ASSOCIATION BETWEEN GLUTATHIONE S-TRANSFERASE M1, T1 POLYMORPHISMS AND CLINICAL OUTCOMES AMONG THAI BREAST CANCER PATIENTS

Phakarat Tangkheunkan
Kitiphong Harncharoen
Somchai Thanasitthichai
Danai Tiwawech

Faculty of Pharmacy
Siam University
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ABSTRACT

Glutathione S-Transferase M1, T1 Polymorphisms and Clinical Outcomes among Thai Breast Cancer Patients

Phakarat Tangkheunkan[1], Kitiphong Harncharoen[2], Somchai Thanasitthichai[3], Danai Tiwawech[3]
[1]Faculty of Pharmacy, Siam University
[2]Faculty of Public Health, Mahidol university
[3]National Cancer Institute
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Glutathione S-transferase M1 and T1 (GSTM1 and GSTT1) are the enzymes that play a key role in the detoxification of a broad range of xenobiotics, including chemotherapeutic drugs. The deletion polymorphisms of GSTM1 and GSTT1 genes are associated with reduced enzyme activity that could be related to clinical outcomes of chemotherapeutic agents in breast cancer. However, they have yielded inconsistent results and there is limited information among Thai patients. Therefore, the objective of this research is to explore the role of GSTM1, GSTT1 polymorphisms on clinical outcomes among Thai patients with breast cancer treated with chemotherapy.

The retrospective study was conducted to evaluate the toxicity and survival among breast cancer patients with chemotherapy. Fifty six patients who had received complete course of anthracycline based chemotherapy were evaluated for hematotoxicity according to the CTCAE v3.0. The prevalence of the grades was as follows: 38 anemia (67.90%), 38 leucopenia (67.90%), 10 neutropenia (17.90%) and 3 thrombocytopenia (5.40%) and grade 4 hematotoxicity and toxic deaths were not observed in this study. There were no significant association between GSTM1 and GSTT1 polymorphisms and hematotoxicity ($p = 0.36$, 0.34, 0.25 and 0.19). With respect to survival study, the frequency of the GSTM1 and GSTT1 null genotype in 198 breast cancer patients was 65.70% and 33.30%, respectively. The statistically significant association between the GSTM1 null genotype and the tumor stage was found after adjusted for age at diagnosis alone, progesterone receptor status alone and age at diagnosis and progesterone receptor status ($P = 0.043$, 0.047, and 0.037). For GSTT1, statistically significant association between the GSTT1 null genotype and the tumor size was found (OR = 0.51, 0.03). The overall survival at 1, 3, 5 years was 95.00%, 83.00%, 71.00% respectively. The log rank test and Cox proportional hazards revealed a significant different in the 5-year overall survival according to lymph node metastasis and tumor stage ($P = 0.014$ and $P < 0.001$). No associations between overall survival and GSTM1 or GSTT1 genotype were found in single genotype or combined genotypes analyses ($P = 0.76$, 0.15). This result provided the epidemiological and pharmacogenetic information to use in developing treatment guideline and prognostic of hematotoxicity and survival of chemotherapy.

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BC	breast cancer
GST	glutathione S-transferase enzyme
GSTM1	glutathione S-transferase mu1 enzyme
GSTT1	glutathione S-transferase theta1 enzyme
GST	glutathione S-transferase gene
GSTM1	glutathione S-transferase mu1 gene
GSTT1	glutathione S-transferase theta1 gene
NCI	National Cancer Institute
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
ER	estrogen receptor
PR	progesterone receptor
HER2	human epidermal growth factor receptor2
OR	odds ratio
χ^2	chi-squares
95 % CIs	95 % confidence intervals
PCR	polymerase chain reaction

LIST OF ABBRIVIATIONS (Continued)

mm	millimeters
mg/m^2	milligrams per square meter
µL	microliter

CHAPTER I
INTRODUCTION

1.1 Background and Rationale

Breast cancer is a dangerous tumor that has developed from cells in the breast. Usually breast cancer can starts at the epithelium cells of the lobules and/or the the mammary glands ducts and can invade nearby healthy breast tissue, lymph nodes, and other distant organs such as liver, bone and brain (1-3). Therefore, breast cancer can cause a worse quality of life in patients (4,5), a burden to their family, caregivers (6,7) and society, including national economic loss (8).

Globally, including Thailand breast cancer is the common cancer and one of the leading causes of mortality among female (9). In 2018, the GLOBOCAN project reported that there were 2,088,849 new cases and 626,679 deaths of breast cancer among women worldwide, which were a higher percentage than other types of cancer. In Thailand, according to National Cancer Institute' s data, breast cancer was the most leading cancer among women in 2017 follow by cervix uteri cancer. Mean annual age-standardized incidence rate is 28.5 per 100,000 in women with breast cancer and 0.5 per 100,000 in men (10). The overall survival rates at 1, 3 and 5 years were 83.3%, 59.9% and 42.9%, respectively (11).

To cope with breast cancer, there are many therapeutic methods such as chemotherapy, radiotherapy, hormone therapy and targeted therapy which are the general treatments. Chemotherapy is usually used as a therapeutic method as neoadjuvant (before surgery of breast cancer) or adjuvant (after surgery of breast cancer). In breast cancer treatment, chemotherapy is the leading beneficial therapeutic method to stop the proliferation and growth of cancer cell (12). Unfortunately, many studies reports that there were a high incidence of adverse events (13,14). Consequently, adverse events may affect to home and work activities, they may interfered in survival and quality of life of breast cancer patients (15,16). However, clinical outcomes to chemotherapy is inconstant and cannot be predicted in patients with breast cancer.

Previous research reported that there were a high chemotherapy drugs resistance among patients with breast cancer and a risk of toxicity have been frequently occurred after chemotherapy treatment (17- 21) . Therefore, the clinical outcomes prediction to chemotherapy treatment seem to be significant to successful method.

Genetic variations of drug metabolizing enzymes have been revealed to be related with the chemotherapy response. Glutathione S-transferase M1 and T1 (GSTM1 and GSTT1) are the enzymes that can detoxify the several xenobiotics, including chemotherapeutic drugs or their metabolites in human. The deletion polymorphisms of *GSTM1* and *GSTT1* genes are related with reduced enzyme function that may reduce the effectiveness of the cytotoxins detoxification created by chemotherapeutic agents in breast cancer treatment (22) . Recently, several research indicated that the genetic polymorphisms of *GSTM1*, and *GSTT1* provided a stronger scientific data for chemotherapy response (23-25). In addition many studies in vitro have showed that the expression of *GSTM1*, and *GSTT1* had a crucial relation with chemotherapy resistance (26, 27) nevertheless, they have yielded inconsistent results (28-31).

Because of the limited information among Thai patients with breast cancer and controversial results on the association of *GSTM1* and *GSTT1* polymorphisms with clinical outcomes and toxicity among Thai patients with breast cancer patients treated with chemotherapy. Consequently, the objective of our research is to explore the role of *GSTM1*, *GSTT1* polymorphisms on clinical outcomes among Thai patients with breast cancer.

1.2 Research Questions

The research question guiding this study is the following:

How does the association between *GSTM1* and *GSTT1* polymorphisms and clinical outcomes among breast cancer patients?

1.3 Research Objectives

General Objective:

The objective of this research is to explore the role of *GSTM1, GSTT1* polymorphisms on clinical outcomes among Thai breast cancer patients with chemotherapy.

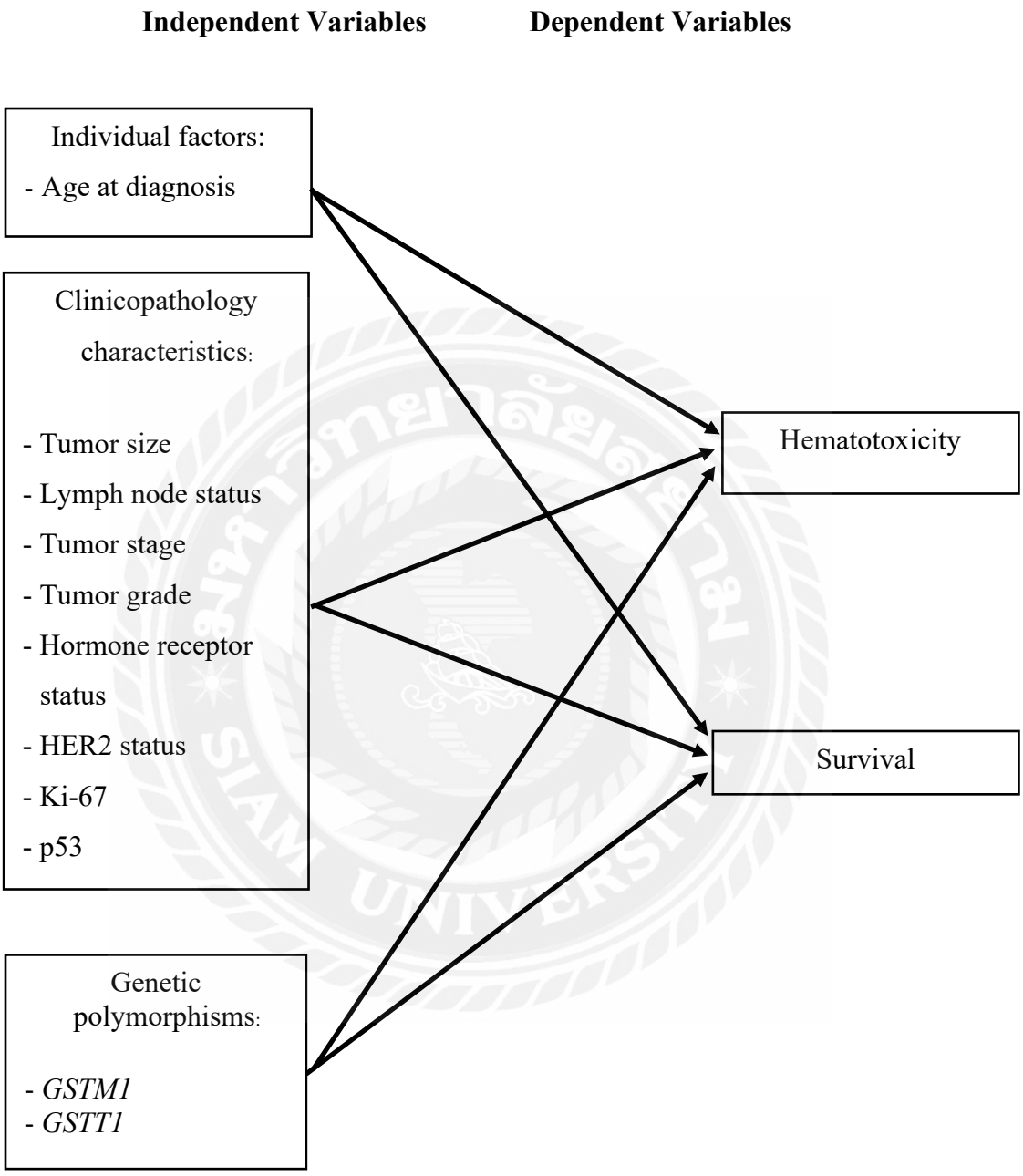
Specific Objectives:

1. To determine the association of *GSTM1* and *GSTT1* polymorphisms with hematotoxicity among Thai breast cancer patients with chemotherapy
2. To determine the association of *GSTM1* and *GSTT1* polymorphisms with survival among Thai breast cancer patients with chemotherapy

1.4 Research Hypotheses

1. *GSTM1* and *GSTT1* polymorphisms associate with hematotoxicity among Thai breast cancer patients with chemotherapy.
2. *GSTM1* and *GSTT1* polymorphisms associate with survival among Thai breast cancer patients with chemotherapy.

1.5 Conceptual Framework



1.6 Operational Definitions

1. Age at diagnosis

Age at Diagnosis is the age of the patient at diagnosis (in number of years) determined by calculating the difference between date of birth and date of diagnosis.

2. Tumor size

Size of breast cancer tumor represents the widest points of the breast cancer tumor. It is used to indicate the stage of the breast cancer according to TNM classification and it will be estimated in millimeters (mm). According to TNM, tumor size can be classified in seven categories as following.

TX represents the tumor that can't be measured.

T0 represents no any evidence of the primary tumor.

Tis represents that the cancer is "in situ" which is no cancer in the healthy breast tissue.

T1, T2, T3 and T4 represent the numbers that are depended on the tumor size and the extent to which it has grown into adjacent breast tissue. The T4 shows the bigger cancer size and/or the more it may have grown into the breast tissue more than T3, T2 and T1 respectively.

3. Lymph node status

Lymph node status represents to the number of neighboring lymph nodes that invaded by cancer. According to TNM classification, lymph node status can be classified into five categories including NX, N0, N1, N2 and N3.

NX represents tumor in neighboring lymph nodes that cannot be determined.

N0 represents no cancer in adjacent lymph nodes.

N1, N2 and N3 represents to the number and site of lymph nodes that invaded by cancer. N3 shows the higher number and the more lymph nodes that invaded by cancer more than N2 and N1 respectively.

In this study, lymph node status was categorized into 2 groups including, none which mean there is no lymph node metastasis, and present which mean there are at least 1 lymph node metastasis.

4. Tumor stage

Based on TNM classification, tumors are classified according to invasive tumor size (T), lymph node involvement (N) and distant metastases (M).

5. Tumor grade

Tumor grade is classified after observed under a microscope based on the abnormal of the tumor cells and the tumor tissue. It can show of how rapidly grow and spread of tumor. The general system for a tumor grade is the following as below.

GX represents the grade that undetermined

G1 represents the well differentiated or low grade

G2 represents the moderately differentiated or intermediate grade

G3 represents the poorly differentiated or high grade

G4 represents the undifferentiated or high grade

6. Hormone receptor status

Estrogen receptor positive (or ER+) cancer refers to breast cancers that have estrogen receptors determined by using the immunohistochemistry (IHC).

Progesterone receptor positive (or PR+) cancer refers to breast cancers that have progesterone receptors determined by using the IHC.

7. Human epidermal growth factor receptor 2 (HER2) status

HER2 gene involves in the breast cancer development. The Immunohistochemistry test was used to examine the over express of HER2 protein level in tumor cells. The IHC test result can be 0 or negative, 1+ or negative, 2+ or borderline, or 3+ or positive (HER2 protein overexpression).

8. Ki-67

The Ki-67 is a superb marker protein for cell growth in human body. It occurs while cells in body is in the active stage including G1, S, G2, and mitosis and miss while cells in the resting stage or G0 which makes it. Ki-67 as the proliferation marker of many tumors because involves in proliferation of cells. In this study, Ki-67 was classified to + (positive) and - (negative).

9. p53

p53 gene encodes for a tumor protein that operates the cell functions and cell cycle by suppression of cancer growth which is very important for multicellular organisms to suppress cancer by preventing genome mutation. In this study, p53 was classified to + (positive) and - (negative).

10. *GSTM1*, *GSTT1* polymorphisms

Genotyping of GSTM, GSTT1 were performed using real time PCR and classified to *GSTM1* present genotype or wild genotype, *GSTM1* null, *GSTT1* present genotype or wild genotype, and *GSTT1* null.

11. Hematotoxicity

The chemotherapy toxicity was assessed and graded following to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 3.0. Adverse events attribute to chemotherapy was recorded; consist of hematologic toxicities; anemia, leukopenia, neutropenia and thrombocytopenia.

12. Survival

Cumulative 5-year survival and hazard ratios was determined starting from the diagnosis date by Cox's proportional hazard and Kaplan-Meier survival analysis.

1.7 Expected Outcomes

This research result provided the information to use in developing treatment guideline and prognostic of hematotoxicity and survival of chemotherapy and provide the basic for the further studies of epidemiological and pharmacogenetic on population diversity among Thai breast cancer patients for policy makers.

CHAPTER II
LITERATURE REVIEW

In this part, the literature review provides related information of this research including the definitions of breast cancer, the burden situation in Thailand, chemotherapy treatment, clinicopathology characteristics, genetic polymorphisms and *GSTM1*, *GSTT1* and clinical outcomes and setting in this study.

2.1 Breast Cancer

Among women worldwide, breast cancer is the common cancer and the foremost causes of mortality, including Thailand. Breast cancer is a tumor that originates from cells in the breast; start by cells in the mammary gland or ducts of the mammary gland. It can invade nearby tissue, normal breast cell and metastasis to the lymph nodes and other distant organs in the patients; thus, this makes the symptoms worse and difficult to treat. To cope with breast cancer, epidemiological data relate to the distribution of breast cancer and risk factors will be useful for planning programs or strategies to control breast cancer and as a basis for future research.

According to GLOBOCAN report, which collects epidemiological data about the importance of cancer in 185 countries throughout the world. Breast cancer is the common cancer and the foremost causes of mortality associated to cancer in women worldwide in 2018. The new cases of breast cancer patients were 2,088,849 people. The number of people who die from breast cancer was 626,679 and the five-year prevalence of breast cancer was 6,232,108 people (36).

Globally, not only in developed but also developing countries, breast cancer is a crucial public health problem. In the developing region, the number of new cases was 883,000 people, while the developed region was 794,000 people. The incidence of breast cancer is different almost four times over the world from 92 per 100,000 population in North America and 27 per 100,000 population in Africa, the Middle East and East Asia (37).

Respect with woman, breast cancer is the most common cancer and the leading cause of cancer death, followed by lung cancer and colorectal cancer. However, depending on the economic development status, social and life style factors affect to the extremely differ over countries and within each county (36).

2.2 Breast Cancer in Thailand

With respect to the report from the National Cancer Institute in 2015, cancer with the high incidence rate among men were liver and intrahepatic bile ducts cancer, trachea, bronchus and lung cancer, and colon and rectum cancer, respectively. While among women, cancer with the highest incidence rate was breast cancer followed by liver and intrahepatic bile ducts cancer, cervix uteri cancer and colon and rectum cancer, respectively. Among men, incidence rate of breast cancer was very low (0.5 per 100,000 population) (38). Breast cancer had the highest incidence of women at 2015 and is expected to remain the highest in 2025 (39).

The breast cancer's incidence rate varied by geography in Thailand. The highest age-standardized incidence rate per year was in the central region (33.9 per 100,000 population), followed by the East (33.4 per 100,000 population), the North (32.4 per 100,000 population), South (27.4 per 100,000 population) and the Northeast. (19.4 per 100,000 population), respectively. In Bangkok, incidence rates standardized average age per year was 35.1 per 100,000 population. The province with the highest incidence rate standardized average age per year was Chonburi (36.0 per 100,000 population) while Ubon Ratchathani was the lowest incidence rate standardized average age per year in Thailand (17.0 per 100,000 population) (38).

Considering the age of the women in Thailand, the incidence of breast cancer began at 15 years old and increased with increasing age. Among 50-70 years old of Thai women, the breast cancer's incidence rate per year is relatively high and varied by regions. In addition, the study of the distribution of age in breast cancer among the population from the register since the 2002-2011 period, showed that during 10 years, the number of breast cancer patients was 7,711 people; in addition, the incidence of breast cancer under 40 years old was relatively low (4.13 per 100,000 population); in contrast, the breast cancer incidence at 40 years old and older was relatively high (39.2 per 100,000 population) (10).

Respect with the tissue type of breast cancer, the almost tissue type were ductal carcinoma (breast cancer cells caused the cells lining the milk ducts), follow by lobular carcinoma (cancer cells in the mammary gland that arise from the epithelial cells) and other tissue types. Considering the stage of breast cancer, that can be divided into three stages; including, the local stage (cancer cells locate within the breast), regional stage (cancer cells invade to the adjacent lymph nodes in the human body such as the axillary lymph nodes), and distance stage (cancer cells spread to other organs in the body). The breast cancer stage differs across the province in Thailand. The majority of breast cancer stage were in the regional stage and there were some patients who do not know in any stage (38).

2.3 Breast Cancer Treatment

Breast cancer is primarily treated with surgery, either by means of modified radical mastectomy, whereby the complete breast is removed, or by means of breast-conservative surgery, whereby only part of the tissue is removed. In addition, there are other therapeutic methods such as radiotherapy, hormone therapy, chemotherapy and targeted therapy are the common treatment in breast cancer (40-43).

Chemotherapy

Chemotherapy is the leading and useful treatment method not only before breast cancer surgery (neoadjuvant chemotherapy) but also after breast cancer surgery (adjuvant chemotherapy) and it play a crucial role to suppress the proliferation and growth of breast cancer cell (12).

Adjuvant chemotherapy

Chemotherapy will be used as an adjuvant treatment (ATC) in early stage of cancer after primary breast cancer surgery. Adjuvant cytotoxic chemotherapy regimens have developed from single chemotherapy agents to polychemotherapy regimens consolidating anthracyclines and/or taxanes. Since predicting usefulness from chemotherapy has been more aspiring, adjuvant treatments are guided by some predictive factors. Previous studies show the progression to use several parameter gene

expression assays that may more accurate to select patients for benefit from adjuvant chemotherapy (24, 44, 45).

Generally, several reports reveal a high incidence of toxicity of chemotherapy (13, 14). Consequently, adverse events may directly interfere home and work life, they may involve in quality of life and survival of patients with breast cancer (15, 16). However, clinical response, toxicity, and treatment outcome to chemotherapy is differ in individual patient and do not predictable the outcomes for breast cancer patients. Several studies show that there are a crucial risk of toxicity and higher risk of chemotherapy drug resistance in breast cancer patients (17-21). Consequently, the predictors of clinical response, toxicity, and treatment outcome to chemotherapy could be importance for successful individualizing treatment.

Chemotherapy regimens

Chemotherapy drug such as anthracycline-based chemotherapy (epirubicin or doxorubicin). In this study, anthracycline-based chemotherapy composes of doxorubicin and cyclophosphamide.

Doxorubicin

Doxorubicin was the first anthracycline to be used in clinical practice and epirubicin was the 4'-epi-isomer of doxorubicin. Doxorubicin counteract to DNA by inhibits topoisomerase II, consequently, inhibit DNA replication and ultimately, interfering with RNA and protein synthesis. Furthermore, it also produces toxic free-radical intermediates and involves lipid peroxidation (14). There are many adverse drug events of anthracycline; however, cardiotoxicity has been a major concern (14,46).

Cyclophosphamide

The mechanism of action of this agent for anticancer activity is an alkylating agent. The phosphoramide mustard, the active metabolite of cyclophosphsmide, forms a highly reactive cyclic aziridinium cation, which can react with the N of the guanine and with cytidine from the DNA. Moreover, its mechanism of action was cell cycle independent. Nevertheless, same as all alkylating agents, quickly proliferating breast cancer cells are sensitive to cyclophosphamide. In addition, another mechanism was an

immunosuppressive effect which suppress the natural immune response of cancer patients (47, 48). There were several adverse effects of cyclophosphamide administration including gastrointestinal side effects. Nausea and vomiting are common adverse effects with intermediate and high doses. Furthermore, there are several toxicities include hematologic toxic, cardiac toxic, gonadal toxic, bladder toxic had also been associated with cyclophosphamide therapy (48).

2.4 Clinicopathology Characteristics

Clinicopathology characteristics include tumor stage, tumor size, lymph node status, histological grade, age at diagnosis, human epidermal growth factor receptor type 2, Ki-67 status, p53 status, and hormone receptor factors (estrogen receptor, and progesterone receptor).

Tumor stage

With respect to TNM classification (49), tumors are classified according to the tumor size, lymph node status and distant metastases. T0 represents no sign of primary tumor. T is stands for carcinoma in situ. It is a pre-invasive cancer where the cancer cells are proliferating in an uncontrolled manner but have not invaded through the basal membrane into the surrounding normal tissue. T1-3 represents different sizes of the tumor and T4 represents a tumor that has grown into the chest wall or involves the skin, independently of its size. Tumor size can be utilized as a significant predictor in breast cancer patients for 15-year survival. An increase of tumor size was significantly associated with the increasing in breast cancer mortality (50). N0 represents no spread to the lymph nodes. N1 tumors have spread to the axillary lymph nodes. Distant metastases are denoted by M, where M0 represents no distant metastases and M1 distant metastases. Breast cancer can be classified in four main stages based on the TNM classification, but the use of this classification varies from country to country and the cancers are sometimes referred to as early- and later-stage breast cancer, or simply node-negative or node positive.

Tumor grade

The histological grade of the tumor is based on the evaluation of tubular differentiation, nuclear pleomorphism, and mitotic count. The score of each three

morphologic characteristics is in between 1 and 3. By sum-up the score of each three characteristic, the overall histological grade is obtained which have the total score in between 3 and 9. A score of between 3 and 5, between 6 and 7 and between 8 and 9 denotes a tumor grade 1, grade 2 and grade 3, respectively. The histological grade is representative of the aggressiveness of the tumor, grade 1 tumor seem to be less aggressive than grade 2 and grade 3 tumors. To cope with breast cancer, the grade is very important to guide for choosing the treatment options in breast cancer patients (51).

Age at diagnosis

Age is a significant risk factor in breast cancer. The older breast cancer patients show less severity of disease than the younger patients. For overall survival and breast cancer specific survival, middle-aged patients show higher survival than young and elderly patients; however, after adjustments for confounders breast cancer specific survival rates are similar to those of young patients. One of the important independent predictor for a poor prognosis was an age of 60 years or more (52).

Human epidermal growth factor receptor type 2 (HER2)

In breast cancer, a tyrosine kinase receptor; HER2, can be found to be overamplified about 20-30%. The patients with breast cancer with HER2 positive generally have more severity of disease, higher recurrence rate and mortality rate. It is not only a prognostic but also a treatment predictive factor for the response to the monoclonal antibody trastuzumab which is the standard treatment of HER2 positive breast cancer (53). Lapatinib which has the mechanism of action to inhibit both ErbB1 (EGFR) and ErbB2 (HER2) receptor tyrosine kinases has been used orally for metastatic breast cancer treatment. In trastuzumab-refractory MBC, Lapatinib can consider to be used with an acceptable adverse event profile (54).

Estrogen receptor (ER) and progesterone receptor (PR) status

ER and PR are weak prognostic factors. In the luminal B HER2 negative group, ER- or PR-negative subgroup indicated the worse prognosis than ER- and PR-positive subgroup so the negativity of ER or PR could be considered as prognostic marker in luminal B HER2-negative subtype of breast cancer (55). They are mainly

treatment predictive factors. Receptor-positive tumors are more sensitive to endocrine treatment. Breast cancer patients with ER- or PR-positive subgroup could have the long-term benefit from adjuvant tamoxifen (56).

2.5 Genetic Polymorphisms

Recently pharmacogenomic studies have revealed that the genetic variation can influence on chemotherapy response, therefore, giving a scientific basis to optimize the chemotherapy treatment for individual patient with breast cancer (19, 57-60).

Glutathione S-transferases gene (*GSTs*) are the member of a superfamily of ubiquitous, multifunctional dimeric cytosolic enzymes which function by conjugating reactive intermediates with glutathione to produce less reactive water-soluble compounds which is the process of human biotransformation Phase II pathway and can protect cell damage against a wide array of xenobiotic, chemotherapeutic agents and carcinogens as show in figure 1 (61, 62).

In human, GST enzymes have been identified and characterized into eight classes based on the homology of sequences and the specificity of substrate. The eight classes compose of alpha (α), mu (μ), omega (ω), pi (π), sigma (σ), theta (θ), zeta (ς) and kappa K (κ). Each classes is encoded by a separate gene or gene family (respectively are GSTA, GSTM, GSTO, GSTP, GSTS, GSTT, GSTZ and GSTK genes) (62).

There are several studies focus on GSTT1, GSTP1, and GSTM1 showed the potential of those genetic polymorphisms to benefit for various diseases (63) including breast cancer. It can imply that there is not only an relation between GST polymorphism and the breast cancer risk but also the relation between GST polymorphism and chemotherapeutic drugs response. GSTs polymorphism may also affect to breast cancer risk in certain ethnic groups (28, 64, 65).

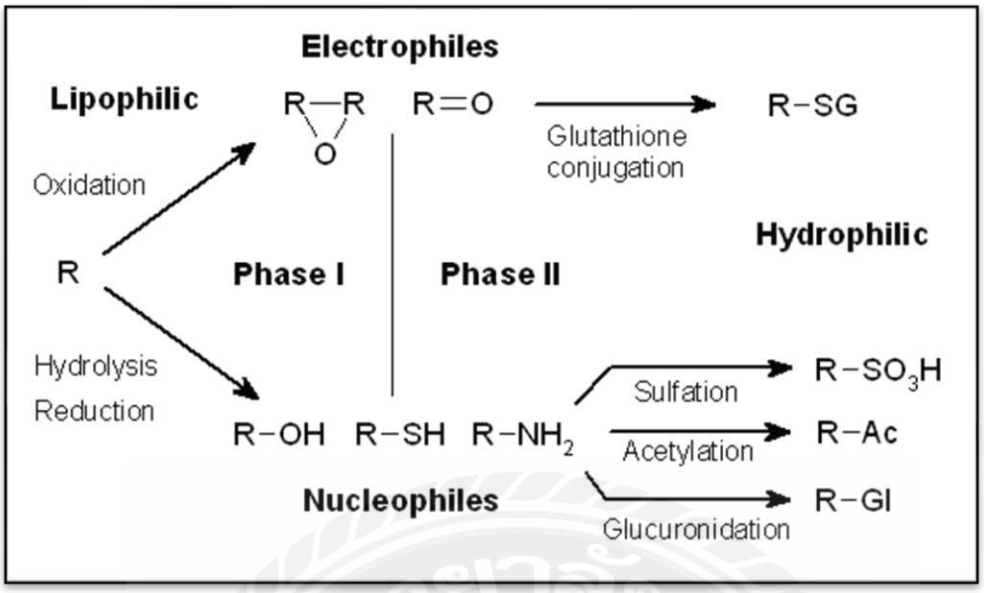


Figure 2.1 Phase I and phase II xenobiotic metabolism

Source: https://en.m.wikipedia.org/wiki/File:Xenobiotic_metabolism.png

Glutathione-S-transferase T1

GSTT1 gene is a haplotype-specific and placed at chromosome 22q11.2 which translate to enzyme called glutathione S-transferase theta 1 (*GSTT1*). It is also a member of a superfamily of GST that catalyze the conjugation reaction to transform glutathione to electrophilic and hydrophobic compounds. There are many subtypes of *GSTT* such as *GSTT1, GSTT2, and GSTT2B.* Both *GSTT1* and *GSTT2/GSTT2B* subtypes share more than 50% of amino acid sequence identity which involve in human carcinogenesis. The *GSTT1* gene is absent from 38% of the population and there are several transcript variants happen after having the alternative splicing of *GSTT1*. GSTT1 gene is a polymorphic and shows two alleles, *GSTT1*1* wild type and the *GSTT1*0* mutant gene or null genotype. *GSTT1* null gene is a non- functional allele because of null genotype or wild type cannot synthesis the *GSTT1* enzyme (66). The frequency of GSTT1 null genotypes is vary across country in in Asians (35-48%) Europeans (13.31%) and Africans (14-57%) (67-70).

Glutathione-S-transferase M1

The *GSTM1* gene is placed at chromosome 1p13.3. Interestingly, the fully gene is homozygous deleted (null polymorphism) cause the complete absence of the

GSTM1 enzyme activity varying in the different populations. There are three polymorphisms; *GSTM1*0* or null genotype is a deletion variant that results in a lack of functional enzyme and the other two polymorphisms are *GSTM1a* and *GSTM1b* diverge by a Cytosine → Guanine substitution at base position 534, resulting in a Lysine → Asparagine by replacement at amino acid 172 position. With regard to vary ethnicities, a wide range of variation in *GSTM1* null polymorphism are vary globally approximately 20-67% (61). The *GSTM1* null alleles distribute mostly in Asians (41-63%) and Europeans (42-60%) followed by Africans (16-36%)(68-70). Therefore the absent of functional *GSTM1* enzyme, the null allele cannot function efficiently to the biotransformation reaction and the succeeding elimination of toxic metabolites by bile and urine; subsequently, the accumulation of toxic products in cells will lead to carcinogenesis (28,71).

2.6 *GSTM1*, and *GSTT1* and Chemotherapy

Several evidences have revealed that drug-metabolizing enzymes function an crucial part in therapeutic response variations among in interindividual breast cancer patients (72). The glutathione S-transferases (GSTs) are a superfamily of enzyme that play a key role in detoxifying by catalyzing the reduction reaction of chemotherapeutic drugs or their metabolites by the way of their conjugation with glutathione. Therefore the GSTT1 and GSTM1 genetic polymorphisms could decrease the effectiveness of the detoxification of xenobiotics and cytotoxic agents generated by chemotherapeutic drugs in the breast cancer treatment (22). Many research have showed the relation between GSTT1 and GSTM1 and overall survival (OS) of patients with breast cancer, but they have produced inconsistent results (30, 31, 73). For instance, in China, the study aimed to determine the function of *GSTM1* polymorphism, *GSTT1* polymorphism and *GSTP1* IIe105Val polymorphism in the clinical outcome to chemotherapy and survival of breast cancer patients found that there is no significant relation of the *GSTT1* and *GSTP1* polymorphisms and chemotherapy response and OS in breast cancer patients by using the Cox proportional hazard model. The hazard ratio for overall survival (OS) in patients with breast cancer harboring the *GSTM1* null genotype was 0.57, 95%CI 0.32-0.98 by the non-null genotype as the reference factors (74).

2.7 Setting

The National Cancer Institute of Thailand (NCI) is under the Department of Medical Services under the Ministry of Public Health. NCI is accountable for technology in cancer treatment include examine, investigate, analyze, develop and expand the cancer therapeutic agents. Moreover, provide the training for healthcare professionals. Moreover, this setting is also function for diagnostic and treatment services of all cancer types that will help to do research and gain the knowledge for coping cancer burden in Thailand. In addition, NCI provides the monograph "Cancer in Thailand", which contains the available up-to-date data on incidence and distribution of different cancers starting from the first volume in 1993. Therefore, an up-to-date picture of the cancer situation in Thailand will be useful for planning and monitoring cancer control strategies as well as for different areas of cancer research in Thailand.

NCI is located on Bangkok, center of Thailand, NCI provides many services as follows:

1. Screening cancer risk and cancer in early stage by health check- up service.
2. Diagnosis of curious cancer symptoms for instances;
    - Abnormality in excretion including bleeding or black feces
    - Difficult to swallow, colic, or abdominal distension
    - Gruffness or chronic cough
    - Abnormal leukorrhoea, such as smelling or bleeding
    - Chronic wound
    - Abnormal in wart or mole
    - Cyst in the body part
    - Other curious symptoms
3. Diagnostic of additional cancer for instance the gastrointestinal endoscopy and ultrasound.
4. Monitor the prospective cancer patients for instance; patients with Hepatitis B Virus, patients with Human Papillomavirus, patients with abnormal changes in cervical cells.
5. Treatment of cancer
6. Hospitalization during and after treatments for instance;

- Pain Suppression Clinic
- Nutrition Clinic
- Rehabilitation

In this research, the association between *GSTM1* and *GSTT1* polymorphisms and hematotoxicity and survival was determined among Thai patients with breast cancer. This study result provided the epidemiological and pharmacogenetic information to future research and policy makers to develop the treatment guideline and prognostic of hematotoxicity and survival of chemotherapy.

CHAPTER III
MATERIALS AND METHODS

To investigate the prognostic role of *GSTM1*, *GSTT1* polymorphism in the hematotoxicity and survival among Thai patients treated with chemotherapy. In this study, the retrospective study was conducted to evaluate the hematotoxicity and survival among breast cancer patients.

Therefore, the processes were composed of three parts to find the appropriate information as follows:

Part 1: Study population

Part 2: Data collection

Part 3: Data analysis

In addition, this chapter provide information of study population, inclusion-exclusion criteria, genotyping protocol, and ethical consideration as well.

Part 1: Study Population

To evaluation the hematotoxicity and survival among breast cancer patients with chemotherapy, the retrospective cohort study was conducted based on data collected from hospital-based cancer registry of the National Cancer Institute, Thailand. The registry did regular follow-up of patients by 2 methods: 1) passive follow up by patient revisit to the hospitals they were treated; 2) checking against the death registry of the Ministry of Interior.

According to the 638 DNA available samples in this study, the total participants recruited in this study are 198 patients with breast cancer at the National Cancer Institute (NCI). We recruited all patients who met in inclusion and exclusion criteria which more than the sample size that was calculated in Appendix A. The participants were divided in 2 groups; 56 participants for evaluating hematotoxicity of chemotherapy and 198 participants for evaluating survival as show in Figure 2. All

patients were treated at NCI. The chemotherapy includes anthracycline-based chemotherapy consists of cyclophosphamide and doxorubicin.

This study was approved by the Ethical Review Committee of Human Research, Faculty of Public Health, Mahidol University (protocol number 179/2560) as show in Appendix B and the Research Committee of National Cancer Institute (project number 195_2017T_OUT525) as show in Appendix C.

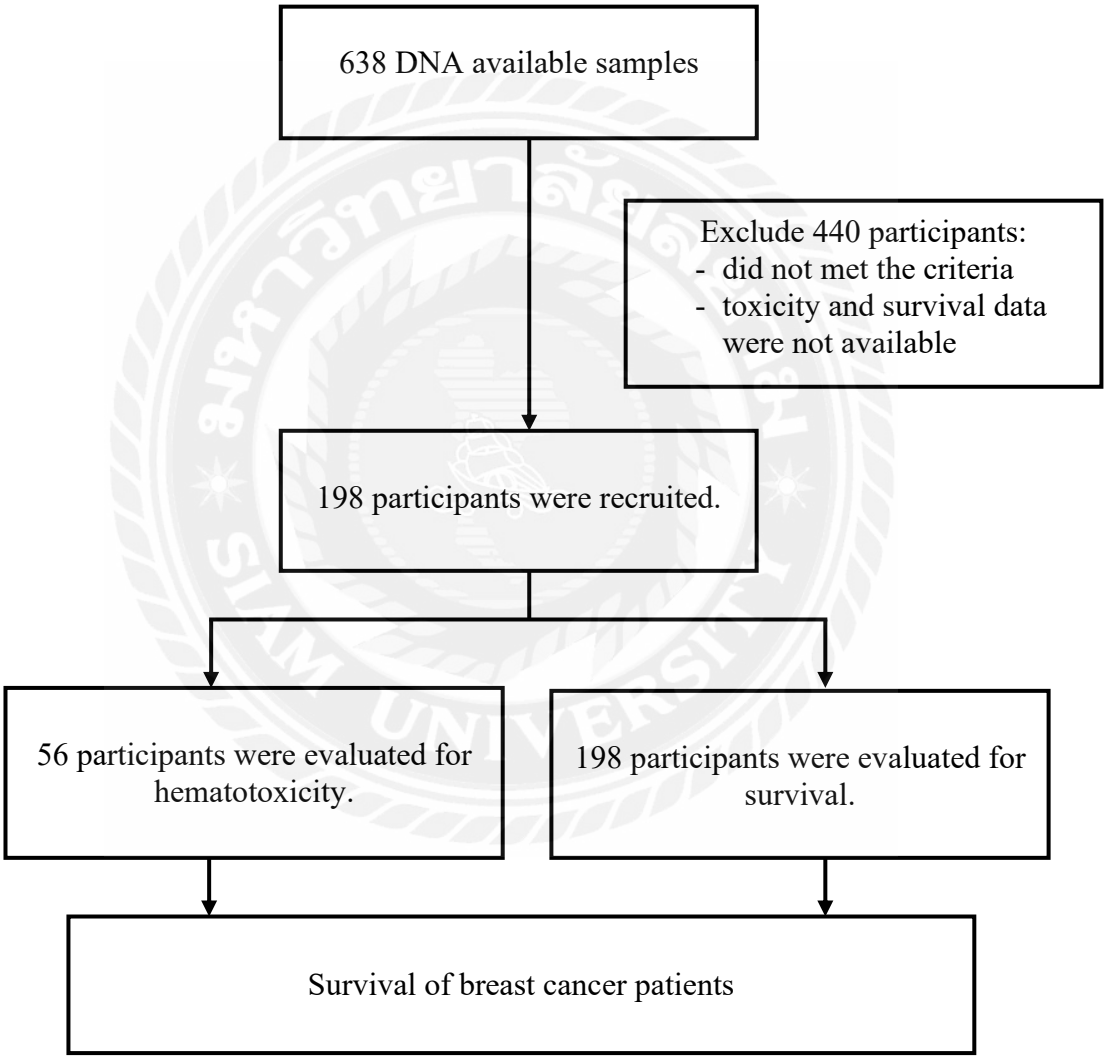


Figure 3.1 Flowchart indicating the overall study plan for hematotoxicity and survival study

Study population for hematotoxicity study:

Breast cancer patients who were admitted and registered in NCI during January 1st, 2010 to December 31st, 2011.

Inclusion criteria:

1. Women with age 18 years or older
2. Newly diagnosis of breast cancer and histologically confirmed

Exclusion criteria:

1. Previously diagnosed of other cancers
2. Any serious concomitant systemic disorder
3. Hematotoxicity data did not available
4. Tissue or blood for genotyping are not available

Study population for survival study:

Breast cancer patients who were admitted and registered in NCI during January 1st, 2010 to December 31st, 2011.

Inclusion criteria:

1. Women with age 18 years or older
2. Newly diagnosis of breast cancer and histologically confirmed

Exclusion criteria:

1. Previously diagnosed of other cancers
2. Any serious concomitant systemic disorder
3. Tissue or blood for genotyping are not available

Part 2: Data Collection

Secondary data were collected. Demographic data and clinicopathology characteristics such as tumor size, tumor grade, tumor stage, HER2 receptor, Ki-67 status, p53 status and hormone receptors were collected from hospital base registry data and medical records.

Hematotoxicity

Hematotoxicity was assessed and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 3.0. Adverse events attribute to chemotherapy were recorded; consist of,

- Hematologic toxicities: anemia, leukopenia, neutropenia, and thrombocytopenia

All patients were treated with combination chemotherapy containing an anthracycline drug; doxorubicin, along with cyclophosphamide. The highest-grade hematotoxicity that occurs during the course of treatment of an individual patient was used for the analysis.

Survival

Date of death was confirmed by hospital records and the national death registry. Other medical data were available in cancer registry database. In addition, genetic polymorphism data were obtained by real-time PCR after DNA extraction from collected participant's blood or tissue.

Part 3: Data Analysis

Descriptive statistics of patients were presented as mean and standard deviations for continuous measures, whereas frequencies were used for categorical measures. The power of the study is set at 80 %. The statistical significance of differences in genotype frequencies between participants were estimated by the Chi-square ($\chi 2$) test. Binary logistic regression was used for all analysis variables to estimate risk as odds ratios (ORs) with 95 % confidence intervals (95 % CIs). ORs were adjusted for confounding variables like age, tumor stage, tumor grade, hormone receptor and HER2 status.

In calculating survival, cumulative 5-year survival rates were calculated starting from the date of diagnosis. Survival time was determined from cancer diagnosis to the end of follow-up, with vital status of alive or dead. Cases whose vital status was unknown at 5 years after diagnosis was assumed to be alive as of the last known date of living. Survival estimated was determined by Kaplan Meier method and differences in survival was compared by the log-rank test. A Cox regression model was used to

calculate the hazards ratio of death, take into account the genetic polymorphism of *GSTM1*, and *GSTT1* and other factors.

All the tests were set at significance level of 95%. All statistical analysis was performed using SPSS version 10 (2007).

Genotyping Protocol

Genetic polymorphism data were obtained by real time PCR after DNA extraction from collected participant's buffy coat or tissue.

GSTM1 and GSTT1 genotyping by multiplex qualitative real-time PCR method

The extracted DNA from buffy coat or paraffin embedded samples collected from 198 breast cancer patients were kept at -80ºC prior to analysis. A multiplex qualitative real-time PCR method was used to detect the presence or absence of the GSTM1 and GSTT1 gene in the genomic DNA samples of the participants. The assay was performed in the StepOnePlus Real-Time PCR System (Applied Biosystems, U.S.A.). The Express SYBR Greener qPCR Super Mix Universal (Invitrogen, U.S.A.) was used as the master mix. All primers were ordered from Macrogen, Korea. Determination of the null GSTM1 polymorphism was performed using the following primers 5'- GAA CTC CCT GAA AAG CTA AAG C-3' and 5'- GTT GGG CTC AAA TAT ACG GTG G-3', for GSTT1, using primers 5'-TCT CCT TAC TGG TCC TCA CAT CTC-3', 5'-TCA CCG GAT CAT GGC CAG CA-3'. The internal control was performed by the human β-globin using primers 5'-AAC TTC ATC CAC GTT CAC C-3' and 5'-GAA GAG CCA AGG ACA GGT AC-3'. The protocol was slightly modified from previous study (14), briefly, the reaction mixture (10 µl) was incubated at 95˚C for 10 min prior to the PCR for 40 cycles at 95˚C for 10 sec, 58 ˚C for 5 sec and at 72˚C for 10 sec. The amplicons were identified using melting curve analysis, by increasing the temperature of the reaction mixtures up to 95°C at a rate of 0.1°C/sec, starting at 68°C for 15 sec. The fluorescence signal of SYBR green in each reaction was measured at a wavelength of 530 nm. Later, the melting curves were converted to display the first negative derivative (−dF/dT) versus the temperature. The amplicons of GSTM1, GSTT1 and β-globin had melting points of 82.5 and 87.5˚C, respectively (Figure 2). DNA of breast

cancer patient who has GSTM1+ /GSTT1+ was used as the positive control and DDW (UltraPure, Invitrogen, U.S.A.) was used as the negative control.

Ethical Consideration

For ethical consideration, this study protocol was submitted and obtained an approval from the Ethical Review Committee of Human Research, Faculty of Public Health, Mahidol University (protocol number 179/2560) as show in Appendix A and the Research Committee of the National Cancer Institute, Bangkok (project number 195_2017T_OUT525) as show in Appendix B. The approval had to be obtained in order to protect the rights, comfort, and well-being of the participants as research subjects before data collection begin.

CHAPTER IV
RESULTS

This chapter presented the results into 2 aspects as follows:

4.1 Association between hematotoxicity of anthracycline based chemotherapy and *GSTM1* and *GSTT1* polymorphisms

4.1.1 The distribution for general and clinicopathological characteristics of hematotoxicity data

4.1.2 The distribution of *GSTM1* and *GSTT1* polymorphisms of hematotoxicity data

4.1.3 Prevalence of hematotoxicity of anthracycline based chemotherapy

4.1.4 Association between *GSTM1* and *GSTT1* polymorphisms and hematotoxicity data

4.2 Association between survival of patients with breast cancer and *GSTM1* and *GSTT1* polymorphisms

4.2.1 The distribution for general and clinicopathological characteristics of survival data

4.2.2 The distribution of *GSTM1* and *GSTT1* polymorphisms of survival data

4.2.3 The association between GSTM1 and GSTT1 polymorphisms and clinicopathological characteristics

1) The association between *GSTM1* polymorphisms and clinicopathological characteristics

2) The association between *GSTT1* polymorphisms and clinicopathological characteristics

4.2.4 Association between overall survival and clinicopathological characteristics among invasive ductal carcinoma

4.2.5 The Kaplan-Meier curve of overall survival by clinicopathological characteristics

4.2.6 The association between survival and *GSTM1* and *GSTT1* polymorphisms

4.2.7 The Kaplan-Meier curve of overall survival by *GSTM1* and *GSTT1* polymorphisms

4.1 Association between hematotoxicity of anthracycline based chemotherapy and *GSTM1* and *GSTT1* polymorphisms

4.1.1 The distribution for general and clinicopathological characteristics of hematotoxicity data

Among the total participants in this study, there were 56 patients with breast cancer with primary invasive ductal carcinoma who had the completed data available for hematotoxicity evaluation as show in figure 3. The distribution of general and clinicopathological characteristics of 56 patients was shown in Table 4.1.

According to the general and clinicopathological characteristics of participants at diagnosis, there was 46.40% of participants who were above 50 years old, and age between 27 to 75 years old with a median age of 48.50 years. With regards to the tumor size, 40.90% of participants had a tumor with diameter 2.5 cm or less, while 59.10% of participants had a tumor size larger than 2.5 cm. The result showed that 62.50% of participants had lymph node metastasis. As regard to the tumor grade, there were 5.40 %, 33.90 % and 60.70% of participants who had tumor grade I, II, and III respectively. For tumor stage, mostly of participants were in stage I and II (67.90 %).

About hormone receptors status, participants had 58.90% estrogen receptor status positive and 36.40% progesterone receptor status positive. With regards to HER-2 receptor status, there are 12.70% of participants who had HER-2 receptor positive. For the proliferation marker Ki-67 status and tumor suppressor p53 status in this study, there were 77.40% and 83.00% respectively.

There were 4 groups with respect to treatment method, in this study, composed of participants who treat with chemotherapy alone, chemotherapy with hormone, chemotherapy with radiation, and chemotherapy with hormone and radiation which were 37.50%, 28.57%, 21.43% and 12.50%, respectively.

Table 4.1 General and clinicopathological characteristics of hematotoxicity data (N = 56)

Characteristics	No. of patients	Percent
All patients		
Median age = 48.50 Range = 27 - 75	56	100.00
Age		
< 50 years	30	53.60
≥ 50 years	26	46.40
Tumor size		
≤ 2.5 cm	18	40.90
>2.5 cm	26	59.10
LN metastasis		
None	21	37.50
Present	35	62.50
Tumor Grade		
I	3	5.40
II	19	33.90
III	34	60.70
Tumor stage		
I - II	38	67.90
III - IV	18	32.10
Estrogen receptor		
Positive	33	58.90
Negative	23	41.10
Progesterone receptor		
Positive	20	36.40
Negative	35	63.60

Table 4.1 General and clinicopathological characteristics of hematotoxicity data (N = 56) (continued)

Characteristics	No. of patients	Percent
HER2 receptor		
Positive	7	12.70
Negative	48	87.30
Ki-67		
Positive	41	77.40
Negative	12	22.60
P53		
Positive	39	83.00
Negative	8	17.00

4.1.2 The distribution of *GSTM1* and *GSTT1* polymorphisms of hematotoxicity data

The distribution of *GSTM1* and *GSTT1* polymorphisms

From peripheral blood leucocytes, the extracted DNA collected from 56 breast cancer patients particularly invasive ductal carcinoma were performed in the StepOnePlus™ Real-Time PCR model (Applied Biosystems, U.S.A.). The median age of participants in this study was 48.50 years. Genotypes and allele distributions of *GSTM1* and *GSTT1* polymorphisms in patients with breast cancer are summarized as show in Table 4.2. The frequency of the *GSTM1* and *GSTT1* null genotype in breast cancer patients was 71.43% and 30.36%, respectively observed in this study.

Table 4.2 Genotype and allele frequencies of glutathione S-transferase M1 and T1 genes polymorphisms in breast cancer patients for hematotoxicity data (N = 56)

Genotype	Frequencies	N (%)
GSTM1	Present	16 (28.57)
	Null	40 (71.43)
GSTT1	Present	39 (69.94)
	Null	17 (30.36)

4.1.3 Prevalence of hematotoxicity of anthracycline based chemotherapy

In this study, all treatment regimen was anthracycline based chemotherapy as follows: cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2 gave to all patients every 21 days, for 4 cycles. Prior each chemotherapy cycle was administered, physical examination and a full blood counts were collected. Moreover, renal function and hepatic function tests were examined before administered the first cycle of treatment as the baseline data as show in appendix A. Particular patients with breast cancer who had obtained complete course of chemotherapy were evaluated for hematotoxicity. Hematotoxicity was scored every 3 weeks based on the Common Terminology Criteria for Adverse Events v3.0 (CTCAE).

Respect with all hematotoxicity events that occurred this study, the prevalence and grade of hematotoxicity were summarized in Table 4.3. There were total 214 hematotoxicities (all grade) occurred in this study composed of 115 anemia, 79 leucopenia, 14 neutropenia and 6 thrombocytopenia.

For anemia, grade 1-2 and grade 3-4 toxicity were 110 and 5 events respectively and no toxicity was 109 events.

For leucopenia, grade 1-2 and grade 3-4 toxicity were 79 and 0 events respectively and no toxicity was 145 events.

For neutropenia, grade 1-2 and grade 3-4 toxicity were 13 and 1 events respectively and no toxicity was 210 events.

For thrombocytopenia, grade 1-2 and grade 3-4 toxicity were 6 and 0 events respectively and no toxicity was 218 events.

Table 4.3 Prevalence and grade of all hematotoxicity events (N = 224)*

Hematotoxicity N (%)	**Total**			
	No toxicity	**Grades 1-2**	**Grades 3-5**	**All grades**
Anemia	109 (48.67)	110 (49.11)	5 (2.23)	115 (51.34)
Leucopenia	145 (64.73)	79 (35.27)	0 (0.00)	79 (35.27)
Neutropenia	210 (93.75)	13 (5.80)	1 (0.45)	14 (6.25)
Thrombocytopenia	218 (97.32)	6 (2.68)	0 (0.00)	6 (2.68)

*based on the NCI- CTCAE Version 3.0

Respect with the highest-grade hematotoxicity events that occurs while individual patients were administer the course of treatment.

The result showed that all patients were evaluated for the hematotoxicity and were summarized in Table 4.4. Among patients who developed hematotoxicity, the prevalence of any grade was as follows: 38 anemia (67.90%), 38 leucopenia (67.90%), 10 neutropenia (17.90%) and 3 thrombocytopenia (5.40%).

According to anemia, the most patients suffered grade1 toxicity (53.6%) follow by grade 2 (8.9%) and grade 3 (5.4%); likewise, leucopenia, the most patients experienced grade1 toxicity (58.90%) follow by grade 2 (8.9%).

In contrast to neutropenia, the most patients suffered grade 2 toxicity (12.50%) follow by grade 1 (3.60%) and grade 3 (1.80%). In this study, few patients experienced only grade 1 thrombocytopenia (5.40%); furthermore, grade 4 hematotoxicity and toxic deaths were not observed in this study.

Table 4.4 Prevalence and the highest-grade hematotoxicity events in an individual patient (N = 56)*

Hematotoxicity N (%)	**Toxicity grade**					
	No toxicity	**1**	**2**	**3**	**4**	**5**
Anemia	18 (32.10)	30 (53.60)	5 (8.90)	3 (5.40)	0	0
Leucopenia	18 (32.10)	33 (58.90)	5 (8.90)	0	0	0
Neutropenia	46 (82.10)	2 (3.60)	7 (12.50)	1 (1.80)	0	0
Thrombocytopenia	53 (94.60)	3 (5.40)	0	0	0	0

*based on the NCI- CTCAE Version 3.0

4.1.4 Association between *GSTM1* and *GSTT1* polymorphisms and hematotoxicity data

Associations between *GSTM1* and *GSTT1* polymorphisms and hematotoxicity were reported as show in Table 4.5-4.8. There was no significant relation between *GSTM1* and *GSTT1* polymorphisms and hematotoxicity including anemia, leucopenia, neutropenia and thrombocytopenia.

In table 4.5, patients with breast cancer with *GSTM1* present and *GSTM1* null genotype had the same probability of developing anemia in any grade (OR = 1.06, 95% CI: 0.30-3.69, p = 0.928). On the other hand, patients with breast cancer with *GSTT1* present genotype had a lower developing anemia risk than *GSTT1* null genotype although there was no statistical significance difference (OR = 0.55, 95% CI: 0.15-2.01, p = 0.362).

In table 4.6, patients with breast cancer with *GSTM1* present and *GSTM1* null genotype had the same developing leucopenia risk in any grade (OR = 1.06, 95% CI: 0.30-3.69, p = 0.928). On the other hand, patients with breast cancer with *GSTT1* present genotype had a higher developing leucopenia risk than *GSTT1* null genotype although there was no statistical significance difference (OR = 1.78, 95% CI: 0.54-5.87, p = 0.339).

In table 4.7, patients with breast cancer with *GSTM1* present genotype had a higher developing neutropenia risk than *GSTM1* null genotype (OR = 3.18, 95% CI: 0.78-13.07, p = 0.129). Similarly, patients with breast cancer with *GSTT1* present genotype had a higher developing neutropenia risk than *GSTT1* null genotype although there was no statistical significance difference (OR = 4.80, 95% CI: 0.56-41.34, p = 0.253).

In table 4.8, patients with breast cancer with *GSTM1* present genotype had a higher developing thrombocytopenia risk than *GSTM1* null genotype (OR = 5.57, 95% CI: 0.47-66.33, p = 0.193).

Table 4.5 Association among *GSTM1, GSTT1* polymorphisms and risk of anemia of any grade (grade 1-5 vs no toxicity) (N = 56)

Genotype	Anemia				
	No toxicity	**Toxicity**	**OR**	**95% CI**	**P value[a]**
GSTM1					
Null	13	27	reference		
Present	5	11	1.059	0.304-3.687	0.928
GSTT1					
Null	4	13	reference		
Present	14	25	0.549	0.150-2.011	0.362

[a]P value from Pearson Chi-Square

Table 4.6 Association among *GSTM1, GSTT1* polymorphisms and risk of leucopenia of any grade (grade 1-5 vs no toxicity) (N = 56)

Genotype	Leucopenia				
	No toxicity	**Toxicity**	**OR**	**95% CI**	**P value[a]**
GSTM1					
Null	13	27	reference		
Present	5	11	1.059	0.304-3.687	0.928
GSTT1					
Null	7	10	reference		
Present	11	28	1.782	0.541-5.865	0.339

[a]P value from Pearson Chi-Square

Table 4.7 Association among *GSTM1, GSTT1* polymorphisms and risk of neutropenia of any grade (grade 1-5 vs no toxicity) (N = 56)

Genotype	Neutropenia				
	No toxicity	**Toxicity**	**OR**	**95% CI**	**P value[a]**
GSTM1					
Null	35	5	reference		
Present	11	5	3.182	0.775-13.067	0.129
GSTT1					
Null	16	1	reference		
Present	30	9	4.800	0.557-41.341	0.253

[a]P value from Fisher's Exact test

Table 4.8 Association among *GSTM1, GSTT1* polymorphisms and risk of thrombocytopenia of any grade (grade 1-5 vs no toxicity) (N = 56)

Genotype	Thrombocytopenia				
	No toxicity	**Toxicity**	**OR**	**95% CI**	**P value[a]**
GSTM1					
Null	39	1	reference		
Present	14	2	5.571	0.468-66.325	0.193
GSTT1					
Null	17	0	-	-	-
Present	36	3			

[a]P value from Fisher' Exact test

4.2 Association between survival of breast cancer patients and *GSTM1* and *GSTT1* polymorphisms

4.2.1 The distribution for general and clinicopathological characteristics of survival data

Total participants in this study included 198 patients with breast cancer with primary invasive ductal carcinoma who had admitted at the NCI, Thailand between 2011 and 2013. The distribution of general and clinicopathological characteristics of survival data is shown in Table 4.9. On the day of the censored point which was May 31, 2018, it was found that 28.80%t of the total patients had died. As of May 2013, the median follow-up time was 60.89 months (SD = 19.76) with range 3.50 to 87.40 months.

According to the general and clinicopathological characteristics of participants at diagnosis, there was 57.00% of participants who were above 50 years old, and age between 24 and 84 years old with a median age of 50.50 years (SD = 11.77). With regards to the tumor size, 43.43% of participants had a tumor with diameter 2.5 cm or less, while 56.57% of participants had a tumor size larger than 2.5 cm. The result showed that 55.05% of participants had lymph node metastasis. As regard to the tumor grade, there are 6.06%, 48.48% and 45.46% of participants who had tumor grade I, II, and III respectively. For tumor stage, mostly of participants were in stage II (56.57%) and stage III (34.34%), the number of participants in each stage were shown in table 4.9.

About hormone receptors status, participants had 61.11% estrogen receptor status positive and 47.47% progesterone receptor status positive. Consider with HER-2 receptor status, there are 15.66% of participants who had HER-2 receptor positive. For the proliferation marker Ki-67 status and tumor suppressor p53 status in this study, the frequency of Ki-67 positive and p53 positive were 74.24% and 68.69% respectively.

Table 4.9 General and clinicopathological characteristics of survival data (N = 198)

Characteristics	Number	Percent
Total	198	100.00
Status (at the end of study)		
Dead	57	28.80
Alive	141	71.20
Age at diagnosis (years)		
< 40	33	16.70
40 - 49	52	26.30
50 - 59	64	32.30
≥ 60	49	24.70
Median = 51.50 SD = 11.77		
Range 24 - 84 years		
Tumor size		
≤ 2.5cm	86	43.43
>2.5cm	112	56.57
LN metastasis		
None	89	44.95
Present	109	55.05
Tumor grade		
I	12	6.06
II	96	48.48
III	90	45.46
Tumor stage		
I	18	9.10
II	7	3.50
IIA	68	34.40
IIB	37	18.70
III	4	2.00
IIIA	43	21.70
IIIB	21	10.60
IV	0	0.00

Table 4.9 General and clinicopathological characteristics of survival data (N = 198) (continued)

Characteristics	Number	Percent
Estrogen receptor		
Positive	121	61.11
Negative	70	35.35
Unknow	7	3.54
Progesterone receptor		
Positive	94	47.47
Negative	91	45.96
Unknow	13	6.57
HER-2 receptor		
Positive	31	15.66
Negative	140	70.71
Unknow	27	13.63
Ki-67		
Positive	147	74.24
Negative	24	12.12
Unknow	27	13.64
p53		
Positive	136	68.69
Negative	27	13.64
Unknow	35	17.67

4.2.2 The distribution of *GSTM1* and *GSTT1* polymorphisms of survival data

From peripheral blood leucocytes collected from 198 breast cancer patients particularly invasive ductal, the extracted DNA were analyzed in the StepOnePlus™ Real-Time PCR System (Applied Biosystems, U.S.A.). The median age of participants in current study was 51.50 years. Among breast cancer patients, genotypes and allele distributions of *GSTM1* and *GSTT1* polymorphisms are summarized as show in Table 4.10. The *GSTM1* and *GSTT1* null genotype frequency among breast cancer patients was 65.70% and 33.30%, respectively observed in this current study. The frequency of

GSTM1present/GSTT1present, *GSTM1 present/GSTT1 null*, *GSTM1null/GSTT1present*, and *GSTM1null/GSTT1null* were 22.20%, 12.10%, 44.40%, and 21.20% respectively.

Table 4.10 Genotype and allele frequencies of GSTM1 and GSTT1 genes polymorphisms in patients with breast cancer (N = 198)

Gene	Frequencies	N (%)
GSTM1	Present	68 (34.30)
	Null	130 (65.70)
GSTT1	Present	132 (66.70)
	Null	66 (33.30)
GSTM1 and *GSTT1* combined	*GSTM1+/GSTT1+*	44 (22.20)
	GSTM1+/GSTT1-	24 (12.10)
	GSTM1-/GSTT1+	88 (44.40)
	GSTM1-/GSTT1-	42 (21.20)

4.2.3 The association between *GSTM1* and *GSTT1* polymorphisms and clinicopathological characteristics

1) The association between *GSTM1* polymorphisms and clinicopathological characteristics

Among patients with breast cancer to determine the potential role of *GSTM1* null genotype in the breast cancer development and progression, the clinicopathological parameters of the breast cancer patients with *GSTM1* null genotype and *GSTM1* present genotype were revealed as show in Table 4.11.

The univariate analysis result showed that the association with all characteristics was no statistically significant such as the lymph node metastasis, histopathological grade, progesterone receptor status, estrogen receptor status, HER2 receptor, p53 gene status and Ki-67 status. However, breast cancer patients harboring *GSTM1* null genotype had higher tendency to be in stage III and IV than *GSTM1* present genotype (OR = 1.85, P = 0.059).

Consider with the multivariate analysis, there was significant association between clinical stage of the cancer and the *GSTM1* null genotype after adjusted for progesterone receptor status alone and age at diagnosis alone and simultaneous

progesterone receptor status and age at diagnosis (P = 0.043, 0.047, and 0.037) as show in Table 4.12.

After adjusted for progesterone receptor status and age at diagnosis, the result showed that breast cancer patients with stage III and stage IV had about 2-fold frequency of *GSTM1* null genotype compare with *GSTM1* present genotype (OR = 2.09, P = 0.037). In contrast, there was no statistically significant association according to the other characteristics including the histopathological grade, lymph node metastasis, progesterone receptor status, estrogen receptor status, HER-2 receptor, lymph nodes status, Ki-67 and p53 gene status.

Table 4.11 Association of *GSTM1* polymorphism and clinicopathological characteristics in breast cancer patients (N = 198)

Parameters	***GSTM1***		**Univariate**		
	null	**present**	**OR**	**95% CI**	**P value**
Age					
< 50 years	60	25	ref		
≥ 50 years	70	43	0.678	0.372-1.238	0.205
Tumor size					
≤ 2.5cm	61	32	ref		
>2.5cm	69	36	1.005	0.559-1.810	0.986
LN metastasis					
None	55	34	ref		
Present	75	34	1.364	0.757-2.458	0.301
Tumor grade					
I	7	5	ref		
II	65	31	1.498	0.440-5.097	0.518
III	58	32	1.295	0.380-4.412	0.680
Tumor stage					
I - II	78	50	ref		
III - IV	52	18	1.852	0.974-3.522	0.059

Table 4.11 Association of *GSTM1* polymorphism and clinicopathological characteristics in breast cancer patients (N = 198) (continued)

Parameters	*GSTM1*		Univariate		
	null	present	OR	95% CI	P value
Estrogen receptor					
Positive	79	42	ref		
Negative	48	22	0.862	0.460-1.616	0.643
Progesterone receptor					
Positive	63	31	ref		
Negative	61	30	0.999	0.541-1.845	0.999
HER2 receptor					
Positive	17	15	1.924		
Negative	109	50	ref	0.890-4.158	0.096
Ki-67					
Positive	102	45	1.619		
Negative	14	10	ref	0.669-3.919	0.282
p53					
Positive	88	48	ref		
Negative	19	8	0.772	0.315-1.894	0.571

Table 4.12 Association of *GSTM1* polymorphism and clinicopathological characteristics in breast cancer patients: multivariate analysis (N = 198)

Parameters	Univariate			Multivariate		
	OR	95% CI	P value	OR	95% CI	P value
Stage						
I - II	ref			ref		
III - IV	1.852	0.974-3.522	0.059	1.962	1.023-3.766	0.043 [a]
				2.004	1.010-3.975	0.047 [b]
				2.092	1.047-4.179	0.037 [c]

[a] Adjusted for age at diagnosis

[b] Adjusted for progesterone receptor status

[c] Adjusted for age at diagnosis and progesterone receptor

2) The association between *GSTT1* polymorphism and the clinicopathological characteristics

To examine the potential function of *GSTT1* null genotype in the development and progression of breast cancer, the clinicopathological parameters of the breast cancer patients with *GSTT1* null genotype were compared to the parameters of patients that had breast cancer with *GSTT1* present genotype as show in Table 4.13.

The univariate analysis result revealed that there was statistically significant relation of the *GSTT1* null genotype and the tumor size (OR = 0.51, P = 0.026). Patients with the size of tumor greater than 2.5 cm showed the lower proportion of *GSTT1* null genotype than *GSTT1* present genotype. Furthermore, there was no statistically significant association with the other parameters; for example, the histopathological grade, lymph node metastasis, progesterone receptor status, estrogen receptor status, HER-2 receptor status, lymph nodes status, Ki-67 and p53 gene status.

Table 4.13 Association of *GSTT1* polymorphism and clinicopathological characteristics in breast cancer patients (N = 198)

Parameters	***GSTT1***		**Univariate**		
	null	**present**	**OR**	**95% CI**	***P* value**
Age					
< 50 years	26	59	ref		
≥ 50 years	40	73	1.243	0.382-2.269	0.477
Tumor size					
≤ 2.5cm	36	50	ref		
>2.5cm	30	82	0.508	0.279-0.925	0.026
LN metastasis					
None	32	57	ref		
Present	34	75	0.808	0.446-1.461	0.479
Tumor grade					
Grade 1 well diff	4	8	ref		
Grade 2 mod diff	31	65	1.048	0.293-3.749	0.942
Grade 3 poor diff	31	59	0.952	0.265-3.411	0.939

Table 4.13 Association of *GSTT1* polymorphism and clinicopathological characteristics in breast cancer patients (N = 198) (continued)

Parameters	*GSTT1*		Univariate		
	null	present	OR	95% CI	*P* value
Tumor stage					
I - II	45	85	ref		
III - IV	21	47	0.844	0.450-1.583	0.597
Estrogen receptor					
Positive	42	79	ref		
Negative	21	49	1.241	0.658-2.338	0.505
Progesterone receptor					
Positive	28	66	ref		
Negative	33	58	0.746	0.403-1.379	0.349
HER-2 receptor					
Positive	20	12	1.333	0.596-2.982	0.483
Negative	109	50	ref		
Ki-67					
Positive	48	99	1.455	0.543-3.899	0.455
Negative	6	18	ref		
p53					
Positive	46	90	ref		
Negative	7	20	1.460	0.576-3.705	0.424

4.2.4 Association of overall survival with clinicopathological characteristics among primary invasive ductal carcinoma

Cox's proportional hazard model was employed for checking the effect of all prognosis clinicopathological characteristics on overall survival of breast cancer patients and to calculate the hazard ratios of all characteristics. Associations between overall survival and prognosis clinicopathological characteristics was showed in Table 4.13. Among total, 57 deaths were determined in this study participants of 198 breast cancer patients. The probability of overall survival at 1, 3, 5 years was 0.95, 0.83, 0.71 respectively. After analyzing by the log rank test and Cox's proportional hazards model,

the results showed a significant different in the probability of overall survival at 5 years according to lymph node status and stage of tumor (P = 0.014 and P < 0.001).

Patients without lymph node involvement had better 5 years overall survival probability than patients with lymph node involvement (P = 0.014). The probability of survival at 5 years among patients without lymph node involvement and patients with lymph node status were 0.83 and 0.67, respectively. Moreover, after analyzing with Cox's proportional hazard, patients with lymph node involvement had around 2-fold-higher risk of death compared with patients without lymph node involvement (HR = 2.105, 95% CI = 1.148 - 3.859, P = 0.016) as show in Table 4.14.

According to early-stage tumor (stage I and II) patients had greater overall survival probability at 5 years than patients with advanced-stage tumor (stage III and stage IV) (P < 0.001). The probability of survival at 5 years among patients with early-stage tumor and patients with advanced-stage tumor were 0.80 and 0.56, respectively. Moreover, after analyzing with Cox's proportional hazard, patients with advanced-stage tumor had nearly 3-fold-increased risk of death compared with patients with early-stage tumor (HR = 2.782, 95% CI = 1.587 - 4.875, P < 0.001).

With respect with p53 status, patients with p53 status positive had tendency better overall survival probability at 5 years than patients with p53 status negative although, there was no statistical significance different (P = 0.052). The 5 years probability of survival among patients with p53 status positive and patients with p53 status negative were 0.75 and 0.54, respectively. Moreover, after analyzing with Cox's proportional hazard, patients with p53 status negative had an almost 2-fold-increased risk of death compared with patients with p53 status positive although, there was no statistical significance different (HR = 1.960, 95% CI = 0.981 - 3.913, P = 0.057).

Furthermore, no significant association between survival probability at 5 years and other characteristics such as age at diagnosis, tumor grade, tumor size, progesterone receptor status, estrogen receptor status, HER2 receptor status, Ki-67 and p53 status.

Table 4.14 Overall survival probability at 5 years by clinicopathological characteristics (N = 198)

Characteristics	No. of patients	No. of deaths	Probability of survival at 5 years	P value[a]
All patients	198	57	0.71	-
Age				
< 50 years	74	21	0.72	
≥ 50 years	101	29	0.75	0.830
Tumor size				
≤ 2.5cm	72	15	0.79	
>2.5cm	103	35	0.66	0.142
LN metastasis				
None	77	15	0.83	
Present	98	35	0.67	0.014
Tumor gTrade				
I	10	1	0.90	
II - III	161	49	0.72	0.170
Tumor stage				
I - II	112	22	0.80	
III - IV	63	28	0.56	<0.001
Estrogen receptor				
Positive	111	28	0.75	
Negative	60	21	0.65	0.123
Progesterone receptor	85	23	0.73	
Positive	80	24	0.70	0.561
Negative				
HER-2 receptor				
Positive	28	10	0.64	
Negative	142	38	0.76	0.288

Table 4.14 Overall survival probability at 5 years by clinicopathological characteristics (N = 198) (continued)

Characteristics	No. of patients	No. of deaths	Probability of survival at 5 years	P value[a]
p53				
Positive	119	30	0.75	
Negative	24	11	0.54	0.052
Ki-67				
Positive	130	37	0.72	
Negative	23	5	0.78	0.560

[a] P values from the log rank test

Table 4.15 Unadjusted hazard ratios by clinicopathological characteristics (N = 198)

Characteristics	Unadjusted HR[a] (95% CI)	P value
Age		
< 50 years	reference	
≥ 50 years	1.064 (0.606-1.865)	0.830
Tumor size		
≤ 2.5cm	reference	
>2.5cm	1.769 (0.966-3.240)	0.065
LN metastasis		
None	reference	
Present	2.105 (1.148-3.859)	0.016
Tumor grade		
I	reference	
II - III	3.651 (0.504-26.450)	0.200
Tumor stage		
I - II	reference	
III - IV	2.782 (1.587-4.875)	<0.001
Estrogen receptor		
Positive	reference	
Negative	1.558 (0.883-2.746)	0.126

Table 4.15 Unadjusted hazard ratios by clinicopathological characteristics (N = 198) (continued)

Characteristics	Unadjusted HR[a] (95% CI)	P value
Progesterone receptor		
Positive	Ref.	
Negative	1.185 (0.668-2.103)	0.562
HER-2 receptor		
Positive	1.456 (0.725-2.925)	
Negative	Ref.	0.291
Ki-67		
Positive	1.319 (0.518-3.358)	
Negative	Ref.	0.561
p53		
Positive	Ref.	
Negative	1.960 (0.981-3.913)	0.057

[a] HRs from a Cox' s proportional hazards model

4.2.5 The Kaplan-Meier curve of 5 years overall survival by clinicopathological characteristics

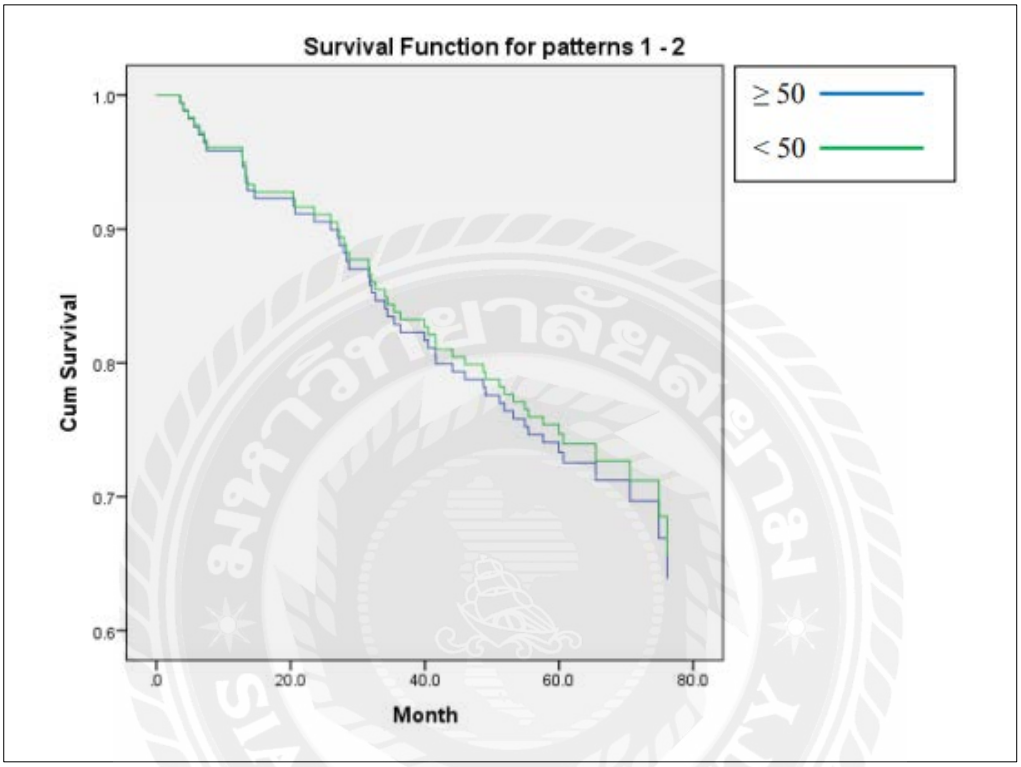


Figure 4.1 Kaplan-Meier curve of 5 years overall survival by age at diagnosis

This figure presents the univariate Kaplan-Maier curves for the dichotomized age (< 50 years and ≥ 50 years). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among patients with breast cancer aged below 50 years and those 50 years and older were 72% and 71% respectively. The Kaplan-Maier curves for breast cancer patients aged below 50 years and those 50 years and older were very close and did not differ significantly (HR = 1.064, p = 0.830).

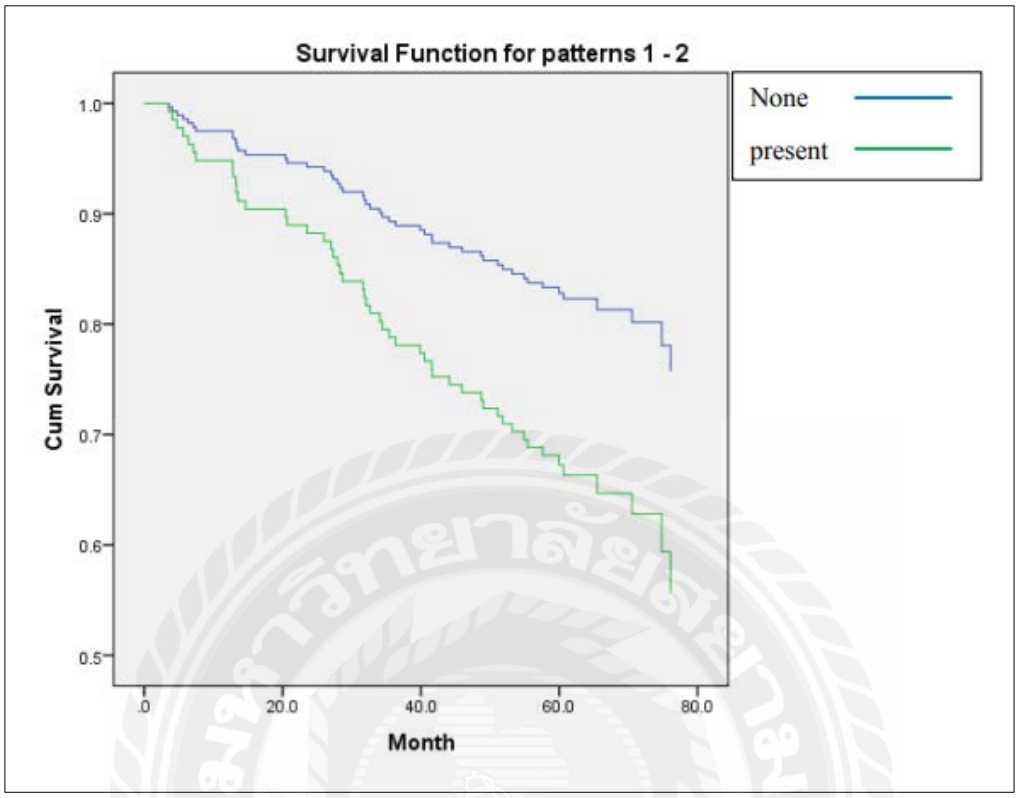


Figure 4.2 Kaplan-Meier curve of 5 years overall survival by lymph node metastasis

This figure presents the univariate Kaplan-Maier curves for the dichotomized lymph node metastasis (none and present). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among breast cancer patients who were none and present lymph node metastasis were 83% and 67% respectively. The Kaplan-Maier curves for breast cancer patients who were none and present lymph node metastasis was significantly different (HR = 2.105, p = 0.016). The result showed that patients without lymph node metastasis had relatively better survival chance compared with patients with lymph node metastasis.

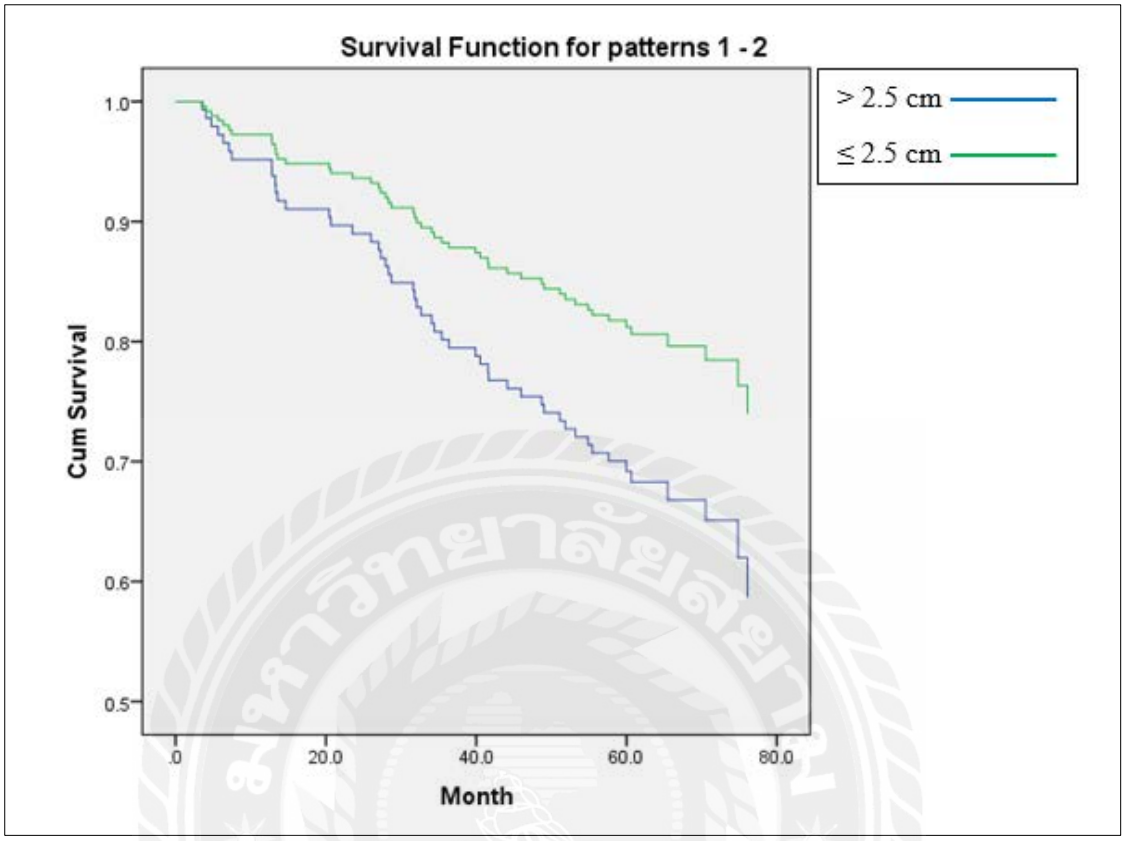


Figure 4.3 Kaplan-Meier curve of 5 years overall survival by tumor size

This figure presents the univariate Kaplan-Maier curves for the dichotomized tumor size (≤ 2.5 cm and > 2.5 cm). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among patients with breast cancer aged below 50 years and those 50 years and older were 79% and 66% respectively. The Kaplan-Maier curves for breast cancer patients who had tumor size ≤ 2.5 cm and > 2.5 cm was significantly different. (HR = 1.769, p = 0.065). The curves showed that patients with tumor size ≤ 2.5 cm had relatively better survival chance compared with patient with tumor size > 2.5 cm.

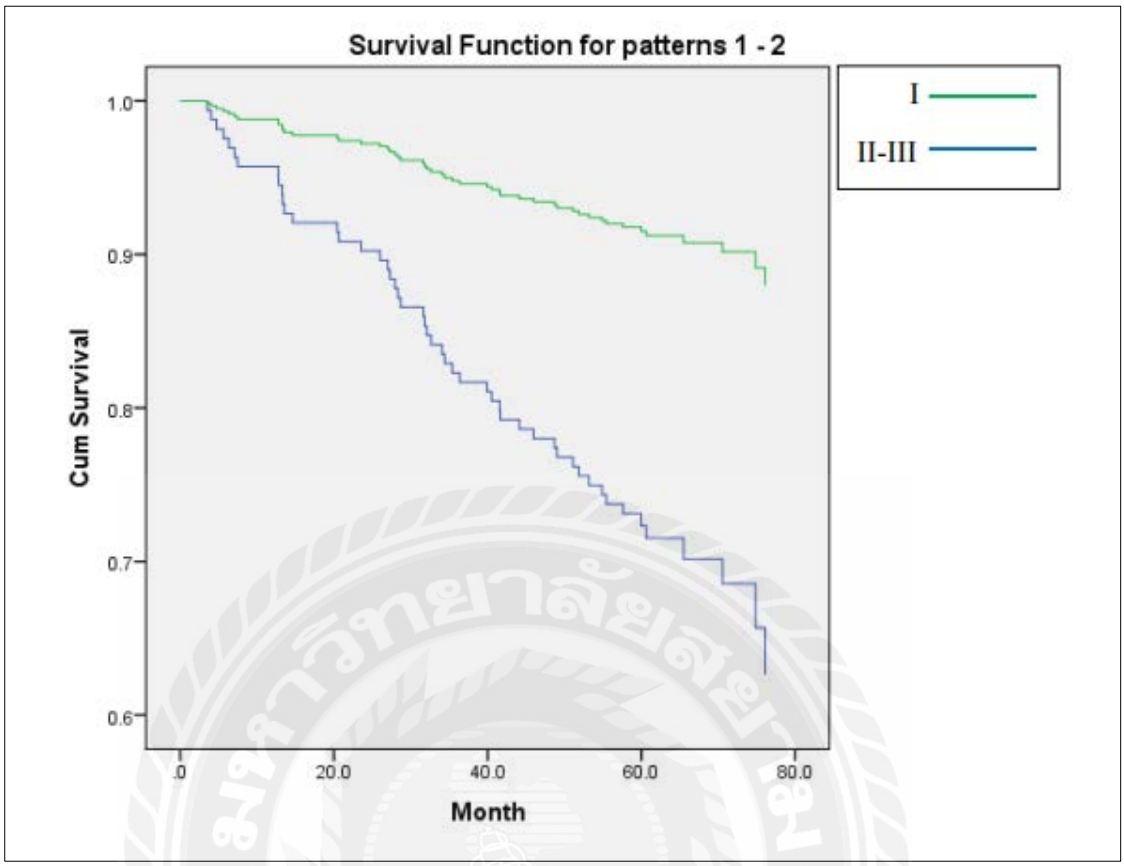


Figure 4.4 Kaplan-Meier curve of 5 years overall survival by tumor grade

This figure presents the univariate Kaplan-Maier curves for the dichotomized tumor grade (I and II - III). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among breast cancer patients with tumor grade I and tumor grade II-III were 90% and 72% respectively. The Kaplan-Maier curves showed that patients with tumor grade I had relatively better survival chance compared with patient with tumor grade II - III but the different did not significantly different (HR = 3.651, p = 0.200).

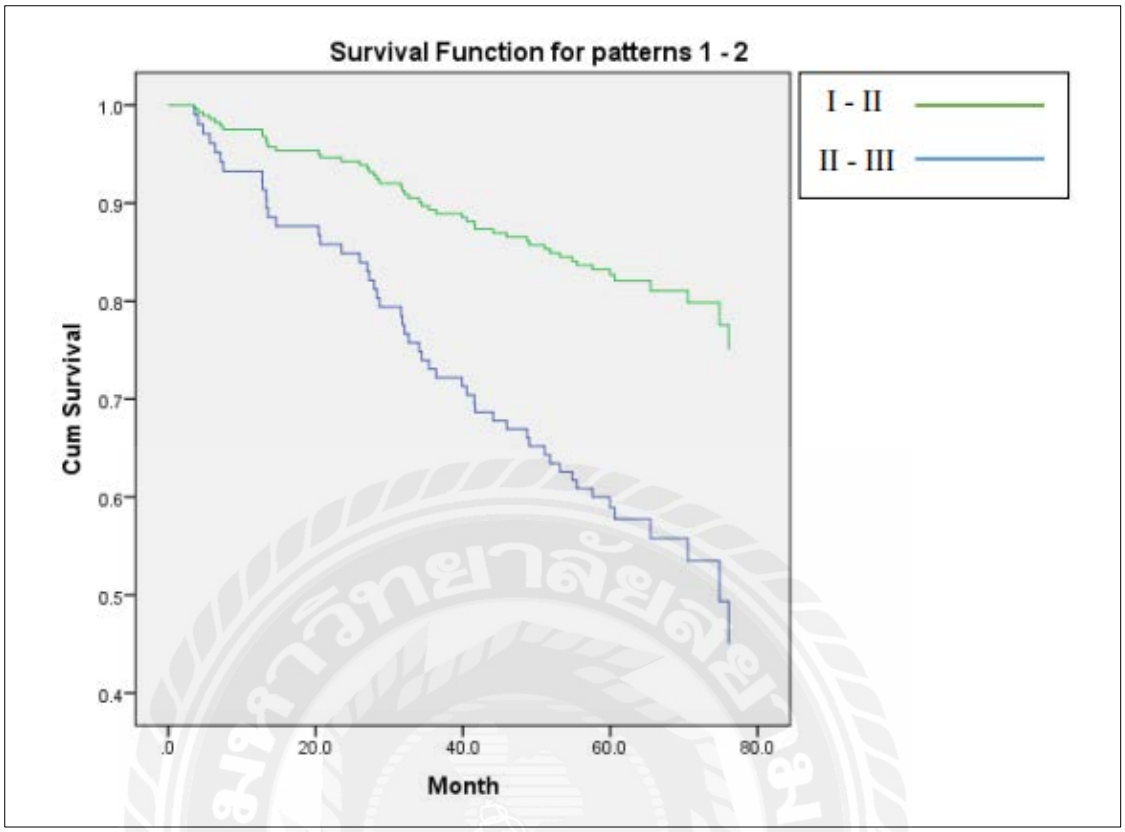


Figure 4.5 Kaplan-Meier curve of 5 years overall survival by tumor stage

This figure presents the univariate Kaplan-Maier curves for the dichotomized tumor stage (early-stage tumor and advance-stage tumor). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among breast cancer patients with tumor stage I - II (early-stage tumor) and stage III - IV (advance-stage tumor) were 80% and 56% respectively. The Kaplan-Maier curves for breast cancer patients who had early-stage tumor and advance-stage tumor was significantly different. (HR = 2.782, $p < 0.001$). The result show that patients with early-stage tumor had relatively better survival chance compared with patient with advance-stage tumor.

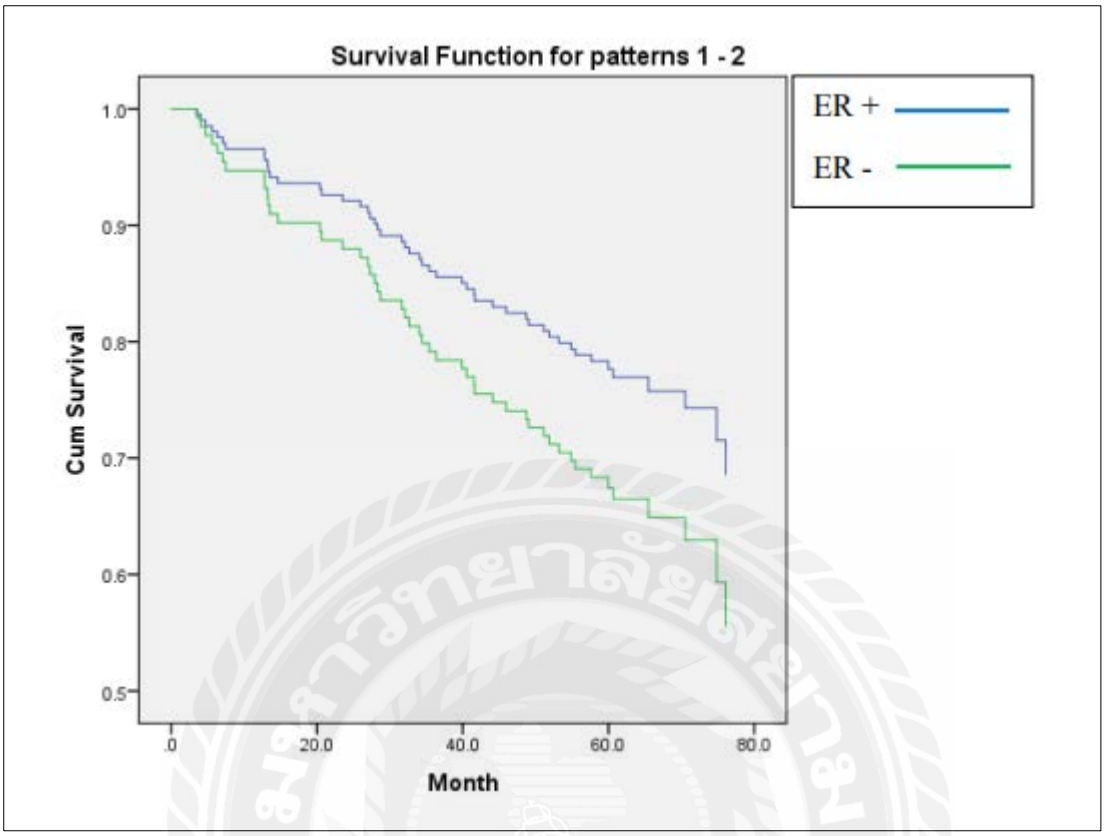


Figure 4.6 Kaplan-Meier curve of 5 years overall survival by estrogen receptor status

This figure presents the univariate Kaplan-Maier curves for the dichotomized estrogen receptor status (positive and negative). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among breast cancer patients with estrogen receptor positive and estrogen receptor tumor negative were 75% and 65% respectively. The Kaplan-Maier curves showed that patients with estrogen receptor positive had relatively better survival chance compared with patient with estrogen receptor negative but the different did not significantly different (HR = 1.558, p = 0.126).

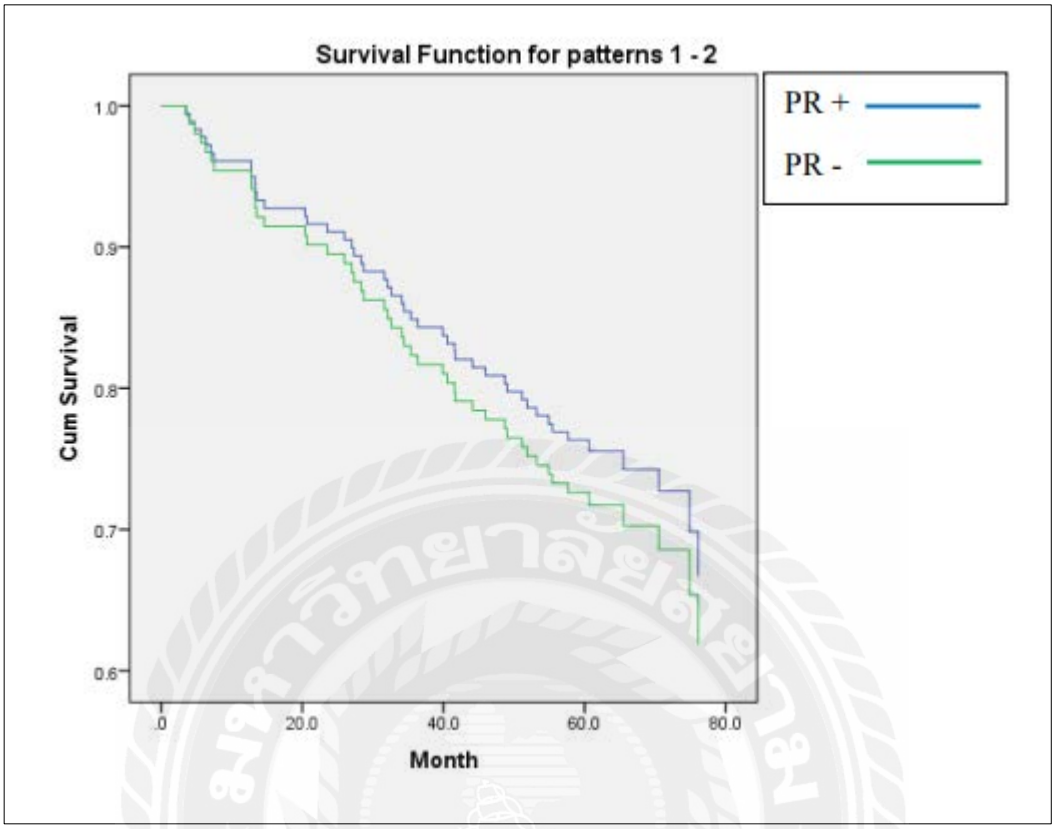


Figure 4.7 Kaplan-Meier curve of 5 years overall survival by progesterone receptor status

This figure presents the univariate Kaplan-Maier curves for the dichotomized progesterone receptor status (positive and negative). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among patients with breast cancer with positive progesterone receptor status and progesterone receptor status negative were 73% and 70% respectively. The Kaplan-Maier curves showed that patients with positive progesterone receptor status had rather same survival chance compared with patient with negative progesterone receptor status but the different did not significantly different (HR = 1.185, p = 0.562).

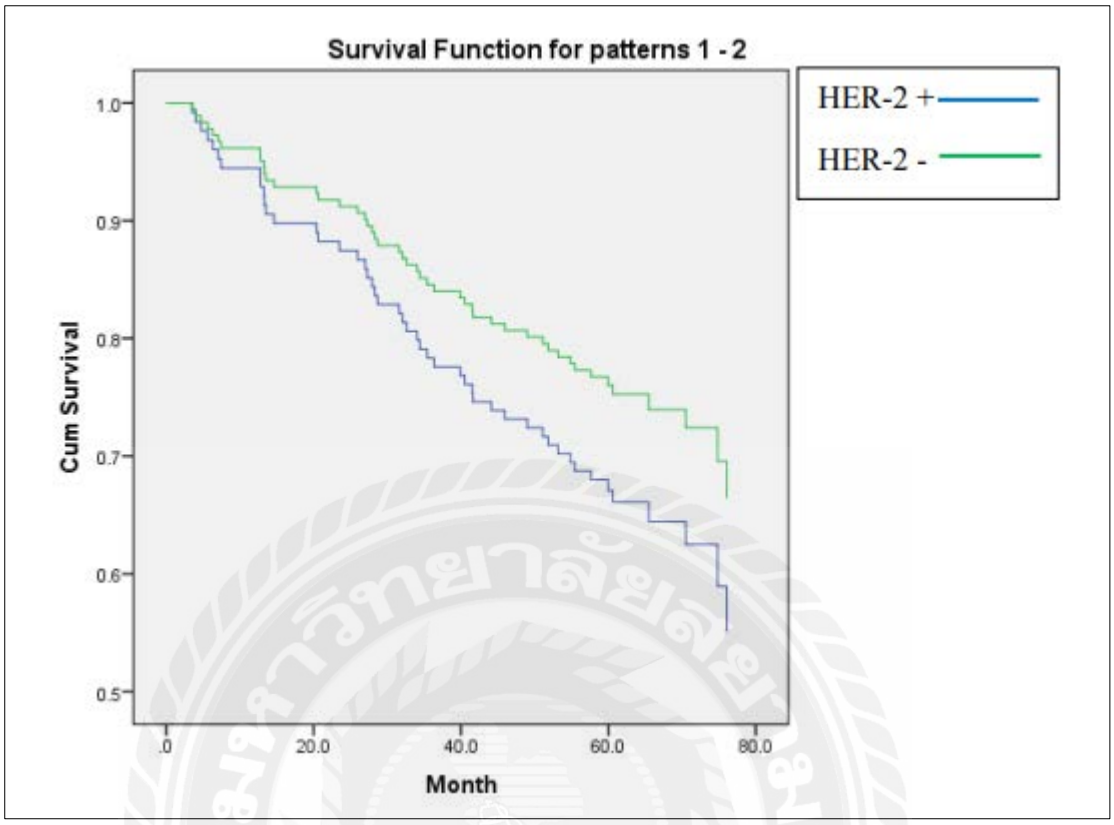


Figure 4.8 Kaplan-Meier curve of 5 years overall survival by HER2 receptor status

This figure presents the univariate Kaplan-Maier curves for the dichotomized progesterone receptor status (positive and negative). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among breast cancer patients with positive HER2 receptor status and negative HER2 receptor status were 64% and 76% respectively. The Kaplan-Maier curves showed that patients with negative HER-2 receptor status had a bit relatively better survival chance compared with patient with positive HER-2 receptor status but the different did not significantly different (HR = 1.456, p = 0.291).

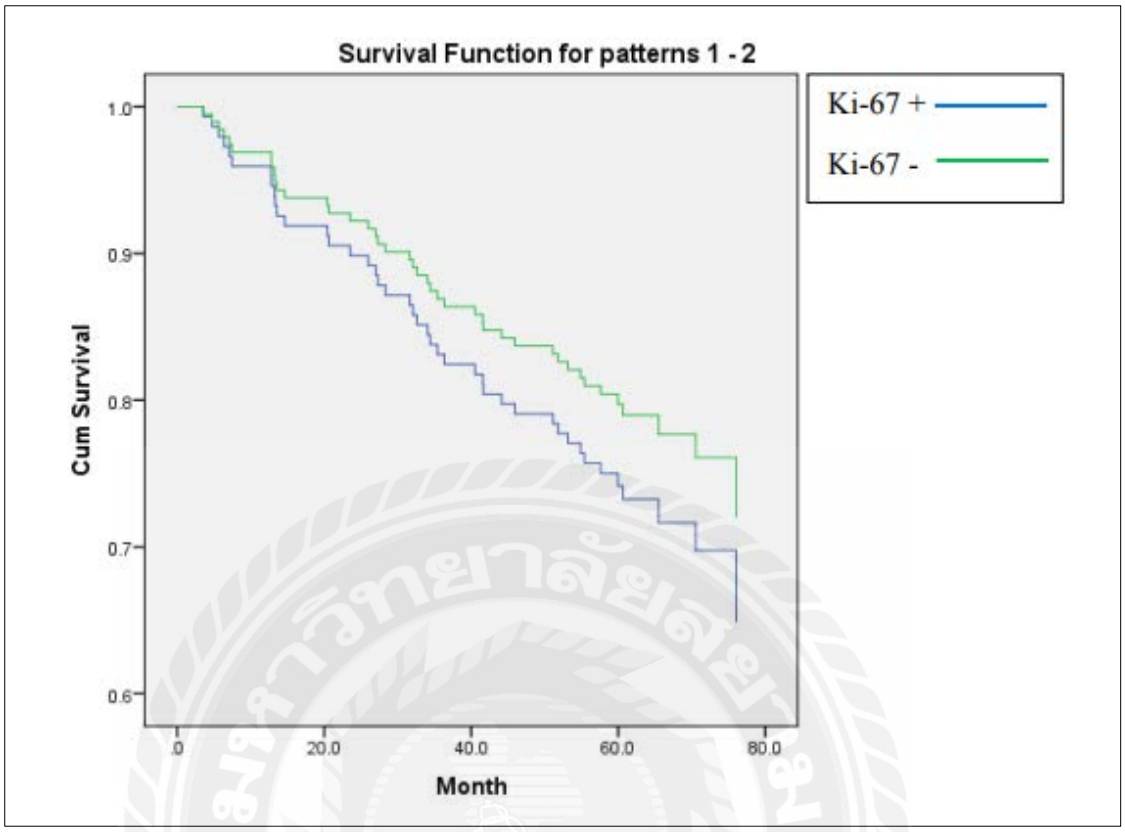


Figure 4.9 Kaplan-Meier curve of 5 years overall survival by Ki-67 status

This figure presents the univariate Kaplan-Maier curves for the dichotomized Ki-67 status (positive and negative). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among breast cancer patients with positive Ki-67 status and negative Ki-67 status were 72% and 78% respectively. The Kaplan-Maier curves showed that patients with positive Ki-67 status had relatively same survival chance compared with negative Ki-67 status (HR = 1.319, p = 0.561).

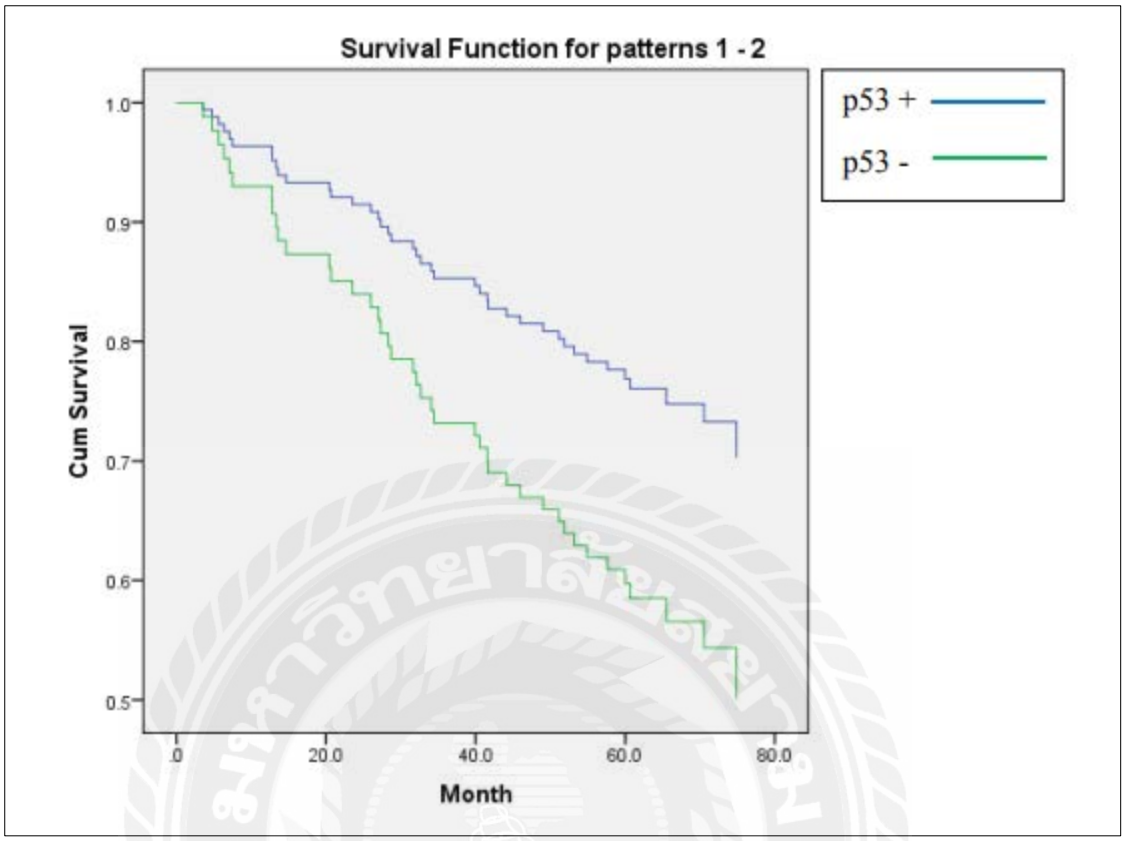


Figure 4.10 Kaplan-Meier curve of 5 years overall survival by p53 status

This figure presents the univariate Kaplan-Maier curves for the dichotomized p53 status (positive and negative). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among breast cancer patients with positive p53 status and negative p53 status were 75% and 54% respectively. The Kaplan-Maier curves showed that patients with positive p53 status had a relatively better survival chance compared with patient with negative p53 status but the different did not significantly different (HR = 1.960, p = 0.057).

4.2.6 The association between survival and *GSTM1* and *GSTT1* polymorphisms

In table 4.16 and 4.17, there was no relations of overall survival and *GSTM1* or *GSTT1* genotype in single genotype or combined genotypes analyses.

Regard with *GSTM1* polymorphisms, breast cancer patients with *GSTM1* null genotype and patients with *GSTM1* present genotype appeared to have almost the same survival. The probability of survival at 5 years among *GSTM1* null genotype and

GSTM1 present genotype were 0.71 and 0.73 respectively (P value = 0.760). Likewise, after Cox's proportional hazard analyzing, comparison of patients harboring *GSTM1* null genotype and patients harboring *GSTM1* present genotype had same risk of death (HR = 1.097, 95% CI = 0.605 - 1.987, P = 0.761).

Regard with *GSTT1* polymorphisms, breast cancer patients who had *GSTT1* null genotype showed the improved survival compared with patients who had *GSTT1* present genotype. The probability of survival at 5 years among *GSTT1* null genotype and *GSTT1* present genotype were 0.78 and 0.68 respectively. This statistically insignificant difference (P = 0.151) was maybe because of the limited number of patients with the null genotype (n = 59). However, after Cox's proportional hazard analyzing, patients harboring *GSTT1* null genotype had a lower risk of death compared with patients harboring *GSTT1* present genotype (HR = 0.632, 95% CI = 0.336 - 1.189, P = 0.154).

With respect to combined genotypes analyses, the probability of survival at 5 years among patients with *GSTM1+/GSTT1+*, *GSTM1+/GSTT1-, GSTM1-/GSTT1+* and *GSTM1-/GSTT1-* were 0.72, 0.80, 0.69 and 0.81, respectively. This statistically insignificant difference (P = 0.459) was maybe because of the limited number of patients in this current study. Moreover, after Cox's proportional hazard analyzing, the hazard ratios among patients with *GSTM1+/GSTT1+*, *GSTM1+ /GSTT1-, GSTM1-/GSTT1+* compared with *GSTM1-/GSTT1-* were 1.481, 1.220 and 1.838 respectively nevertheless, there was no statistically significant difference (P = 0.398, 0.696, 0.132). Patients with both present genotypes had a greater risk of death compared with patients with both null genotypes (HR = 1.481, 95% CI = 0.595 - 3.681, P = 0.398), likewise, patients with *GSTM1-/GSTT1+* had a greater risk of death compared with patients with both null genotypes (HR = 1.838, 95% CI = 0.832 - 4.063, P = 0.132).

Moreover, in this study, the Cox's proportional hazard ratio was analyzed and calculated for adjusted hazard ratios as show in Table 4.18 and 4.19.

After adjusted for tumor grade and progesterone receptor status, patients harboring *GSTT1* null genotype had a lower risk of death compared with patients with *GSTT1* present genotype (HR = 0.630 and 0.612) nevertheless, there was no statistically significant difference (P = 0.152 and 0.143).

With respect to combined genotypes analyses, after adjusted for progesterone receptor status, the hazard ratios among patients with *GSTM1+/GSTT1+*, *GSTM1+*

/GSTT1-, GSTM1-/GSTT1+ compared with *GSTM1-/GSTT1-* were 1.837, 1.653 and 2.018 respectively nevertheless, there was no statistically significant difference (P = 0.209, 0.393, 0.104). Patients with both present genotypes had a greater risk of death compared with patients with both null genotypes (HR = 1.837, 95% CI = 0.712 - 4.743, P = 0.209), likewise, patients with *GSTM1-/GSTT1+* had a greater risk of death compared with patients with both null genotypes (HR = 2.018, 95% CI = 0.866 - 4.698, P = 0.104).

Table 4.16 Overall survival at 5 years by *GSTM1* and *GSTT1* polymorphisms (N = 198)

Genotype	**No. of patients**	**No. of deaths**	**Probability of survival at 5 years**	**P value[a]**
GSTM1				
Present	59	16	0.73	
Null	116	34	0.71	0.760
GSTT1				
Present	116	37	0.68	
Null	59	13	0.78	0.151
GSTM1 and *GSTT1*				
combined	39	11	0.72	
GSTM1+/GSTT1+	20	5	0.80	0.459
GSTM1+/GSTT1-	77	26	0.69	
GSTM1-/GSTT1+	39	8	0.81	
GSTM1-/GSTT1-				

[a] P values from the log rank test

Table 4.17 Unadjusted hazard ratios by *GSTM1* and *GSTT1* polymorphisms (N = 198)

Genotype	Unadjusted HR[a] (95% CI)	P value
GSTM1		
Present	reference	
Null	1.097 (0.605-1.987)	0.761
GSTT1		
Present	reference	
Null	0.632 (0.336-1.189)	0.154
***GSTM1* and *GSTT1* combined**		
GSTM1+/GSTT1+	1.481 (0.595-3.681)	0.398
GSTM1+/GSTT1-	1.250 (0.409-3.822)	0.696
GSTM1-/GSTT1+	1.838 (0.832-4.063)	0.132
GSTM1-/GSTT1-	reference	reference

[a] HRs from a Cox' s proportional hazards model

Table 4.18 Adjusted hazard ratios by *GSTT1* polymorphisms (N = 198)

Genotype	Adjusted HR[a] (95% CI)	P value	Adjusted HR[b] (95% CI)	P value
GSTT1				
Present	reference		reference	
Null	0.630 (0.335-1.186)	0.152	0.612 (0.318-1.180)	0.143

[a] adjusted for tumor grade

[b] adjusted for progesterone receptor status

Table 4.19 Adjusted hazard ratios by *GSTM1* and *GSTT1* combined polymorphisms (N = 198)

Genotype	Adjusted HR[b] (95% CI)	P value
GSTM1 and *GSTT1* combined		
GSTM1+/GSTT1+	1.837 (0.712-4.743)	0.209
GSTM1+/GSTT1-	1.653 (0.521-5.539)	0.393
GSTM1-/GSTT1+	2.018 (0.866-4.698)	0.104
GSTM1-/GSTT1-	reference	

[b] adjusted for progesterone receptor status

4.2.7 The Kaplan-Meier curve of overall survival by GSTM1 and GSTT1 polymorphisms

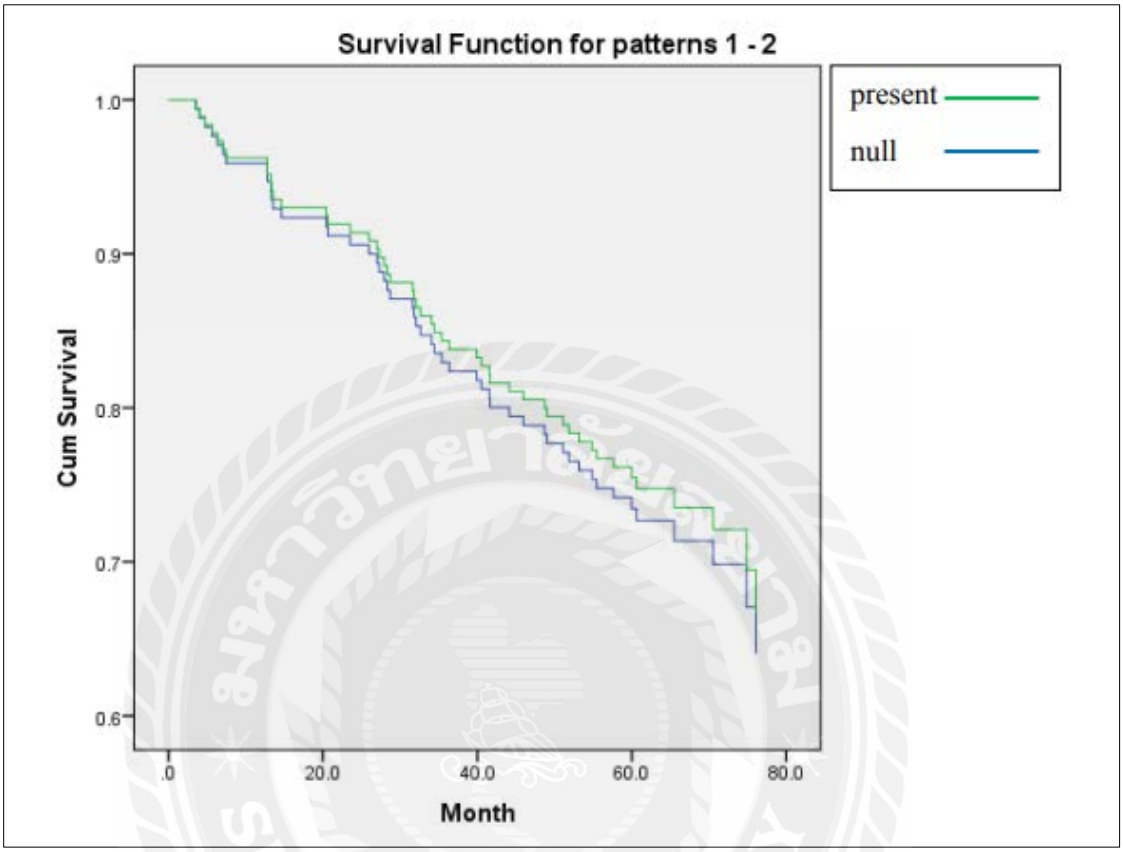


Figure 4.11 Kaplan-Meier curve of 5 years overall survival by *GSTM1* polymorphism

This figure presents the univariate Kaplan-Maier curves for the dichotomized *GSTM1* polymorphisms (null genotype and present genotype). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among breast cancer patients with *GSTM1* null genotype and *GSTM1* present genotype were 71% and 73% respectively. The Kaplan-Maier curves indicated that patients with *GSTM1* null genotype had a rather same survival chance compared with patient with *GSTM1* present genotype (HR = 1.097, p = 0.761).

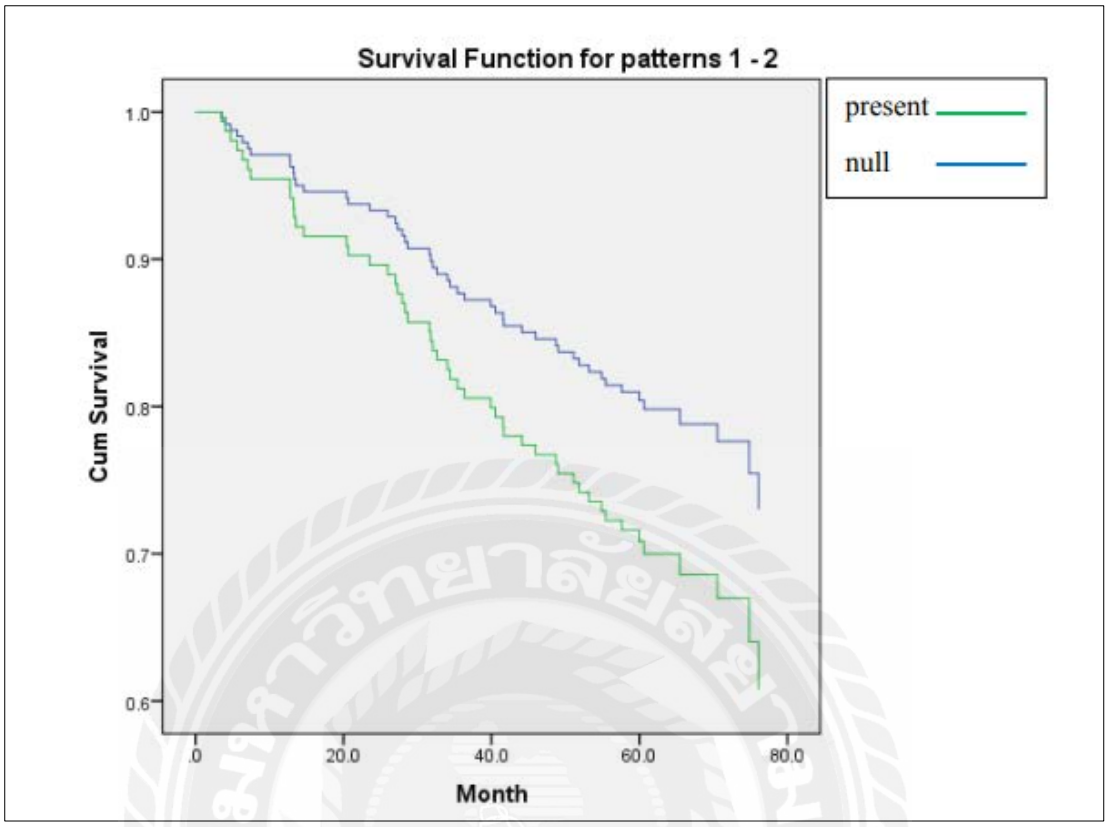


Figure 4.12 Kaplan-Meier curve of 5 years overall survival by *GSTT1* polymorphism

This figure presents the univariate Kaplan-Maier curves for the dichotomized *GSTT1* polymorphisms (null genotype and present genotype). The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among breast cancer patients with *GSTT1* null genotype and *GSTT1* present genotype were 78% and 68% respectively. The Kaplan-Maier curves showed that patients with *GSTT1* null genotype had a relatively better survival chance compared with patient with *GSTM1* present genotype but the different did not significantly different (HR = 0.632, p = 0.154).

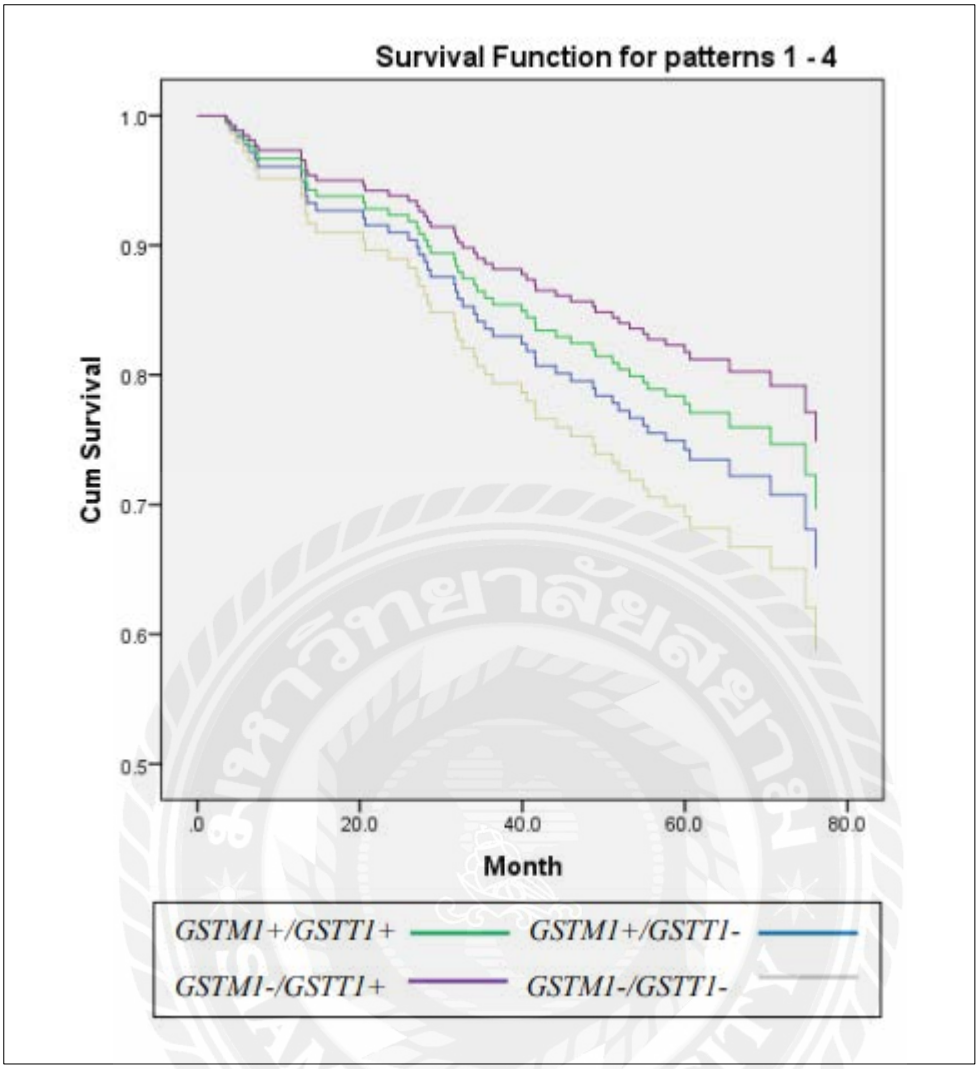


Figure 4.13 Kaplan-Meier curve of 5 years overall survival by *GSTM1* and *GSTT1* combined polymorphisms

This figure presents the univariate Kaplan-Maier curves for *GSTM1* and *GSTT1* combined polymorphisms. The median survival time could not observe because the 5 years overall survival was more than 50%. The 5 years overall survival among patients with breast cancer with *GSTM1+/GSTT1+, GSTM1+/GSTT1-, GSTM1-/GSTT1+* and *GSTM1-/GSTT1-* were 72%, 80%, 69% and 81% respectively. The Kaplan-Maier curves showed that patients with both present genotypes had a greater risk of death compared with patients with both null genotypes (HR = 1.481, 95% CI = 0.595 - 3.681, P = 0.398), likewise, patients with *GSTM1-/GSTT1+* had a greater risk of death compared with patients with both null genotypes (HR = 1.838, 95% CI = 0.832 - 4.063, P = 0.132).

CHAPTER V
DISCUSSION AND CONCLUSION

This chapter discussed on research findings to cope with breast cancer as well as limitation of this study. The discussion of this study is divided into two parts as follows:

5.1 The association of *GSTM1* and *GSTT1* polymorphisms with hematotoxicity among breast cancer patients with chemotherapy

5.2 The association of *GSTM1* and *GSTT1* polymorphisms with survival among breast cancer patients with chemotherapy

5.1 The Association of *GSTM1* and *GSTT1* Polymorphisms with Hematotoxicity Among Breast Cancer Patients with Chemotherapy

Anthracycline based chemotherapy regimen is generally used for treatment of breast cancer patients both in adjuvant and neoadjuvant chemotherapy. Anthracycline based chemotherapy regime that studied in this research composed of doxorubicin and cyclophosphamide. Doxorubicin inhibit topoisomerase 2 alpha and generate reactive oxygen species, which affects to cell apoptosis, while cyclophosphamide is an alkylating agent used to treat the several types of cancer. Glutathione S transferase (GSTs) crucially participate in a variety of drugs detoxification as well as anthracyclines. Inter-individual variations in GSTs in the population may have a powerful effect on the treatment outcomes of breast cancer and toxicities. The absence of enzymes among *GSTM1* null genotype and *GSTT1* null genotype is related with the decreasing rate of metabolism and detoxification of doxorubicin and cyclophosphamide. Consequently, the absence of GSTs function affect the level of drug concentration in blood concentration to elevate and improve the therapeutic effect meanwhile, increase the risk of toxicity (75).

Besides the objective of this study we also found (1) prevalence of hematotoxicity during treatment with anthracycline based chemotherapy regime and (2) potential associations between polymorphisms in genes encoding for *GSTM1* and *GSTT1* and hematotoxicity of anthracycline based chemotherapy regime in breast cancer patients.

Results showed the prevalence of hematotoxocity in any grade was as follows: 38 anemia (67.90%), 38 leucopenia (67.90%), 10 neutropenia (17.90%) and 3 thrombocytopenia (5.40%). According to anemia and leucopenia, the most patients suffered grade1 toxicity 53.6% and 58.90%, respectively. In contrast to neutropenia, the most patients suffered grade 2 toxicity (12.50%) and few patients experienced only grade 1 thrombocytopenia (5.40%). In addition, grade 4 hematotoxicity and toxic deaths were not observed in this current study.

In conclusion, the present genotype of *GSTM1* and *GSTT1* trended to correlate greater risk of neutropenia and *GSTM1* present genotype trended to correlate higher risk thrombocytopenia which was not expected as describe above although the different did not statistically significant.

Similarly, Tulsyan et al. (24) both *GSTM1* and *GSTT1*, all hematotoxicities was often occurred among patients with present genotype, even though the differ did not statistical significance (p=0.269, p=0.397). Furthermore, they found the significant relation of Ile/Val and Ile/Val + Val/Val genotypes of the *GSTP1* $Ile^{105}Val$ polymorphism with grade 2-4 anemia.

Khrunin et al. (76) determined about the side effect risk of cisplatin and cyclophosphamide treatment in ovarian cancer patients. The result showed that the *GSTM1* null genotype can lower the risk of hematotoxicity such as thrombocytopenia, anemia and neurotoxicity compared with *GSTM1* present genotype with statistically significant [OR = 0.13 for thrombocytopenia (95% CI = 0.03-0.62, P = 0.005) and OR = 0.29 for anemia (95% CI = 0.13-0.66, P = 0.003)].

Tecza et al. (77) determined the clinical and genetic risk factors of 5-fluorouracil, doxorubicin and cyclophosphamide (FAC). Among 324 breast cancer patients, specific genes and their polymorphisms such as *GSTM1, GSTP1, GSTT1* and other genes were selected and examined for chemotherapy-related toxicities. They found that together with null genotype of *GSTM1* and *GSTT1* genes augmented the

nephrotoxicity risk and anemia, on the other hand, the independent gene, the present genotype of *GSTM1* and *GSTT1* genes increased the nephrotoxicity risk and anemia.

Because of limited published data considering *GSTM1* and *GSTT1* polymorphisms and toxicity of chemotherapy. However several studies focused on the pharmacogenetic approach to chemotherapy toxicity in breast cancer patients (59,78–82).

According to GSTs crucially participate in the detoxification of a variety drugs and one of them is anthracyclines. Therefore, it can imply that *GSTM1* and *GSTTI* null genotypes have higher drug availability while having lower enzyme activity. Consequently, lead to improve clinical outcomes and maybe lead to higher risk of toxicities. However, the true significance of *GSTM1* and *GSTT1* polymorphisms in the toxicity prognosis to chemotherapy remains obscure because of the combination effect of these variants was not well evaluated. In addition, pharmacokinetic drug-drug were not taken into consideration, especially in the elderly, is due to the use of concomitant drugs such as antihypertensive drugs, antidiabetic drugs, and antihyperlipidemic drugs that could increase or decrease toxicity of chemotherapy (83).

5.2 The Association of *GSTM1* and *GSTT1* Polymorphisms with Survival Among Breast Cancer Patients with Chemotherapy

In this study, the overall survival at 1, 3, 5 years was 95.00%, 83.00% and 71.00% respectively. There were some studies conducted in Thailand to determine the survival of breast cancer patients. In 1995, Sriamporn et al., (84) indicated the 5 years survival of breast cancer from the population-based study in Khon Kaen province registered in the period 1985-1992 was 48.10%. Then in 2000, Amornsak et al. (11) conducted the study at a teaching university in northeast of Thailand among 340 female breast cancer patients and followed-up until the end of 2006 and the 5 years survival was 42.9%. Moreover, Apichat et al. (85) conducted the study in 1999-2009 to evaluate the relation of molecular subtypes and survival among breast cancer patients treated with radiotherapy and the 5 years overall survival was 59.2%.

This could be explained that nowadays breast cancer patients in Thailand trended to improve the survival due to the early screening policy and/or the current standard treatment that allowed the effective treatment outcome and low toxicity.

The survival of breast cancer patients varied among countries (86-90). The survival of breast cancer patients in developed countries is higher than we found in this study. The SEER 5-year survival rate in United States (2008-2014) was 89.7% in females and 83% in males. The study in England & Wales reported by Cancer Research UK Cancer Survival Group indicated that the survival rate at 1 and 5 years were 96.0% and 86.6% respectively. Data collected in developing countries showed either the same or vary of the 5 years survival (91-94). The international diversity of survival in breast cancer was not easy to interpret may be due to many factors such as the knowledge and awareness of patients, the early detection, availability of effective treatment and health services accessibility which may be different between developed countries and developing countries.

Furthermore, in this study, Cox's proportional hazards model and the log rank test results revealed a significant different in the 5 years overall survival probability according by lymph node metastasis and tumor stage ($P = 0.014$ and $P < 0.001\%$). The 5 years overall survival among breast cancer patients who have none and present lymph node metastasis were 83% and 67% respectively. Patients with lymph node metastasis had around 2-fold-higher risk of death compared with patients without lymph node metastasis ($HR = 2.105$, $P = 0.016$). Moreover, the overall survival at 5 years among breast cancer patients with tumor stage I - II (early-stage tumor) and stage III - IV (advance-stage tumor) were 80% and 56% respectively. patients with advanced-stage tumor had around 3-fold-higher risk of death compared with early-stage disease patients ($HR = 2.782$, $P < 0.001$).

A key prognostic parameter of patients with breast cancer survival is stage at diagnosis (95-98). Breast cancer patients with early-stage showed much higher survival rates than those with late-stage. Several studies showed that survival significantly diverged to stage at diagnosis. Walters et al. (99) investigated the differences in breast cancer survival in developed countries including Canada, Denmark, Norway, Sweden, and the United Kingdom according to the stage at diagnosis. The result showed that approximately 30.1% - 45.2% patients were diagnosed with tumor stage I, 39.0% - 47.7% with tumor stage II, 3.5% - 15.3% with tumor stage

III, and 2.9% - 6.9% with tumor stage IV. In our study population revealed a greater frequency of stage II (56.57%) and stage III (34.34%) compared with those in developed countries which may emphasize the importance for screening program for primary prevention.

Conform to several studies that focused on lymph node metastasis status as the predictive factors for breast cancer survival thus the lymph node metastasis is linked to distant recurrence and survival of patients (100-105). Respect to lymph node status, patients without lymph node metastasis had much greater survival rates than those with lymph node metastasis. In this study, lymph node metastasis was analyzed as the dichotomous variables; none and present, in contrast to some studies analyzed as the number of nodes involved or lymph node ratio (proportion of number of lymph nodes that are positive metastasis to the total number of lymph nodes evaluated). For instances, Mahmood et al. (103) observed that patients with less than 5 nodes metastasis survived for more than 10 years were 16.5% compare with patients with more than 9 nodes metastasis survived were only 5% therefore it can imply that survival decrease while number of nodes metastasis increase. Hung et al. (104) revealed that lymph node ratio was a proper prognosis factor of survival than TNM system from the American Joint Committee on Cancer (AJCC).

Breast cancer is the global public health problem including Thailand. The information about genetic variation in breast cancer in Thailand may be limited due to the small sample size. In this study, 198 breast cancer patients were genotyped, and the result showed that among Thai breast cancer patients, the frequency of the *GSTM1* and *GSTT1* null genotype was 65.70% and 33.30%, respectively. Several studies reported the frequency of the *GSTM1* and *GSTT1* null genotype in breast cancer patients. Nevertheless, Pongtheerat et al. (57) showed the frequencies of the *GSTM1* and *GSTT1* null genotype in Thai patients with breast cancer was (14/40) 35.00% and (18/43) 41.90%, respectively. The findings from our study showed the greater frequency of *GSTM1* null genotype (65.70%) in breast cancer patients than the finding from the former study (35.00%).

Several evidences showed that the genetic polymorphisms of drug transporters, drug-metabolizing enzymes and drug targets are involved in inter-individual diversity of the efficacy and toxicity of chemotherapy and several medicines (106-114). A personalized chemotherapy is proposed to be a promising tool to increase chemotherapy

response, prevent the toxicity and elevate overall survival of patients with breast cancer. As we know that the GST super-family belongs to the phase II biotransformation enzymes, which function a crucial part in the biotransformation or detoxification of a variety of xenobiotics as well as chemotherapeutic agents. However there were several studies focused on the role of GSTs in chemotherapy efficacy and treatment outcome, the results of those studies have indicated the inconsistent association (22, 27, 29-31, 45, 72, 115-119). The purpose of this study determined the association of *GSTM1* and *GSTT1* polymorphisms and the clinical outcomes of chemotherapy in breast cancer patients.

This current study found that patients with breast cancer who harboring *GSTM1* null genotype (71%) appeared to have same 5 years survival compared with patients who harboring *GSTM1* present genotype (73%) (HR = 1.097, 95% CI = 0.605 - 1.987, P = 0.761). Regard to *GSTT1* polymorphisms, patients with breast cancer who harbored *GSTT1* null genotype (78%) appeared to improve survival compared with patients who harbored *GSTT1* present genotype (68%). (HR = 0.632, 95% CI = 0.336 - 1.189, P = 0.154). Regard to combined genotypes analyses, the 5 years survival among patients with *GSTM1+/GSTT1+*, *GSTM1+/GSTT1-, GSTM1-/GSTT1+* and *GSTM1-/GSTT1-* were 72.0%, 80.0%, 69.0% and 81.0%, respectively. The hazard ratios among patients with *GSTM1+/ GSTT1+*, *GSTM1+/GSTT1-, GSTM1-/GSTT1+* compared with *GSTM1-/GSTT1-* were 1.481, 1.220 and 1.838 respectively nevertheless, this difference was not statistically significant (P = 0.398, 0.696, 0.132). Patients with both present genotypes had a higher risk of death compared with patients harboring both null genotypes (HR = 1.481, 95% CI = 0.595 - 3.681, P = 0.398), likewise, patients with *GSTM1-/GSTT1+* had a higher risk of death compared with patients harboring both null genotypes (HR = 1.838, 95% CI = 0.832 - 4.063, P = 0.132).

In multivariate analysis, after adjusted for tumor grade and progesterone receptor status, patients with *GSTT1* null genotype had a lower risk of death compared with patients with *GSTT1* present genotype (HR = 0.630 and 0.612) nevertheless, this difference was not statistically significant (P = 0.152 and 0.143). With respect to combined genotypes analyses, after adjusted for progesterone receptor status, the hazard ratios among patients with *GSTM1+/GSTT1+*, *GSTM1+/GSTT1-, GSTM1-/GSTT1+* compared with *GSTM1-/GSTT1-* were 1.837, 1.653 and 2.018 respectively nevertheless, this difference was not statistically significant (P = 0.209, 0.393, 0.104). Patients with both present genotypes had a higher risk of death compared with patients harboring both

null genotypes (HR = 1.837, 95% CI = 0.712 - 4.743, P = 0.209), likewise, patients with *GSTM1-/GSTT1+* had a higher risk of death compared with patients harboring both null genotypes (HR = 2.018, 95% CI = 0.866 - 4.698, P = 0.104).

Indeed, our results could not find the relation of *GSTM1* and *GSTT1* polymorphisms and the overall survival among patients with breast cancer treated with chemotherapy. Conform to the result from Sarab et al. (119) which found that *GSTM1* polymorphism did not associate with clinicopathology characteristics, clinical outcomes of chemotherapeutic agents in advanced breast cancer. Moreover, Yang et al. (23) indicated that there was no relation to any of the *GSTM1* or *GSTT1* polymorphisms as potential role in prognosis to the clinical outcomes and overall survival of breast cancer patients after chemotherapy. On the other hand, some studies indicated the contrast results, Wang et al. (74) reported that the *GSTM1* null genotype was related with a greater to chemotherapeutic agents response and the odds ratio was 1.78 (95% CI = 1.03 - 3.08) and the hazard ratio for overall survival in patients with the *GSTM1* null genotype was 0.57 (95% CI = 0.32 - 0.98) compare with *GSTM1* present genotype. However, they indicated that there was no statistically significant relation of the *GSTT1* polymorphisms and overall survival among breast cancer patients. Another study from China, Wang et al. (29) found that patients with *GSTM1* null genotype related to worse overall survival of breast cancer patients treated with chemotherapy and the hazard ratio for overall survival was 2.00 (95% CI = 1.15 - 3.48). Furthermore, Bai et al. evaluated the prognostic role of GST gene polymorphisms among patients with breast cancer treated with neoadjuvant chemotherapy and the result showed that patients with the *GSTM1* null genotype had a better survival and statistical significantly lower risk of death than patients harboring *GSTM1* present genotype (HR = 0.66, 95% CI = 0.31- 0.93). These differences results may be cause of methodology, study design, study population and sample size, genotyping methods or chemotherapy regimens.

This study, the retrospective study design and the small sample size are the main limitations of this current study. In retrospective study, toxicity data are less likely to be interpreted correctly than in a prospective study. To limit interpretation bias, we chose to observe only hematotoxicity as the main point. The small sample size in this research may lead to the lower statistical power. Other limitations in our study include, the low statistic power due to limited sample size and the patients from single center may not represent all breast cancer patients in Thailand therefore, further prospective

studies with larger sample size and multicenter should be study to validate these association.

Recommendation for Policy Maker

Chemotherapy is used as the standard treatment for breast cancer and the heterogeneity in the response, toxicity and survival in breast cancer patients was unclear. The knowledge of pharmacogenetics function in chemotherapy biotransformation could describe the differences in breast cancer patients. These genetic polymorphiams could be used as biomarkers to predict the treatment outcome due to personalize chemotherapy regime, consequently, potentially improving the effectiveness and decreasing the chemotherapy toxicities and prolong overall survival of patients with good quality of life. We found no association between *GSTM1, GSTT1* and hematotoxicity and survival among Thai breast cancer patients in this study. In the further study in the large sample size to validate the result and cost effectiveness analysis to determine the *GSTM1* and *GSTT1* polymorphisms as the biomarkers to predict clinical outcomes in chemotherapy treatment should do for assure of the direction of standard treatment.

Recommendation for the Further Studies

This study exposed some knowledges, but there are some limitations in this study as well.

For toxicity study

Pharmacokinetic drug-drug were not taken into consideration, especially in the elderly, is due to the use of concomitant drugs such as antihypertensive drugs, antidiabetic drugs, and antihyperlipidemic drugs that could increase or decrease toxicity of chemotherapy. In this study, the retrospective study design and the small sample size are the main limitations. In retrospective study, toxicity data are less likely to be interpreted correctly than in a prospective study. To limit interpretation bias, we chose to observe only hematotoxicity as the main point. The small sample size in this study may cause to the lower statistical power.

For survival study

The participants included this study were those whom a data was available. The exclusion of participants with incomplete data is potential source of selection bias. The low statistic power maybe the due to limited sample size and the patients from single center may not represent all breast cancer patients in Thailand therefore, further prospective research with larger sample size and multicenter are necessary to validate these association.

REFERENCES

1. Ogata H, Kikuchi Y, Natori K, Shiraga N, Kobayashi M, Magoshi S, et al. Liver metastasis of a triple-negative breast cancer and complete remission for 5 years after treatment with combined bevacizumab/paclitaxel/ carboplatin: case report and review of the literature. Medicine (Baltimore). 2015; 94(42): e1756.
2. Koo T, Kim IA. Brain metastasis in human epidermal growth factor receptor 2-positive breast cancer: from biology to treatment. Radiat Oncol J. 2016; 34(1): 1-9.
3. Kang Y. Dissecting Tumor-Stromal Interactions in Breast Cancer Bone Metastasis. Endocrinol Metab. 2016; 31(2): 206-212.
4. Rahou BH, Rhazi K El, Ouasmani F, Nejjari C, Bekkali R, Montazeri A. Quality of life in Arab women with breast cancer: a review of the literature. Health Qual Life Outcomes [Internet]. 2016; 14: 64. Available from: http://dx.doi.org/10. 1186/s12955-016-0468-9
5. Jankowska-polanska B, Szymanska- A. Quality of life of patients with lung cancer. Onco Targets Ther. 2016; 9: 1023-8.
6. Girgis A, Lambert S, Johnson C, Waller A, Currow D. Physical, Psychosocial, relationship, and economic burden of caring for people with cancer: a review. J Oncol Pract [Internet]. 2013; 9(4): 197-202. Available from: http://jop.ascopubs.org/cgi/doi/10.1200/JOP.2012.000690
7. Kim Y, Given BA. Quality of life of family caregivers of cancer survivors: Across the trajectory of the illness. Cancer. 2008; 112(11 SUPPL.): 2556-68.
8. Wissinger E, Griebsch I, Lungershausen J, Foster T, Pashos CL. The economic burden of head and neck cancer: a systematic literature review. Pharmacoeconomics. 2014; 32(9): 865-82.
9. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. Eur J Cancer [Internet]. 2013; 49(6): 1374-403. Available from: http://dx.doi.org/10.1016/j.ejca.2012.12.027
10. Kotepui M, Chupeerach C. Age distribution of breast cancer from a thailand population- based cancer registry. Asian Pac J Cancer Prev. 2013; 14(6): 3815-7.
11. Poum A, Kamsa-ard S, Promthet S. Survival rates of breast cancer: a hospital-based study from Northeast of Thailand. Asian Pac J Cancer Prev. 2012; 13: 791-4.
12. Twelves C, Jove M, Gombos A, Awada A. Cytotoxic chemotherapy: Still the mainstay of clinical practice for all subtypes metastatic breast cancer. Crit Rev Oncol/Hematol [Internet]. 2016; 100: 74-87. Available from: http://dx. doi.org/10.1016/j.critrevonc.2016.01.021

13. Reinisch M, von Minckwitz G, Harbeck N, Janni W, Kümmel S, Kaufmann M, et al. Side effects of standard adjuvant and neoadjuvant chemotherapy regimens according to age groups in primary breast cancer. Breast Care (Basel). 2013; 8(1): 60-6.
14. McGowan J V., Chung R, Maulik A, Piotrowska I, Walker JM, Yellon DM. Anthracycline chemotherapy and cardiotoxicity. Cardiovasc Drugs Ther. 2017; 31(1): 63-75.
15. Chui PL, Abdullah KL, Wong LP, Taib NA. Quality of life in CAM and Non-CAM users among breast cancer patients during chemotherapy in Malaysia. PLoS One. 2015; 10(10): 1-17.
16. Baulies S, Belin L, Mallon P, Senechal C, Pierga J, Cottu P, et al. Time-varying effect and long-term survival analysis in breast cancer patients treated with neoadjuvant chemotherapy. Br J Cancer. 2015; 113(1): 30-6.
17. Li W-S, Lam WS, Liu K-C, Wang C-H, Chang HC, Jen YC, et al. Overcoming the drug resistance in breast cancer cells by rational design of efficient glutathione S-transferase inhibitors. Org Lett [Internet]. 2010; 12(1): 20-3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19961147
18. Lim E, Tarulli G, Portman N, Hickey TE, Tilley WD, Palmieri C. Pushing estrogen receptor around in breast cancer. Endocr Relat Cancer. 2016; 23(12): T227-41.
19. Tulsyan S, Mittal RD, Mittal B. The effect of ABCB1 polymorphisms on the outcome of breast cancer treatment. Pharmgenomics Pers Med. 2016; 9: 47-58.
20. Lee CK, Stockler MR, Coates AS, Gebski V, Lord SJ, Simes RJ. Self-reported health-related quality of life is an independent predictor of chemotherapy treatment benefit and toxicity in women with advanced breast cancer. Br J Cancer. 2010; 102(9): 1341-7.
21. Zhang M, Wei W, Liu J, Yang H, Jiang Y, Tang W, Li Q, Liao X. Comparison of the effectiveness and toxicity of neoadjuvant chemotherapy regimens, capecitabine/epirubicin/cyclophosphamide vs followed by adjuvant, capecitabine/docetaxel vs docetaxel, in patients with operable breast cancer. Onco Targets Ther. 2016; 9: 3443-50.
22. Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene. 2003; 22(47): 7369-75.
23. Yang G, Shu XO, Ruan ZX, Cai QY, Jin F, Gao YT, et al. Genetic polymorphisms in glutathione-S-transferase genes (GSTM1, GSTT1, GSTP1) and survival after chemotherapy for invasive breast carcinoma. Cancer. 2005; 103(1): 52-8.
24. Tulsyan S, Chaturvedi P, Agarwal G, Lal P, Agrawal S, Mittal RD, et al. Pharmacogenetic influence of GST polymorphisms on anthracycline-based chemotherapy responses and toxicity in breast cancer patients: A multi-analytical approach. Mol Diagnosis Ther. 2013; 17(6): 371-9.
25. Mishra A, Chandra R, Mehrotra PK, Bajpai P, Agrawal D. Glutathione S-transferase M1 and T1 polymorphism and response to neoadjuvant

chemotherapy (CAF) in breast cancer patients. Surg Today. 2011; 41(4): 471-6.
26. Satta T, Isobe K, Nakashima I, Takagi H. Expression of MDR 1 and glutatione s transferase-pi genes and chemosensitivities in human gastrointestinal cancer. Cancer Am Cancer Soc. 1992; 69(4): 941-6.
27. Lorand-metze I, Delamain MT, Miranda ECM, Kameo R, Metze K, Lima CSP. Polymorphisms of glutathione S-transferase mu 1, theta 1, and pi 1 genes and prognosis in Hodgkin lymphoma. Leuk Lymphoma. 2010; 51(December): 2215-21.
28. Tang J, Zhou Q, Zhao F, Wei F, Bai J, Xie Y, et al. Association of glutathione S-transferase T1, M1 and P1 polymorphisms in the breast cancer risk: A meta-analysis in Asian population. Int J Clin Exp Med. 2015; 8(8): 12430-47.
29. Liu J, Luo J, Wang Y, Li L, Yang S. Predictive potential role of glutathione S-transferases polymorphisms on prognosis of breast cancer. Int J Clin Exp Pathol. 2014; 7(12): 8935-40.
30. Duggan C, Ballard-Barbash R, Baumgartner RN, Baumgartner KB, Bernstein L, McTiernan A. Associations between null mutations in GSTT1 and GSTM1, the GSTP1 Ile(105)Val polymorphism, and mortality in breast cancer survivors. Springerplus [Internet]. 2013; 2: 450. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3786079&tool=pmcentrez&rendertype=abstract
31. Oliveira AL, Oliveira Rodrigues FF, dos Santos RE, Rozenowicz RL, Barbosa de Melo M. GSTT1, GSTM1, and GSTP1 polymorphisms as a prognostic factor in women with breast cancer. Genet Mol Res. 2014; 13(2): 2521-30.
32. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell [Internet]. 2011; 144(5): 646-74. Available from: http://dx.doi.org /10.1016/ j.cell.2011.02.013
33. Tachi T, Teramachi H, Tanaka K, Asano S, Osawa T, Kawashima A, et al. The impact of outpatient chemotherapy-related adverse events on the quality of life of breast cancer patients. PLoS One. 2015; 10(4): 1-15.
34. Terry MB. Are global breast cancer incidence and mortality patterns related to country-specific economic development and prevention strategies? J Glob Oncol. 2018; (4)July: 1-16.
35. Ye M, Lu J, Yang F, Wu B. Economic evaluation of letrozole for early breast cancer in a health resource-limited setting. Biomed Res Int [Internet]. 2018. Available from: https://doi.org/10.1155/2018/9282646
36. Bray F, Ferlay J, Soerjomataram I. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. 2018; 394-424.
37. Yip CH, Taib NA. Breast health in developing countries. Climacteric. 2014; 17 Suppl 2: 54-9.
38. Imsamran W, Chiawiriyabunya I, Pattatang A, Supattagorn P. Cancer in Thailand. Bangkok; 2015.

39. Virani S, Bilheem S, Chansaard W, Id IC, Id KD, Khuanchana S, et al. Incidence of cancer in Thailand: assessing cancers with the highest burdens. Cancers (Basel). 2017; 108(9): 1-27.
40. Ayoub NM, Al-shami KM, Yaghan RJ. Immunotherapy for HER2-positive breast cancer: recent advances and combination therapeutic approaches. 2019; 53-69.
41. Boyages J, Baker L. Evolution of radiotherapy techniques in breast conservation treatment. Gland Surg. 2018; 7(4): 576-95.
42. Murugappan K, Saboo A, Kuo L, Ung O. Paradigm shift in the local treatment of breast cancer: mastectomy to breast conservation surgery. Gland Surg. 2018; 7(6): 506-19.
43. Wong NS. Primary medical therapy and breast conservation treatment: the medical oncology perspective. Gland Surg. 2018; 7(6): 560-75.
44. Anampa J, Makower D, Sparano JA. Progress in adjuvant chemotherapy for breast cancer: an overview. BMC Med. 2015; 13(195): 1-13.
45. Kong X, Li Z, Li X. GSTP1, GSTM1, and GSTT1 polymorphisms as predictors of response to chemotherapy in patients with breast cancer: a meta-analysis. Cancer Chemother Pharmacol. 2016; 1-11.
46. Jannazzo A, Hoffman J, Lutz M. Monitoring of anthracycline-induced cardiotoxicity. Ann Pharmacother. 2008; 42(1): 99-104.
47. Ahlmann M, Hempel G. The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. Cancer Chemother Pharmacol. 2016; 78(4): 661-71.
48. Emadi A, Jones RJ, Brodsky RA. Cyclophosphamide and cancer: golden anniversary. Nat Rev Clin Oncol. 2009; 6(11): 638-47.
49. American Cancer Society. Breast Cancer Staging 7th Edition. Am Jt Comm Cancer [Internet]. 2010;1-2. Available from: cancerstaging.org
50. Narod SA. Tumour size predicts long-term survival among women with lymph node-positive breast cancer. Curr Oncol. 2012; 19(5): 249-53.
51. Rakha EA, Reis-filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, et al. Breast cancer prognostic classify cation in the molecular era: the role of histological grade. Breast Cancer Res. 2010; 12(207): 1-12.
52. Chen H, Zhou M, Tian W, Meng K, He H. Effect of age on breast cancer patient prognoses: a population-based study using the seer 18 database. Plos One. 2016; 11(10).
53. Mitri Z, Constantine T, Regan RO. The HER2 receptor in breast cancer: pathophysiology, clinical use, and new advances in therapy; Chemother Res Pract. 2012; 2012: 743193.
54. Ito Y, Tokudome N, Sugihara T, Takahashi S, Hatake K. Does lapatinib, a small-molecule tyrosine kinase inhibitor, constitute a breakthrough in the treatment of breast cancer? Breast Cancer [Internet]. 2007; 14(2): 156-62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17485900
55. Park C, Park K, Kim J, Sin Y, Park I, Cho H, et al. Prognostic values of negative estrogen or progesterone receptor expression in patients with luminal B

HER2-negative breast cancer. World J Surg Oncol [Internet]. 2016; 1-9. Available from: http://dx.doi.org/10.1186/s12957-016-0999-x

56. Fohlin H, Fornander T, Lo B, Nordenskjo A. Progesterone receptor positivity is a predictor of long-term benefit from adjuvant tamoxifen treatment of estrogen receptor positive breast cancer. Breast Cancer Res Treat. 2016; 160(2): 313-22.
57. Westbrook K, Stearns V. Pharmacogenomics of Breast Cancer Therapy: An Update Kelly. Pharmacol Ther. 2013; 139(1): 1-11.
58. Rausch SM, Clark MM, Patten C, Liu H, Felten S, Li Y, et al. Relationship between cytokine gene single nucleotide polymorphisms and symptom burden and quality of life in lung cancer survivors. Cancer. 2010; 116(17): 4103-13.
59. Angelini S, Botticelli A, Onesti CE, Giusti R, Sini V, Durante V, et al. Pharmacogenetic approach to toxicity in breast cancer patients treated with taxanes. Anticancer Res [Internet]. 2017; 37(5): 2633-9. Available from: http://ar. iiarjournals.org/content/37/5/2633.abstract
60. Pullarkat S, Stoehlmacher J, Ghaderi V, Xiong Y-P, Ingles S, Sherrod A, et al. Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. Pharmacogenomics J. 2001; 1: 65-70.
61. Sherratt PJ, Hayes JD. 9 Glutathione S-transferases. Vol. 4, Enzyme systems that metabolise drugs and other xenobiotics. edited by costas ioannides. 2002. 319-352 p.
62. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Rev Lit Arts Am. 2005; 51-88.
63. Tiwawech D, Srivatanakul P, Karalak A, Ishida T. Glutathione S-transferase M1 gene polymorphism in Thai nasopharyngeal carcinoma. Asian Pac J Cancer Prev [Internet]. 2005; 6(3): 270-5. Available from: http://www. ncbi. nlm.nih.gov/ pubmed/16235985
64. Sharma A, Pandey A, Sharma S, Chatterjee I, Mehrotra R, Sehgal A, et al. Genetic polymorphism of glutathione S-transferase P1 (GSTP1) in Delhi population and comparison with other global populations. Meta Gene. 2014; 2(1): 134-42.
65. Pongtheerat T, Treetrisool M, Purisa W. Glutathione S-Transferase polymorphisms in breast cancers of Thai patients. Asian Pacific J Cancer Prev. 2009; 10(1): 127-32.
66. Seidegård J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. Proc Natl Acad Sci U S A. 1988; 85(19): 7293-7.
67. Demir A, Altin S, Demir I, Koksal V, Cetincelik U, Dincer SI. Original communication the frequency of GSTT1 null genotype in Turkish population and lung cancer risk. Indian J Hum Genet. 2005; 11(2): 89-93.

68. Nasseri G, Zahedi T, Mousavi-kazerooni F. Prevalence of null genotypes of glutathione s-transferase t1 (gstt1) and m1 (gstm1) in seven Iranian populations. Iran J Public Health. 2015; 44(12): 1655-61.
69. Alshagga MA, Mohamed N, Suhid AN, Ibrahim IAA, Zakaria SZS. Frequencies of glutathione s-transferase (GSTM1, GSTM3 and GSTT1) polymorphisms in a Malaysian population. Arch Med Sci. 2011; 7(4): 572-8.
70. Gattás GJF, Kato M, Soares-Vieira JA, Siraque MS, Kohler P, Gomes L, et al. Ethnicity and glutathione S-transferase (GSTM1/GSTT1) polymorphisms in a Brazilian population. Brazilian J Med Biol Res. 2004; 37(4): 451-8.
71. Millikan R, Pittman G, Tse C, Savitz DA, Newman B, Bell D. Glutathione S -Transferases M1, T1, and P1 and breast cancer. Am Assoc Cancer Res. 2000; 9(June): 567-73.
72. Arun BK, Granville L a., Yin G, Middleton LP, Dawood S, Kau S-W, et al. Glutathione-S-Transferase-Pi Expression in Early Breast Cancer: Association with outcome and response to chemotherapy. Cancer Invest. 2010; 28(5): 554-9.
73. Bai Y-L, Zhou B, Jing X-Y, Zhang B, Huo X-Q, Ma C, et al. Predictive role of GSTs on the prognosis of breast cancer patients with neoadjuvant chemotherapy. Asian Pac J Cancer Prev. 2012; 13(March 2007): 5019-22.
74. Wang J, Wang T, Yin G, Yang L, Wang Z, Bu X. Glutathione S -transferase polymorphisms influence chemotherapy response and treatment outcome in breast cancer. Genet Mol Res. 2015; 14(3): 11126-32.
75. Lo HW, Ali-Osman F. Genetic polymorphism and function of glutathione S-transferases in tumor drug resistance. Curr Opin Pharmacol. 2007; 7(4): 367-74.
76. Khrunin A V., Moisseev A, Gorbunova V, Limborska S. Genetic polymorphisms and the efficacy and toxicity of cisplatin-based chemotherapy in ovarian cancer patients. Pharmacogenomics J [Internet]. 2010; 10(1): 54-61. Available from: http://dx.doi.org/10.1038/tpj.2009.45.
77. Tecza K, Pamula-Pilat J, Lanuszewska J, Butkiewicz D, Grzybowska E. Pharmacogenetics of toxicity of 5-fluorouracil, doxorubicin and cyclophosphamide chemotherapy in breast cancer patients. Oncotarget. 2018; 9(10): 9114-36.
78. Kim H-J, Im S-A, Keam B, Ham HS, Lee KH, Kim TY, et al. ABCB1 polymorphism as prognostic factor in breast cancer patients treated with docetaxel and doxorubicin neoadjuvant chemotherapy. Cancer Sci. 2015; 106(1): 86-93.
79. Abraham JE, Guo Q, Dorling L, Tyrer J, Ingle S, Hardy R, et al. Replication of genetic polymorphisms reported to be associated with taxane-related sensory neuropathy in patients with early breast cancer treated with paclitaxel. Clin Cancer Res. 2014; 20(9): 2466-75.
80. Marchand V, Angelergues A, Gobaux V, Hajage D, Kirova YM, Campana F, et al. Prospective and comparative evaluation of the toxicity of adjuvant

concurrent chemoradiotherapy after neoadjuvant chemotherapy for breast cancer. Am J Clin Oncol [Internet]. 2013; 36(5): 425-9.
81. Angelini S, Botticelli A, Onesti CE, Giusti R, Sini V, Durante V, et al. Pharmacogenetic Approach to Toxicity in Breast Cancer Patients Treated with Taxanes. Anticancer Res. 2017; 37(5): 2633-9.
82. Bray J, Sludden J, Griffin MJ, Cole M, Verrill M, Jamieson D, et al. Influence of pharmacogenetics on response and toxicity in breast cancer patients treated with doxorubicin and cyclophosphamide. Br J Cancer [Internet]. 2010; 102(6): 1003-9.
83. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: An update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol. 2013; 65(2): 157-70.
84. Sriamporn S, Black RJ, Sankaranarayanan R, Kamsa-ad S, Parkin DM, Vatanasapt V. Cancer survival in Khon Kaen province, Thailand. Int J Cancer. 1995; 61(3): 296-300.
85. Kongsiang A, Tangvoraphonkchai V, Jirapornkul C, Promthet S, Kamsa-ard S, Suwanrungruang K. Survival Time and Molecular Subtypes of Breast Cancer after Radiotherapy in Thailand. Asian Pac J Cancer Prev. 2014; 15: 10505-8.
86. Baghestani AR, Moghaddam SS, Majd HA, Akbari ME, Nafissi N, Gohari K. Survival Analysis of Patients with Breast Cancer using Weibull Parametric Model. Asian Pac J Cancer Prev. 2015; 16(18): 8567-71.
87. Djermoune SO, Oukkal M, Bouzid K, Denine R, Bonnabry P. Cancer survival in fi ve continents: a worldwide population-based study (CONCORD). Pharm Hosp Clin. 2016; 51(1): 40-50.
88. Che Y, You J, Zhou S, Li L, Wang Y, Yang Y, et al. Comparison of survival rates between Chinese and Thai patients with breast cancer. Asian Pac J Cancer Prev. 2014; 15: 6029-33.
89. Mohammad H, Khan R, Saxena A, Gabbidon K, Stewart TS, Bhatt C. Survival analysis for white non-hispanic female breast cancer patients. Asian Pac J Cancer Prev. 2014; 15: 4049-54.
90. Zaha DC, Lazar E, Lazureanu C. Clinicopathologic features and five years survival analysis in molecular subtypes of breast cancer. Rom J Morphol Embryol. 2010; 51(1): 85-9.
91. Zare N, Ghanbari S, Salehi A. Effects of two chemotherapy regimens, anthracycline-based and CMF, on breast cancer disease free survival in the eastern Mediterranean region and Asia: a meta-analysis approach for survival curves. Asian Pacific J Cancer Prev. 2013; 14(3): 2013-7.
92. Bhoo-Pathy N, Hartman M, Yip CH, Saxena N, Taib NA, Lim SE, et al. Ethnic differences in survival after breast cancer in South East Asia. PLoS One. 2012; 7(2).
93. Chuang S-C, Chen W, Hashibe M, Li G, Zhang Z-F. Survival rates of invasive breast cancer among ethnic Chinese women born in East Asia and the United States. Asian Pacific J Cancer Prev. 2006; 7(2): 221-6.

94. Ghanbari S, Ayatollahi SMT, Zare N. Comparing role of two chemotherapy regimens, CMF and anthracycline-based, on breast cancer survival in the eastern Mediterranean Region and Asia by multivariate mixed effects models: A meta-analysis. Asian Pacific J Cancer Prev. 2015; 16(14): 5655-61.
95. Zuo T, Zeng H, Li H, Liu S, Yang L, Xia C, et al. The influence of stage at diagnosis and molecular subtype on breast cancer patient survival: a hospital-based multi-center study. Chin J Cancer. 2017; 36(1): 84.
96. Møller H, Henson K, Lüchtenborg M, Broggio J, Charman J, Coupland VH, et al. Short-term breast cancer survival in relation to ethnicity, stage, grade and receptor status: National cohort study in England. Br J Cancer. 2016; 115(11): 1408-15.
97. Clèries R, Ameijide A, Buxó M, Martínez JM, Marcos-Gragera R, Vilardell ML, et al. Long-term crude probabilities of death among breast cancer patients by age and stage: a population-based survival study in Northeastern Spain (Girona-Tarragona 1985-2004). Clin Transl Oncol. 2018; 20(10): 1252-60.
98. Iqbal J, Ginsburg O, Rochon PA, Sun P, Narod SA. Differences in breast cancer stage at diagnosis and cancer-specific survival by race and ethnicity in the United States. JAMA - J Am Med Assoc. 2015; 313(2): 165-73.
99. Walters S, Maringe C, Coleman MP, Peake MD, Butler J, Young N, et al. Lung cancer survival and stage at diagnosis in Australia, Canada, Denmark, Norway, Sweden and the UK: A population-based study, 2004-2007. Thorax. 2013; 68(6): 551-64.
100. Yang J, Long Q, Li H, Lv Q, Tan Q, Yang X. The value of positive lymph nodes ratio combined with negative lymph node count in prediction of breast cancer survival. J Thorac Dis. 2017; 9(6): 1531-7.
101. Blenman KRM, He T-F, Frankel PH, Ruel NH, Schwartz EJ, Krag DN, et al. Sentinel lymph node B cells can predict disease-free survival in breast cancer patients. Npj Breast Cancer [Internet]. 2018; 4(1): 28. Available from: http://www.nature.com/articles/s41523-018-0081-7
102. Abd-Elhay FAE, Elhusseiny KM, Kamel MG, Low SK, Sang TK, Mehyar GM, et al. Negative lymph node count and lymph node ratio are associated with survival in male breast cancer [Internet]. Clinical Breast Cancer. 2018. Available from: https://doi.org/10.1016/j.clbc.2018.07. 003
103. Mahmood H, Faheem M, Mahmood S, Sadiq M, Irfan J. Impact of age, tumor size, lymph node metastasis, stage, receptor status and menopausal status on overall survival of breast cancer patients in Pakistan. Asian Pacific J Cancer Prev. 2015; 16(3): 1019-24.
104. Hung M, Xu J, Nielson D, Bounsanga J, Gu Y, Hansen AR, et al. Evaluating the prediction of breast cancer survival using lymph node ratio. J breast cancer [Internet]. 2018; 21(3): 315. Available from: https://synapse.koreamed. org/DOIx.php?id=10.4048/jbc.2018.21.e35

105. Rose BS, Jiang W, Punglia RS. Effect of lymph node metastasis size on breast cancer-specific and overall survival in women with node-positive breast cancer. Breast Cancer Res Treat. 2015; 152(1): 209-16.
106. Savas S. Useful genetic variation databases for oncologists investigating the genetic basis of variable treatment response and survival in cancer. Acta Oncol (Madr). 2010; 49(8): 1217-26.
107. Mak KS, Gainor JF, Niemierko A, Oh KS, Willers H, Choi NC, et al. Significance of targeted therapy and genetic alterations in EGFR, ALK, or KRAS on survival in patients with non-small cell lung cancer treated with radiotherapy for brain metastases. Neuro Oncol. 2015; 17(2): 296-302.
108. Rodríguez-Jiménez C, García-Saiz M, Pérez-Tamajón L, Salido E, Torres A. Influence of genetic polymorphisms of CYP3A5 and ABCB1 on sirolimus pharmacokinetics, patient and graft survival and other clinical outcomes in renal transplant. Drug Metab Pers Ther. 2017; 32(1): 49-58.
109. Peng J, Ma W, Zhou Z, Gu Y, Lu Z, Zhang R, et al. Genetic variations in the PI3K/PTEN/AKT/mTOR pathway predict tumor response and disease-free survival in locally advanced rectal cancer patients receiving preoperative chemoradiotherapy and radical surgery. J Cancer. 2018; 9(6): 1067-77.
110. Zhou F, Zhu M, Wang M, Qiu L, Cheng L, Jia M, et al. Genetic variants of DNA repair genes predict the survival of patients with esophageal squamous cell cancer receiving platinum-based adjuvant chemotherapy. J Transl Med. 2016; 14(1): 1-12.
111. Zhang R, Jia M, Xue H, Xu Y, Wang M, Zhu M, et al. Genetic variants in ERCC1 and XPC predict survival outcome of non-small cell lung cancer patients treated with platinum-based therapy. Sci Rep [Internet]. 2017; 7(1): 1-10. Available from: http://dx.doi.org/10.1038/s41598-017-10800-5
112. Sun Y, Wu Y, Li W, Kong Z, Zou X. Genetic polymorphisms in nucleotide excision repair pathway influences response to chemotherapy and overall survival in osteosarcoma. Int J Clin Exp Pathol. 2015; 8(7): 7905-12.
113. Qu F, Qiao Q, Wang N, Ji G, Zhao H, He L, et al. Genetic polymorphisms in circadian negative feedback regulation genes predict overall survival and response to chemotherapy in gastric cancer patients. Sci Rep. 2016; 6(February): 1-11.
114. Pardanani A, Abdelrahman RA, Finke C, Lasho TT, Begna KH, Al-Kali A, et al. Genetic determinants of response and survival in momelotinib-treated patients with myelofibrosis. Leukemia. 2015; 29(3): 741-4.
115. Syamala VS, Sreeja L, Syamala V, Raveendran PB, Balakrishnan R, Kuttan R, et al. Influence of germline polymorphisms of GSTT1, GSTM1, and GSTP1 in familial versus sporadic breast cancer susceptibility and survival. Fam Cancer. 2008; 7(3): 213-20.
116. Egan KM, Cai Q, Shu X, Jin F. The risk for breast cancer: results from the shanghai breast cancer study and meta-analysis genetic polymorphisms

in GSTM1, GSTP1, and GSTT1. Cancer epidem biomar. 2004; 13(February): 197-204.

117. Abbas M, Kushwaha VS, Srivastava K, Banerjee M. Glutathione S-transferase gene polymorphisms and treatment outcome in cervical cancer patients under concomitant chemoradiation. PLoS One. 2015; 10(11): 1-11.
118. Huang J, Tan P-H, Thiyagarajan J, Bay B-H. Prognostic significance of glutathione S-transferase-pi in invasive breast cancer. Mod Pathol. 2003; 16(6): 558-65.
119. Lizard-nacol S, Coudert B, Colosetti P, Riedinger J, Fargeot P, Brunet-lecomte P, et al. Glutathione S-transferase M1 null genotype: lack of association with tumour characteristics and survival in advanced breast cancer. Breast Cancer Res. 1999; 1: 81-7.

APPENDICES

APPENDIX A
SAMPLE SIZE ESTIMATION

In this study, there are 2 outcomes including; hematotoxicity and survival. To calculate the sample size of this study, survival is the most importance outcome therefore the sample size estimation for survival is:

$$n = \frac{(Z\alpha/2 + Z\beta)2}{(\log HR)2\ \pi1\ \pi2}$$

$$= 99$$

Therefore, The total sample size of 99 participants required for this study.

Parameters description:

Z^{β} = standard normal variate for power
(for 80% power it is 0.842)

$Z\alpha/2$ = standard normal variate for level of significance
($\alpha = 0.05$, $Z\alpha/2 = 1.96$)

HR = The ratio of hazard functions, or the expected increased/decreased likelihood to die of Group 1 versus Group 2
= 0.57 (65)

$\pi1$ = The proportion of the sample assigned to Group 1
= 0.5

$\pi2$ = The proportion of the sample assigned to Group 2
= 0.5

APPENDIX B

CERTIFICATE OF APPROVAL ETHICS COMMITTEE MAHIDOL UNIVERSITY

Documentary Proof of Exemption
Ethical Review Committee for Human Research
Faculty of Public Health, Mahidol University

Protocol Title :	ASSOCIATION BETWEEN GLUTATHIONE S-TRANSFERASE M1, T1 POLYMORPHISMS CLINICAL RESPONSE, TOXICITY AND TREATMENT OUTCOME AMONG BREAST CANCER PATIENTS WITH CHEMOTHERAPY
Protocol No. :	179/2560
Principal Investigator :	Miss Phakarat Tangkheunkan
Co-Investigator(s) :	Lect. Dr. Kitiphong Harncharoen Dr. Danai Tiwawech
Affiliation :	Doctor of Public Health (International Program) Faculty of Public Health, Mahidol University

This protocol complies with a "Research with Exemption" category

Date of Issue : 14 September 2017

The aforementioned project have been reviewed and approved according to the Standard Operating Procedures of Ethical Review Committee for Human Research, Faculty of Public Health, Mahidol University.

S. Natthamongkolchai

(Assoc. Prof. Dr. Sutham Nanthamongkolchai)
Chairperson of Ethical Review Committee for Human Research

420/1 Rajvithi Road, Bangkok, Thailand 10400
Tel. +66 2354 8543-9 ext. 1412, 1127 Fax. +66 2640 9854

APPENDIX C
CERTIFICATE OF APPROVAL ETHICS COMMITTEE
NATIONAL CANCER INSTITUTE

Certificate of Approval
Research Committee of National Cancer Institute

Title of Project	Association between Gluthathione S-Transferase M1,T1 Polymorphisms and clinical reponese,Toxicity and treatment outcome among Breast Cancer Patients with Chemotherapy
Project Number	195_2017T_OUT525
Principle Investigator	Miss Phakarat Tangkheunkan
Affiliation	Mahidol University
Date of Approval	December 18,2017

We confirm that the prior mentioned project has been approved by the Research Committee of National Cancer Institute.

Signature of Chairman .....
(Arkom Chaiwerawattana ,M.D.)

Signature of Director .....
(Weerawut Imsamran, M.D.)

APPENDIX D

HEPATIC AND RENAL FUNCTION TESTS BASELINE BEFORE ADMINISTERED THE FIRST CYCLE OF TREATMENT

Parameter	Normal Range	Mean	SD
Body surface area, BSA (m^2)	-	1.60	0.17
Body mass index, BMI (kg/m^2)	-	25.04	4.83
Height (cm)	-	155.20	6.20
Weight (kg)	-	60.16	12.53
BUN (mg/dL)	9-20	11.36	4.62
Creatinine (mg/dL)	0.5-1.2	0.77	0.22
Aspartate transaminase, AST (U/L)	5-40	23.42	15.17
Alanine aminotransferase, ALT (U/L)	5-40	23.35	22.62
Alkaline phosphatase, ALP (U/L)	35-125	72.56	21.25
White blood cell ($10^3/\mu L$)	5.2-12.4	7.59	2.51
Red blood cell ($10^3/\mu L$)	4.2-5.4	4.54	0.53
Hemoglobin, HGB (g/dL)	12-15	12.39	1.46
Hematocrit, HCT (%)	38- 46	36.94	3.96
Platelet, PLT ($10^3/\mu L$)	150-400	273.66	67.85
Neutrophil (%)	5.2-12.4	56.89	9.41
Lymphocyte (%)	4.2-5.4	41.80	57.22
ANC ($10^3/\mu L$)	> 1.5	4.46	1.90

APPENDIX E
MULTIPLEX REAL-TIME PCR AMPLIFIED MELT CURVES

Multiplex-real-time PCR Amplified Products for *GSTM1* and *GSTT1*. Beta-globin was used as internal control. Melting point of *GSTM1*, beta-globin, and *GSTT1* were 80.94˚C, 85.91˚C, and 89.0˚C respectively.

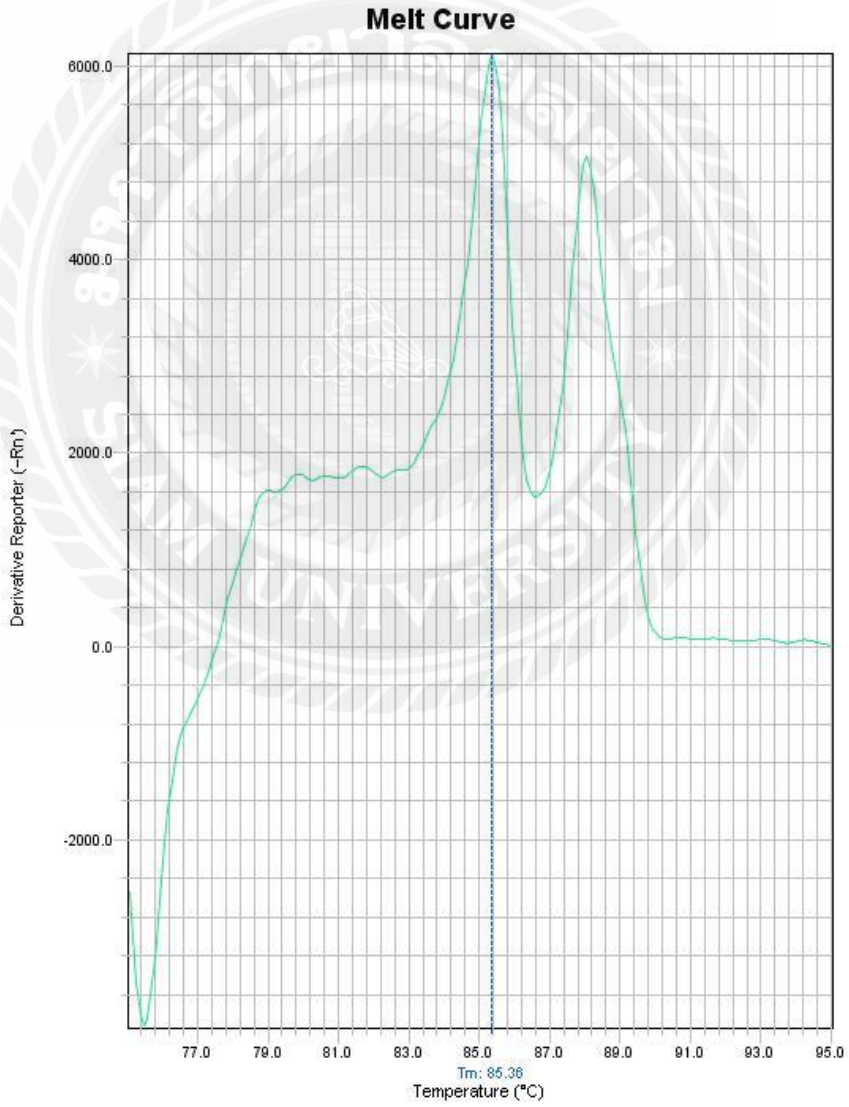


Figure E1 is GSTM1-/ GSTT1+, melting point was found at 85.91˚C and 89.0˚C.

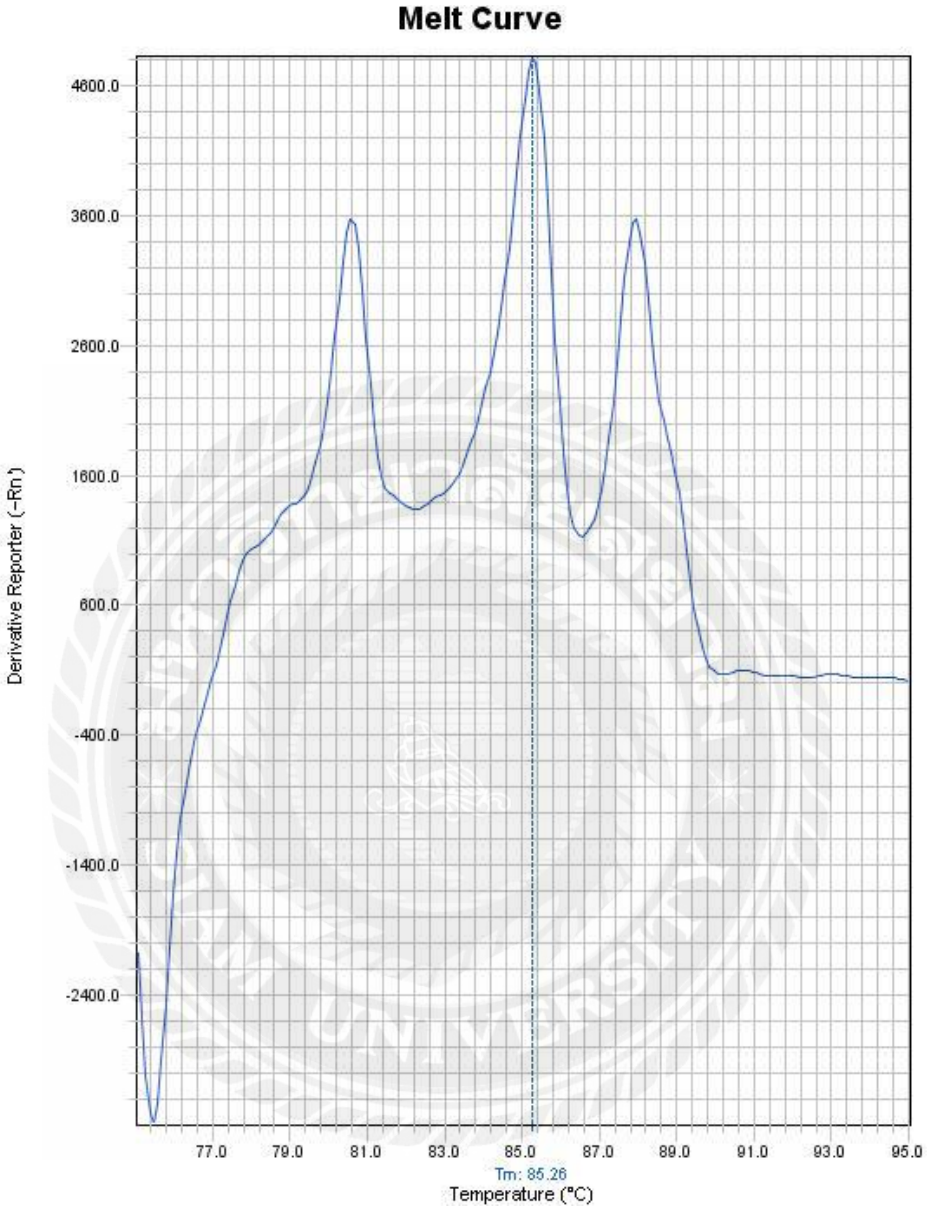


Figure E2 is GSTM1+ / GSTT1+, melting point was found at 80.94˚C, 85.91˚C and 89.0˚C.

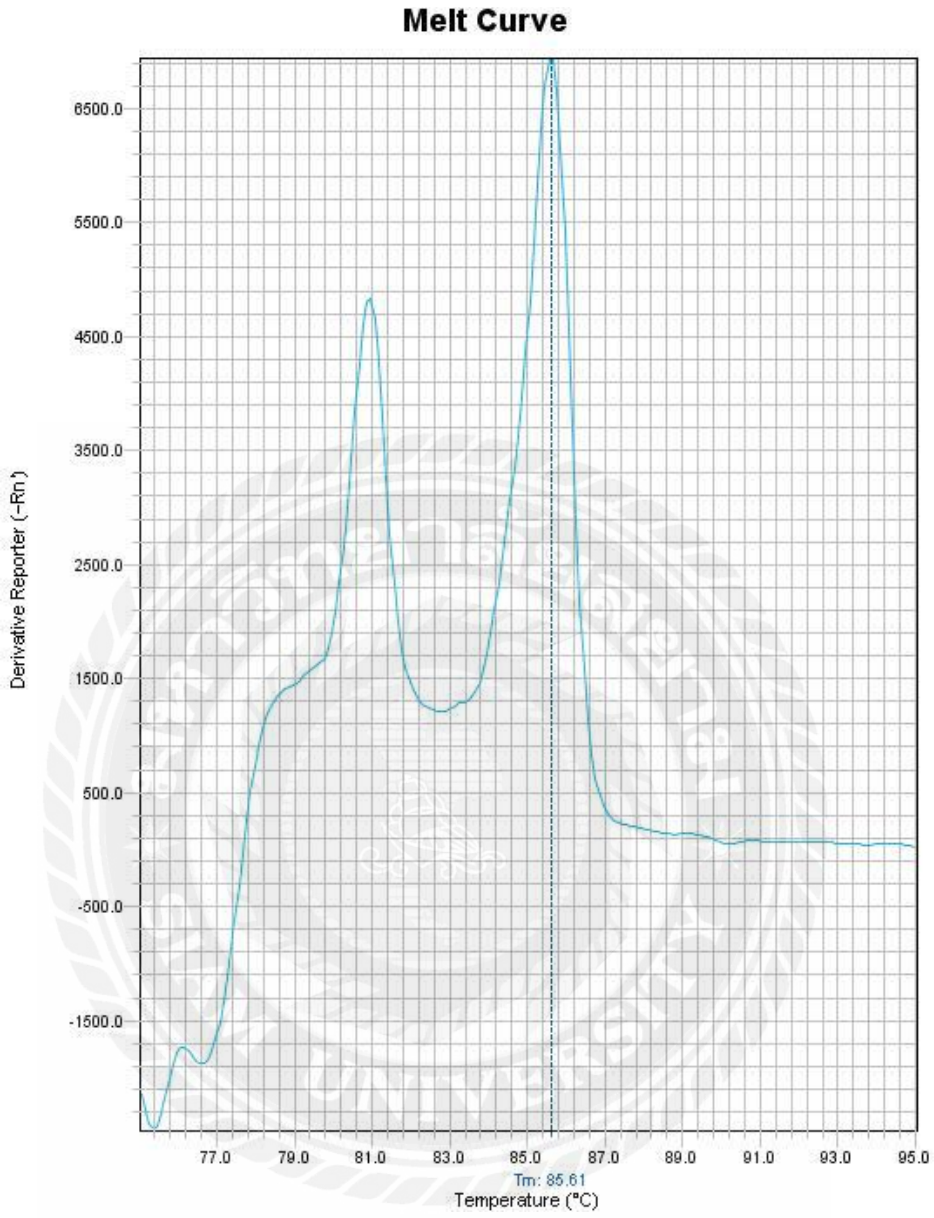


Figure E3 is GSTM1+ / GSTT1-, melting point was found at 80.94˚C and 85.91˚C.

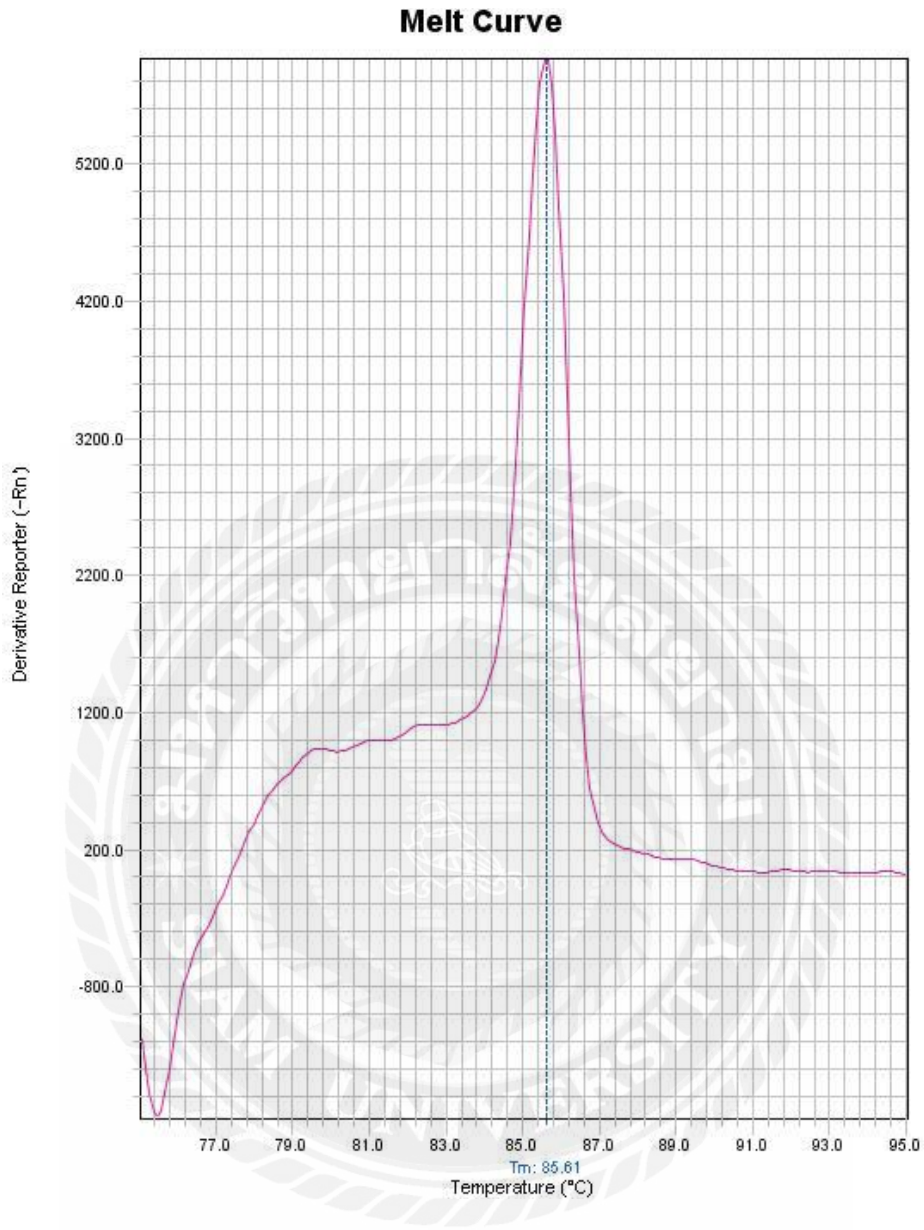


Figure E4 is GSTM1- / GSTT1-, melting point was found at 85.91˚C.