Cold Sweats	4	12.90%	5	19.23%	0.717648
Night sweats	9	29.03%	7	26.92%	0.86249
Vaginal discharge	12	38.71%	11	42.31%	0.777297
Vaginal itching/irritation	7	22.58%	4	15.38%	0.492987
Vaginal bleeding or spotting	5	16.13%	6	23.08%	0.507122
Vaginal dryness	10	32.26%	3	11.54%	0.063252
Pain or discomfort with intercourse*	16	51.61%	1	3.85%	8.48×10^{-5}*
Lost interest in sex	20	64.52%	4	15.38%	0.005461*
Weight gain	20	64.52%	17	65.38%	1
Lightheaded (dizzy)	11	35.48%	9	34.62%	1
Vomiting	2	6.45%	1	3.85%	1
Diarrhea	1	3.23%	0	0.00%	1
Headaches	9	29.03%	14	53.85%	0.057089
Bloating	12	38.71%	12	46.15%	0.571608
Breast sensitivity/tenderness	13	41.94%	14	53.85%	0.371093
Mood swings	23	74.19%	17	65.38%	0.470842
Irritable	18	58.06%	16	61.54%	0.791337
Pain in joints	21	67.74%	13	50.00%	0.173783

*Statistically significant *p*-values were observed between IMs who have received tamoxifen dose adjustment and NMs who took the standard dose

Discussion

We discovered that Indonesian women might be at higher risk of experiencing ineffectiveness of tamoxifen therapy, as evidenced by the high proportion of patients with IM *CYP2D6* phenotype profile (40.7%). Fortunately, we also observed that IMs who received dose increase of tamoxifen to 40 mg daily had positively increased endoxifen levels to a similar level as NMs at baseline, suggesting that tamoxifen dose adjustment can help IM and PM patients achieving better clinical outcomes.

To our surprise, patients with Chinese ethnic background were predominantly IMs, while the Javanese ethnicity group was dominated by NMs (Fig. 4). The Indonesian Chinese IMs' proportion in this study was higher than a similar study conducted on Han Chinese population, which was 45.4% (13). Caucasians have a higher proportion of NMs compared to other races/ethnicities. However, the frequencies vary depending on the geographical location (23–25). To summarize, ethnic differences might represent differences in genetic construction of a population, affecting the proportion of phenotype profiles observed.

Furthermore, *CYP2D6*10*, the most common haplotype in this study, was known to increase the risk of breast cancer recurrence for those taking tamoxifen (26). A study conducted in the Han Chinese population reported 45.7% frequency of *CYP2D6*10* (27), higher than *CYP2D6*10* frequency observed in this study (28.8%). Another important highlight was the relatively high **36* allele frequency observed in this study (25.3%) compared to the observed frequency in the PharmGKB database (1.2%). Compared to other Asian populations, a study conducted in Hong Kong population also recorded a relatively high *CYP2D6*36* frequency which is 34.1% (28). Although some **36* allele contributed to NM status profile, our study observed **10/*36* (which translates to IM phenotype) was the diplotype with highest frequency, suggesting that **36* might play an important role in constructing IM phenotype in Indonesian population. These findings suggested that Indonesian population could be at risk of experiencing ineffectiveness of tamoxifen therapy. It was also supported by the *CYP2D6* IMs high proportion (40.7%) compared to the current known global prevalence (22). In addition, a similar study in Thailand reported a relatively higher IMs frequency (29.1%) compared to the global prevalence, implying that the East Asian population might have relatively higher frequency of IM (29). The NMs frequency observed in this study (54%) was also lower than the current known global prevalence which is between 67-90% (22).

Serum levels of different metabolites of tamoxifen strongly predict tamoxifen's efficacy while lower endoxifen levels in IMs indicates lower efficacy of tamoxifen in preventing recurrence (9). A study conducted in Swedish population found endoxifen level range between 2.3-16 ng/mL (30), while another in Singaporean population displayed a range between 1.74–42.8 ng/mL (31). These showed that similar interventions in different populations may have different ranges of metabolite levels. Differences in metabolite level range may also be due to the measurement or sampling using different techniques. While other studies primarily measure metabolite levels from plasma or serum, our measurement used peripheral whole blood using Volumetric Absorptive Microsampling (VAMS) technique due to its

effectiveness and practicality. This sampling technique also provides more stable condition for storage and transport purposes (18).

IM and PM who received 40 mg of tamoxifen daily all experienced a significant increase across all metabolite levels, suggesting that increasing tamoxifen intake can positively elevate endoxifen levels to increase efficacy. The distribution of endoxifen level in IMs post-dose adjustment was similar to the NMs' at the baseline, implying that increasing tamoxifen dosage to 40 mg daily for IM participants successfully increased endoxifen levels similar to those of NMs. While the findings are significant, further studies with larger sample size and multiple centers need to be conducted to corroborate our observation.

Gynecological side effects such as hot flushes, vaginal dryness, and endometriosis were commonly observed in patients taking tamoxifen (32–34). Despite most participants reported mild to moderate degree of endocrine symptoms, there were no observed dropouts, suggesting that increasing tamoxifen dose does not change side effects of the drug distinctly. The concerning side effects of tamoxifen such as thrombosis, endometriosis, and endometrial cancer (35, 36) were not found, but this might also be underestimated due to the short follow up period. Other studies who have tried to observe tamoxifen side effects in patients with dose increase also concluded that increasing tamoxifen dose did not result in toxicity or short-term increase in side effects (24, 37).

To conclude, our study successfully showed preliminary evidence that tamoxifen dose can be adjusted to improve therapeutic effects without severe adverse events. Although the CPIC guideline recommended the first course of action to switch to aromatase inhibitors, our finding demonstrated that tamoxifen dose adjustment is adequate. In developing countries such as Indonesia, these findings were potentially beneficial to redefine the tamoxifen use for ER+ breast cancer adjuvant therapy, given the lower price of the drug compared to aromatase inhibitor. These preliminary data can potentially be used as an early scientific basis to assess and adjust ER+ breast cancer adjuvant therapy guidelines, giving clinicians more options when determining the most suitable treatment for each patient.

Limitations

One of the several limitations of this study was the subjective measurement of side effects using FACT-ES. Due to the subjective nature of the questionnaire, severity and intensity of symptoms might be interpreted differently. Another limitation of this study was the relatively short period of patient follow up, which may lead to underestimating the number of certain side effects that may not immediately show up after tamoxifen therapy initiation. Further studies with bigger sample size may be required to confirm these findings.

Conclusion

Our study has shown a considerable proportion of *CYP2D6* IMs (40.67%) in Indonesian women with ER+ breast cancer consuming tamoxifen, suggesting possible ineffectiveness of tamoxifen therapy to prevent

recurrence. We found that *CYP2D6*10* was the most common haplotype (28.8%) and **10/*36* (23.6%) was the most frequently observed diplotype, where *CYP2D6*10* has been linked to higher risk of breast cancer recurrence in women taking tamoxifen and **10/*36* is translated as IM. The correlation between genotypes and phenotypes observed were also shown in the significant difference of the endoxifen levels between NMs and IMs. Dose adjustment of tamoxifen can effectively increase the level of endoxifen, the metabolite responsible for the anticancer effect of tamoxifen without observed severe side effects. Implementing *CYP2D6* genotyping prior to tamoxifen therapy will be beneficial to avoid possible inefficacy in preventing breast cancer recurrence. These findings may also be helpful in evaluating tamoxifen use as the first-line adjuvant therapy for ER+ breast cancer.

Future Work

Further follow up on the same breast cancer patient cohort is required to better understand the effect and benefits of *CYP2D6* genotyping and endoxifen level measurement towards clinical outcomes such as relapse rate, disease-free survival, and overall survival. Future work may involve monitoring these patients for a longer period when potential side effects to appear (38).

Declarations

Ethics Approval and Consent to Participate

Institutional Review Board (IRB) approval was granted by MRCCC Siloam Hospitals Semanggi Ethics Review Committee (Jakarta, Indonesia) under IRB Reference Number 001/EA/KEPKK/RSMRCCC/V/2019. Patients' informed consent was obtained before they were enrolled in the study.

Consent for Publication

Not applicable.

Data Availability Statement

The data that supports the findings of this study are available as supplementary materials. Any additional data is available from the corresponding author upon reasonable request.

Competing Interests

KIJ, LLS, CM, MA, G, and AI are employees of Nalagenetics Pte Ltd, Singapore.

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Authors' Contributions

BPM, LLS, AI, YH, H, and SJH contributed to study design. KIJ, MA, and SJH contributed to patient recruitment. BPM and G performed laboratory experiments. KIJ and MA were responsible for patient data management. BPM, KIJ, CM, and AI performed data analysis. KIJ and CM contributed to manuscript writing with supervision from AC, LLS, AI, BPM, and SJH.

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Supplementary Tables

Supplementary Table 1. Number of NMs and IMs in each quintile group of endoxifen measured at baseline.

Quintiles	NMs	IMs	PMs
Q1	8	17	2
Q2	12	12	0
Q3	19	11	0
Q4	15	12	0
Q5	23	5	0

Supplementary Table 2. Number of participants in each ethnicity observed. n=150

Ethnicity		n
Chinese		51
Javanese		38
Others	Ambon	2
	Batak	8
	Betawi	5
	Makassar	1
	Manado	1
	Melayu	1
	Minangkabau	5
	Nias	1
	Padang	1
	Palembang	2
	West Sumatera	1
	Sunda	9
	Timor Leste	1
	Tolaki / Sulawesi	1
	Toraja	1
Multiethnic		16
NA*		6

*NA: data not available

Figures

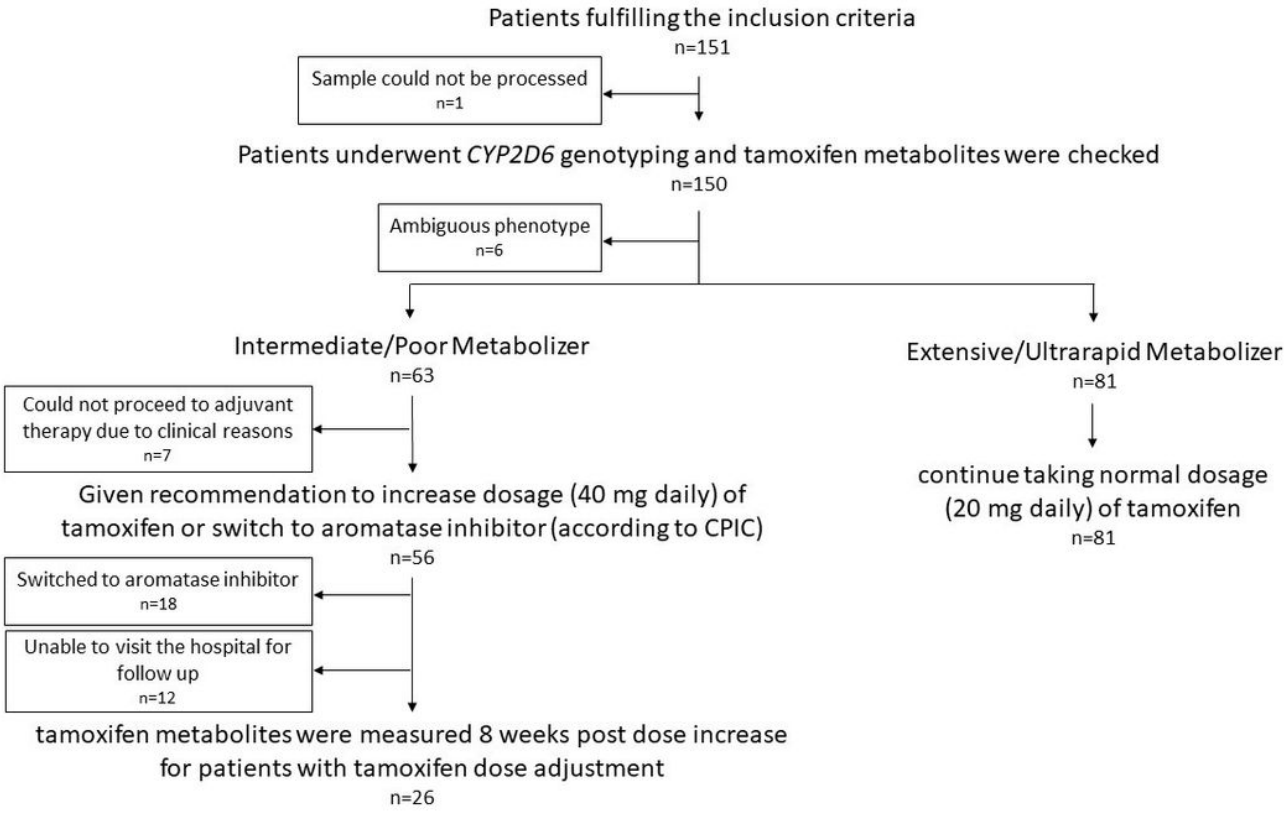

Figure 1

Research flow diagram.

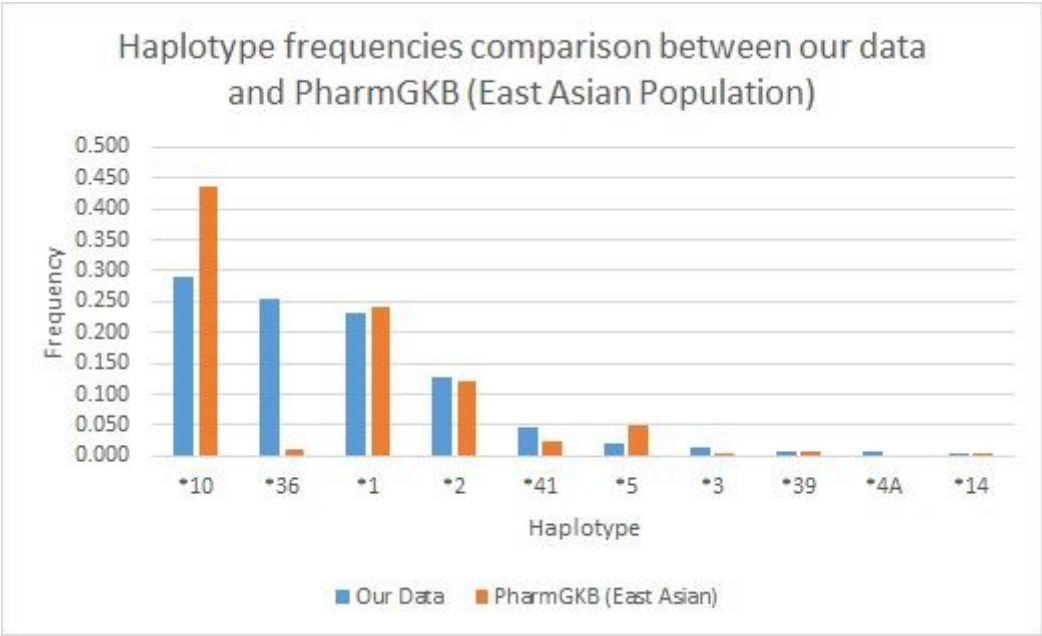

Figure 2

Distribution of haplotype frequencies among Indonesian breast cancer patients. n=288

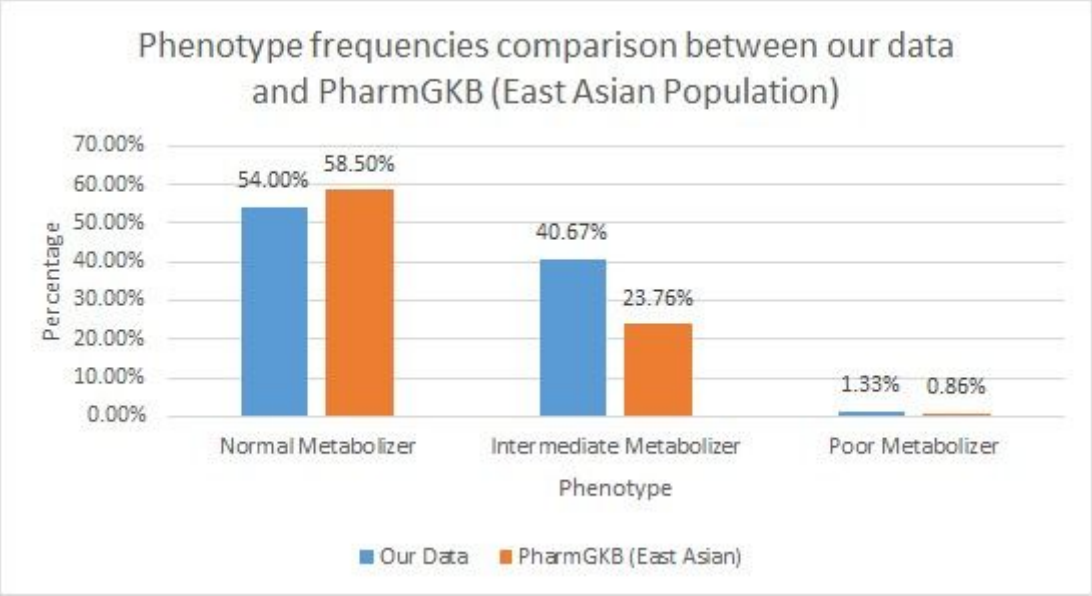

Figure 3

Distribution of phenotype frequencies among Indonesian breast cancer patients. n=144

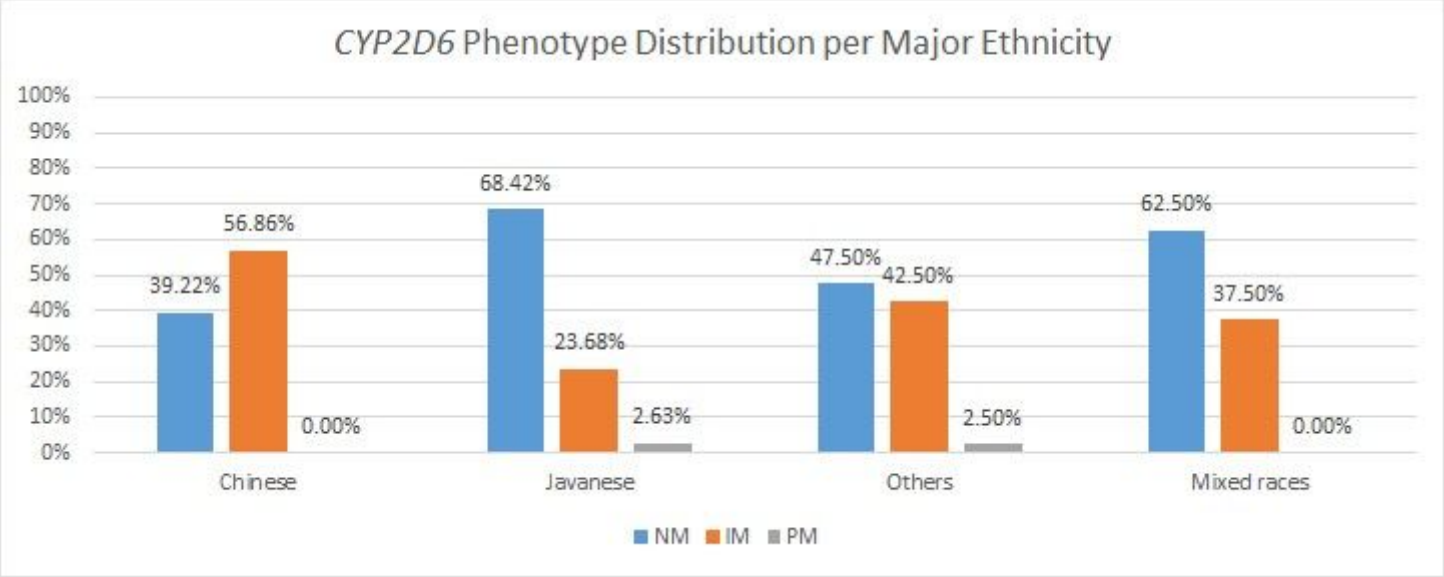

Figure 4

Distribution of phenotype frequencies per major ethnicity among Indonesian breast cancer patients. n=151; among all major ethnicity groups, only the Chinese ethnicity group displayed a greater IM proportion than NM.

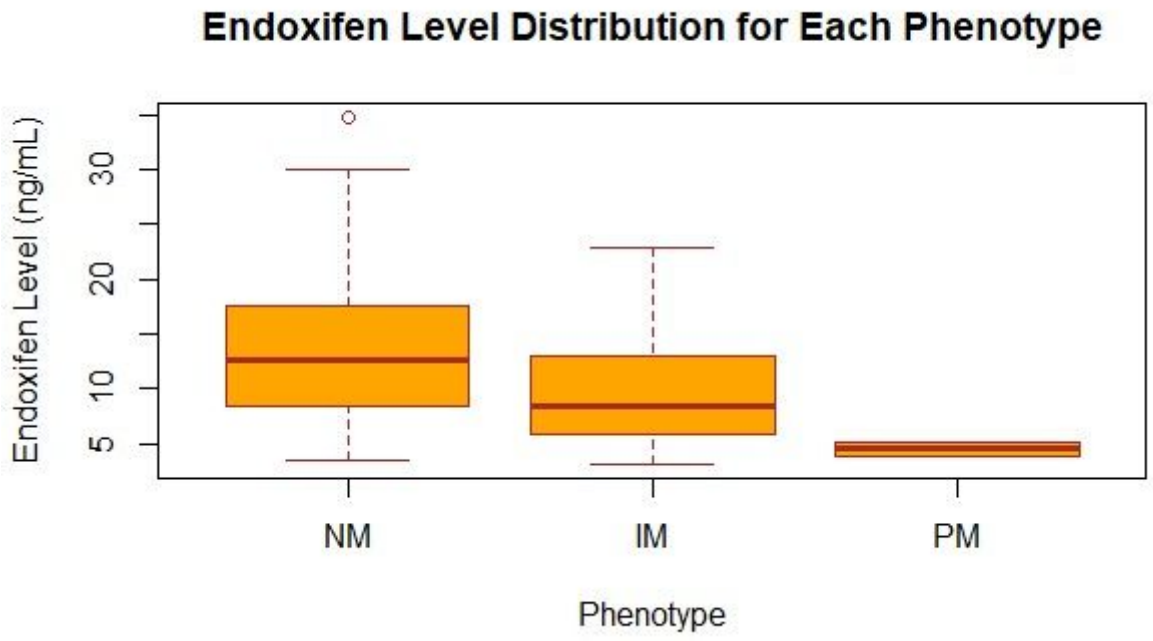

Figure 5

Distribution of endoxifen levels for each observed phenotype at the baseline. Normal metabolizer/NM (n=81), Intermediate metabolizer/IM (n=61), Poor Metabolizer/PM (n=2)

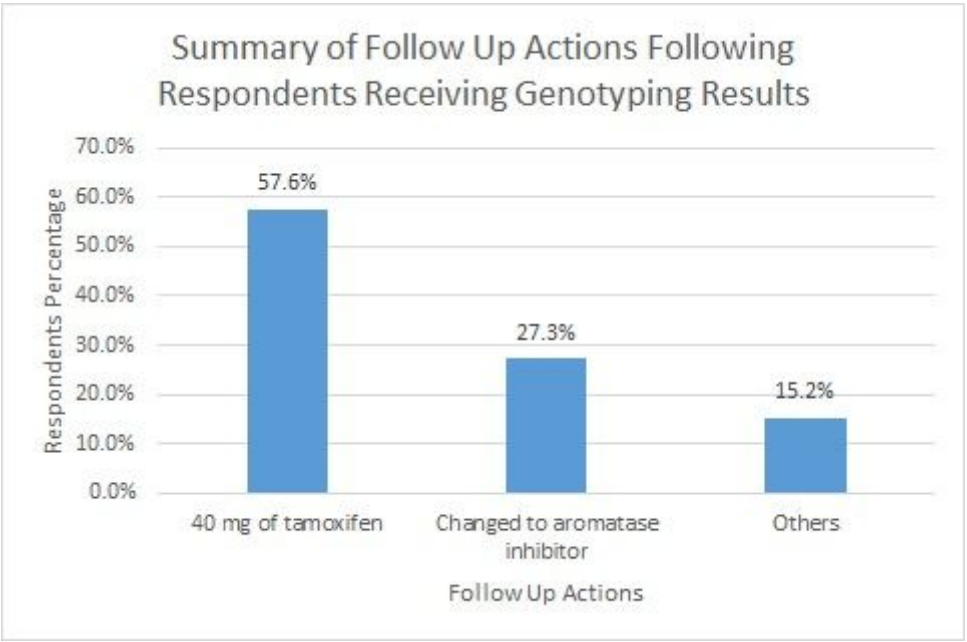

Figure 6

Distribution of the different follow up actions selected by doctors after patient's CYP2D6 profile was characterized through genetic testing. n=66

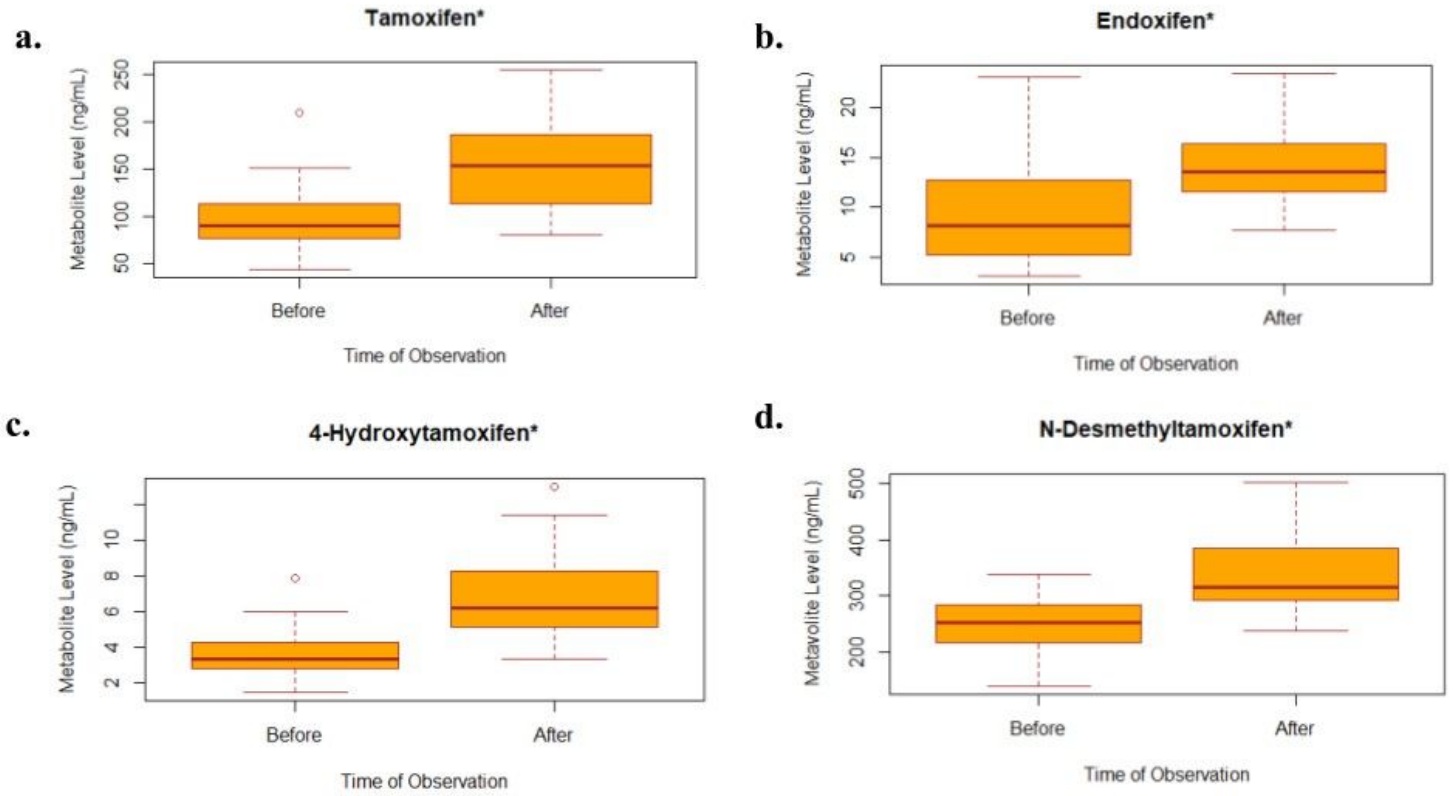

Figure 7

Metabolite levels before and after dose adjustment for IM patients. After dose adjustment, the range of tamoxifen metabolites increased as follows: a.) tamoxifen levels from 14.22-210.39 ng/mL to 80.59-254.96 ng/mL; b.) endoxifen levels from 3.17-22.97 ng/mL to 7.68-23.36 ng/mL; c.) 4-hydroxytamoxifen levels from 1.5-9.31 ng/mL to 3.34-12.99 ng/mL, and d.) N-desmethyltamoxifen levels from 77.61-337.29 ng/mL to 236.8-501.9 ng/mL. *Statistically significant *p*-values were observed between metabolites before and after dose adjustment, n=26

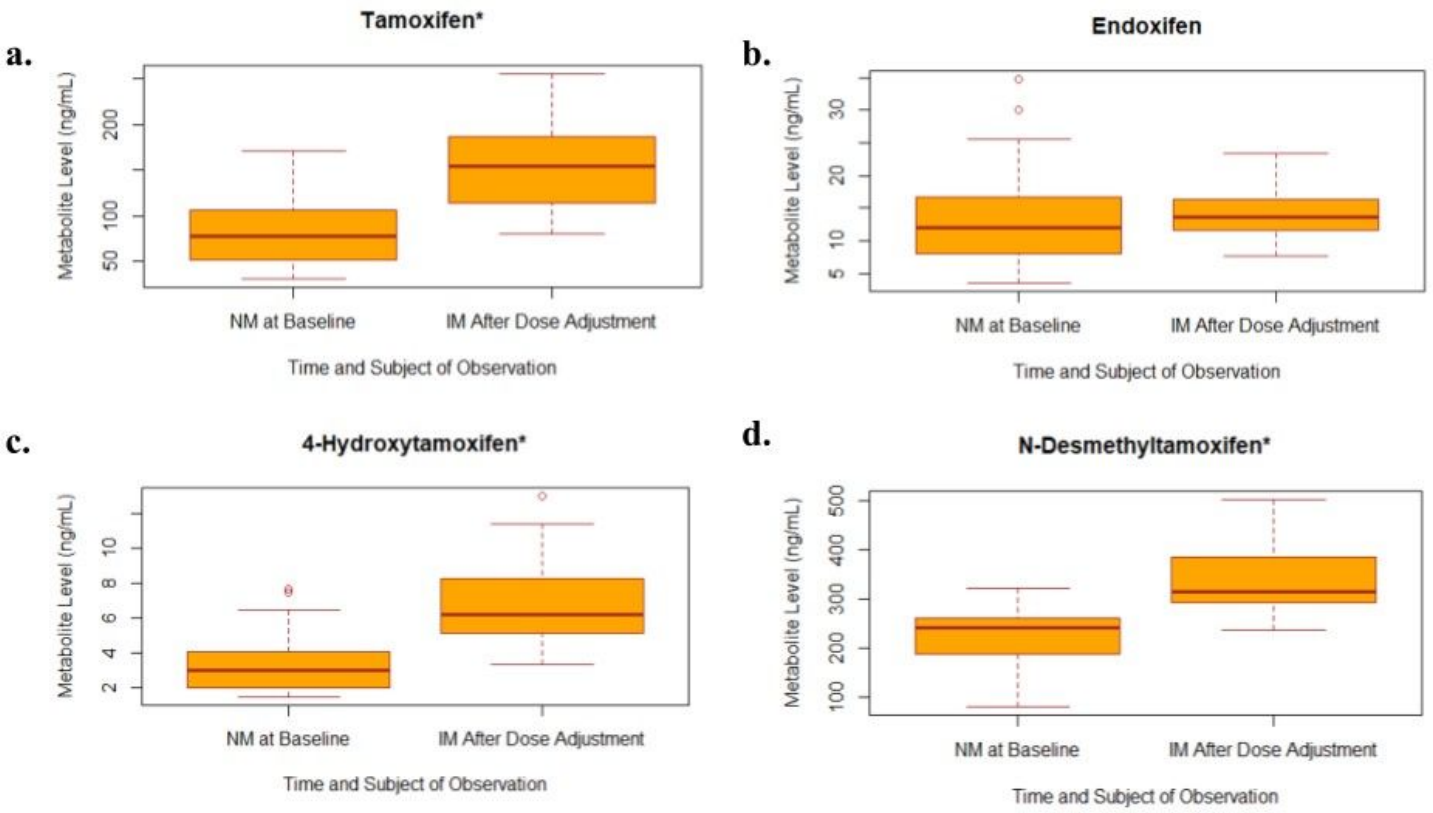

Figure 8

Metabolite levels in IMs after dose adjustment compared to NMs at the baseline. a.) Tamoxifen, b.) endoxifen, c.) 4-hydroxytamoxifen, d.) N-desmethyltamoxifen. *Statistically significant *p*-values were observed, n=81 (NMs), n=26 (IMs). Endoxifen levels in IMs post dose adjustment were statistically similar to NMs at the baseline.