ASSOCIATION BETWEEN VITAMIN D AND OBESITY RELATED ADIPOKINES WITH BREAST CANCER OCCURRENCE AMONG WOMEN PATIENTS IN KELANTAN

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by

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LIST OF ABBREVIATIONS

AACE	Association of Clinical Endocrinologist
ASR	age-standardized incidence rate
BCSC	Breast Cancer Surveillance Consortium
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CAM	Complementary and alternative medicine
CI	Confidence interval
D_2	Ergocalciferol
D_3	Cholecalciferol
DBP	Vitamin D binding protein
DEXA	Dual-energy X-ray Absorptiometry
ECOG	Eastern Cooperative Oncology Group
ER	Estrogen receptor
ES	Endocrine Society
ESCEO	European Society for Clinical and Economic Aspect of Osteoarthritis
GLOBOCAN	Global Cancer Observatory: Cancer Today
HDL-C	High density lipoprotein cholesterol
HER2	human epidermal growth factor receptor 2
HMW	High molecular weight
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HR	Hazard ratio
hs-CRP	High-sensitivity C-reactive Protein
HUSM	Hospital Universiti Sains Malaysia
IDF	International Osteoporosis Foundation
IOM	Institute of Medicine
LMW	Low molecular weight
MMW	Medium molecular weight
NAD	Nicotinamide adenine dinucleotide
Nampt	Nicotinamide phosphoribosyl transferase
NCR	National Cancer Registry
NHANES	National Health and Nutrition Examination Survey

NHMS	National Health and Morbidity Survey
OR	Odds ratio
PR	Progesterone receptor
PBEF	Pre-B cell Colony Enhancing Factor
PTH	Parathyroid hormone
RFLP	Restriction fragment length polymorphism
RR	Relative risk
RXR	Retinoid X receptor
SNPs	Single-nucleotide polymorphisms
SRR	Summary relative risk
TC	Total cholesterol
TG	Triglyceride
TNBC	Triple-negative breast cancer
VDR	vitamin D receptor
U.K.	United Kingdom
U. S. A	United State 0f America
USDA	United States Department of Agriculture
USM	Universiti Sains Malaysia
UVB	Ultraviolet-B
WHO	World Health Organization
WC	Waist circumference
7-DHC	7-dehydrocholesterol
25 (OH) D	25-hydroxyvitamin D
25-OHase	25-hydroxylase
$1,25(OH)_2D$	1,25-dihydroxyitamin D

PERKAITAN ANTARA VITAMIN D DAN ADIPOKIN BERKAITAN OBESITI DENGAN KEJADIAN KANSER PAYUDARA DALAM KALANGAN PESAKIT WANITA DI KELANTAN

ABSTRAK

Kanser Payudara adalah karsinoma yang sering didiagnosis dalam kalangan wanita di seluruh dunia dan juga Malaysia. Banyak kajian dalam menentukan perkaitan antara vitamin D dan obesiti berkaitan adipokin dengan pembentukan kanser payudara, namun kajian yang mengkaji peranan yang mungkin faktor risiko ini masih kurang. Justeru, objektif kajian ini adalah untuk menentukan perkaitan antara serum vitamin D dan obesiti berkaitan adipokin (HMW adiponectin dan visfatin) dengan pembentukan kanser payudara. Kajian ini dijalankan di Kelantan, Malaysia dari Julai 2014 hingga Jun 2017. Satu kajian kawalan kes telah dijalankan sebagai pengambilan peringkat awal dan diteruskan dengan kajian susulan ke atas wanita kanser payudara pada 18 bulan selepas diagnosis. Pada peringkat awal kajian, 78 pesakit kanser payudara yang baru didiagnosis dan 85 wanita sihat mendaftar masuk kajian ini dan berumur dari 21 hingga 59 tahun. Pengumpulan data meliputi sosio-demografi, sejarah reproduktif, antropometri dan pengukuran tekanan darah, serta analisis biokimia klinikal. Analisis multiple logistic regression menunjukkan bahawa cotinin (OR = 1.22; 95% CI: 1.02, 1.48), lilit pinggang (WC) (OR = 1.10; 95% CI: 1.02, 1.20), trigliserida (TG) (OR = 4.93; 95% CI: 1.83, 13.2) dan glukosa semasa berpuasa (OR = 1.90; 95% CI: 1.25, 2.89) mempunyai kaitan yang signifikan dengan peningkatan risiko kanser payudara. Serum Vitamin D (OR = 0.89; 95% CI: 0.83, 0.95) dan Molekul berat tinggi (HMW) adiponektin (OR = 0.76, 95% CI: 0.59, 0.98) mempunyai kaitan yang signifikan dengan penurunan risiko pembentukan kanser payudara. Walau bagaimanapun, tidak terdapat

perkaitan yang signifikan antara polymophism Vitamin D reseptor (VDR) (FokI, BsmI dan TaqI) dengan kanser payudara yang telah diperhatikan. Pada 18 bulan selepas diagnosis, 39.4% mangsa kanser payudara mempunyai berat badan yang stabil dan kira-kira 30% mempunyai kenaikan dan penurunan berat badan masing-masing pada lebih daripada 5%. Model multiple linear regression menunjukkan bahawa BMI mempunyai hubungankait negatif dengan HMW adiponektin serum di peringkat awal (β = -0.35; 95% CI: -0.48, -0.21). Pada 18 bulan selepas diagnosis, hanya perubahan peratus LDL-C mempunyai hubungankait positif yang signifikan dengan perubahan peratus vitamin D (β = 1.43 (95% CI: 1.04, 1.83). Walaupun begitu, perubahan peratus LDL-C dan insulin ketika puasa mempunyai perkaitan dengan perubahan peratus yang lebih rendah dalam HMW adiponectin serum. Kesimpulannya, keputusan ini menunjukkan bahawa pendedahan kepada asap rokok pasif, mempunyai WC yang tinggi, TG yang tinggi dan glukosa ketika puasa yang tinggi merupakan faktor risiko penting untuk kanser payudara pada wanita dalam kajian ini. Di samping itu, serum vitamin D dan HMW adiponektin menunjukkan kesan perlindungan terhadap kanser payudara.

ASSOCIATION BETWEEN VITAMIN D AND OBESITY RELATED ADIPOKINES WITH BREAST CANCER OCCURRENCE AMONG WOMEN PATIENTS IN KELANTAN

ABSTRACT

Breast cancer is a common carcinoma diagnosed among women worldwide as well as Malaysia. Numerous studies reported the association between vitamin D and obesity related adipokines with breast cancer development, yet the possible roles of these risk factors remain scarce. Hence, the objective of this study was to determine the association between serum vitamin D and obesity related adipokines (HMW adiponectin and visfatin) with the development of breast cancer. This study was carried out in Kelantan, Malaysia from July 2014 until June 2017. A case-control study was conducted as a baseline recruitment and continued with follow-up of breast cancer women at 18 months post-diagnosis. At baseline 78 newly-diagnosed breast cancer patients and 85 healthy women aged 21 to 59 years old enrolled in this study. Data collection consisted of socio-demographics, reproductives history, anthropometrics and blood pressure measurement, as well as clinical biochemistry analysis. Multiple logistic regression analysis showed that cotinine (OR = 1.22; 95% CI: 1.02, 1.48), waist circumference (WC) (OR = 1.10; 95% CI: 1.02, 1.20), trigliseride (TG) (OR = 4.93; 95% CI: 1.83, 13.2) and fasting glucose (OR = 1.90; 95% CI: 1.25, 2.89) were significantly associated with increased risk of breast cancer. Serum vitamin D (OR = 0.89; 95% CI: 0.83, 0.95) and high molecular weight (HMW) adiponectin (OR = 0.76, 95% CI: 0.59, 0.98) were significantly related with decreased risk of breast cancer development. However, there was no significant association between Vitamin D receptor (VDR) polymorphism (FokI, BsmI and TaqI) with breast cancer was observed.

At 18 months post-diagnosis, 39.4% of breast cancer survivors had stable weight and approximately 30% of the had gained and loss weight at more than 5% respectively. Multiple linear regression model showed BMI was significantly associated with negative relationship with serum HMW adiponectin at baseline (β = -0.35; 95% CI: -0.48, -0.21). At 18 months post-diagnosis, only percent change of LDL-C showed significant linear positive relationship with percent change of serum vitamin D (β = 1.43 (95% CI: 1.04, 1.83). While, percent change of LDL-C and fasting insulin were significantly associated with lower percent change in serum HMW adiponectin. In conclusion, these results suggest that exposure to passive smoke, high WC, high TG and high fasting blood glucose were important risk factors for breast cancer in women from this study. In addittion, serum vitamin D and HMW adiponectin showed a protective effect towards breast cancer.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The increasing burden of cancer poses a major challenge to global health (Bray et al., 2018; GLOBOCAN, 2020), with epidemiological data linking cardiovascular diseases, diabetes, and obesity to multiple types of cancer, including breast cancer. According to the Global Cancer Observatory: Cancer Today (GLOBOCAN), the incidence of breast cancer has increased from an estimated 1.67 million cases in 2012 to 2.09 million in 2018 and 8 418 new cases were reported in 2020 (Torre et al., 2015; Bray et al., 2018; GLOBOCAN, 2020). Although Indonesia has the highest prevalence of breast cancer among the South-East Asian countries (Bray et al., 2018), the dramatic rise in incidence in Malaysia should be of concern.

In parallel with cancer burden, obesity and its comorbidities also pose challenges to global health and have been identified as a worldwide pandemic (Ng et al., 2014). Globally, the prevalence of overweight and obese adults has risen by 28.0% and is increased among developing countries (Ng et al., 2014). According to the National Health and Morbidity Survey, the prevalence of obesity among Malaysian adults increased from 14.0% in 2006 to 17.7% in 2015, and this growing trend is expected to continue (Institute for Public Health, 2008; Institute for Public Health, 2015).

Vitamin D is traditionally linked to bone homeostasis and mineral metabolism (Holick, 2007). However, over the last two decades, there has been growing interest in the non-skeletal actions of vitamin D and their potential association with chronic diseases

(Wimalawansa, 2018) as vitamin D receptors (VDR) have been discovered in various other tissues such as the kidney, brain, prostate, breast, colon, pancreas, and immune cells (Manson et al., 2012). Furthermore, findings from in-vivo experiments have shown that vitamin D has a novel role in cell differentiation and tumour progression (Hendrickson et al., 2011). Since then, intensive research has been carried out globally to investigate the connection between vitamin D and the occurrence of chronic diseases as well as cancer. Moreover, previous research also suggested that VDR gene polymorphism might be contributed to the development of breast cancer (Shahbazi et al., 2013).

An extensive study by Shanmugalingam et al. (2014) demonstrated a correlation between obesity, insufficient vitamin D, and the incidence of breast cancer, while other studies reported an inverse correlation between the level of vitamin D and the risk of breast cancer (O'Brien et al., 2017; McDonnell et al., 2018) which may be affected by other factors such as menopausal status and obesity. Being obese with vitamin D insufficiency can have an enormous potential impact on many aspects of human health, creating a public health issue.

The function of adipose tissue is typically described as storage and energy regulation, (Poulos et al., 2010) and it is characterised as a complex and metabolically active endocrine organ that secretes several hormones known as adipokines (Ouchi et al., 2014). The common adipokines associated with breast cancer development include leptin, resistin, adiponectin, visfatin, and chemerin (Jarde´ et al., 2011; Dalamaga et al., 2012). A previous study showed that a high circulation level of high molecular weight (HMW) adiponectin significantly reduced the risk of breast cancer (Guo et al., 2015). However, elevated visfatin concentration was revealed to significantly increase the risk of breast

cancer (Dalamaga et al., 2011). In addition, there have been studies reporting that vitamin D may play a role in regulating the function of adipocytes and also take part in the synthesis and modulation of adipokines production (Kong et al., 2013; Walker et al., 2014).

Adipose tissue dysfunction can also contribute to increased sequestration of vitamin D and impair vitamin D release in obese people, which may ultimately lead to the development of insulin resistance (Szymczak-Pajor & Śliwińska, 2019). Obesity is strongly correlated with insulin resistance (Blüher, 2016), with studies showing that the insulin resistance indicators, HOMA-IR, and glucose, were closely associated with the increased risk of breast cancer (Sieri et al., 2012; Hernandez et al., 2014). However, studies examining connections between adipocytes function, obesity-related adipokines, vitamin D status, and insulin resistance to breast cancer development remain scarce and inconclusive, including in Malaysia. Therefore, this study aimed to investigate the connection between vitamin D status, obesity-related adipokines, and insulin resistance to breast cancer development.

1.2 Study Rationales

The drastic increase in breast cancer incidence in parallel with the obesity pandemic (Chooi, Ding & Magkos, 2019) requires urgent attention to illuminate the major obesity-related risk factors that lead to the development of breast cancer. Obesity is known as a modifiable risk factor for breast cancer (Gershuni, Ahima, & Tchou, 2016) and despite larger and improved studies being carried out over time, findings have still been inconclusive, and the complex association of obesity and breast cancer development has

yet to be uncovered. For instance, several studies investigating the association between obesity and breast cancer risk among pre-and postmenopausal women yielded inconclusive results (Biglia et al., 2013; John et al., 2015; Iqbal et al., 2015). This study will therefore provide the necessary information on the correlation between obesity and the risk of breast cancer among women in Malaysia.

Previous studies have also shown that vitamin D deficiency is common among people living in higher latitude countries (Cashman et al., 2016). Surprisingly, a high prevalence of vitamin D deficency is also found in countries near to the equator that have sufficient sunlight all year round, including Malaysia (Moy, 2011; Chin et al., 2014; Thanasitthichai et al., 2014; Neo & Kong, 2016; Smith et al., 2016). In addition, there have been reports that women who have breast cancer have lower serum vitamin D concentrations compared to healthy women (Park et al., 2015; Jamshidinaeini et al., 2016). To date, there has only been a single study that reported on the status of vitamin D among women with breast cancer in Malaysia (Jauhari et al., 2018). However, this study conducted a retrospective nested case control study which consisted of Malaysian Breast Cancer Cohort Study (MyBCC) subjects as the cases and UMMC Mammogram Cohort Study subjects as the hospital control (Jauhari et al., 2018).

There are several factors that have been investigated by other observational studies that affect the vitamin D status included sun exposure, phototype, dietary intake, sociodemographics, anthropometric, lifestyle data, and genetic polymorphisms (Moy, 2011;Thanasitthichai et al., 2014; Touvier et al., 2015; Smith et al., 2016). As factors associated with vitamin D deficiency among Malaysians have not been widely explored,

therefore, this study is expected to help bridge the gap in the current understanding of vitamin deficiency, especially related to breast cancer.

Although there was an epidemiological study that suggested that vitamin D may reduce the risk of breast cancer (O'Brien et al., 2017), the findings in the study were inconsistent, and there were still many gaps that existed in the understanding of these associations, which therefore limited our ability to conclude the existence of a causal association. Some studies have shown a stronger correlation between vitamin D and breast cancer in premenopausal women (Jamshidinaeini et al., 2016; Estébanez et al., 2018), while others displayed contradictory results (Eliassen et al., 2011; Wang et al., 2013). In addition, previous studies have illustrated that there was a possible association between polymorphism in the vitamin D receptor (VDR) gene and breast cancer development (Shahbazi et al., 2013; Li et al., 2016). However, to our knowledge, no studies have been published on VDR gene polymorphism and breast cancer risk among Malaysian women.

There is strong biological evidence that obese women are more likely to have lower levels of vitamin D (Shi et al., 2014), indicating potential interactions between obesity and vitamin D (Acevedo et al., 2016; Walsh, Bowles & Evans; 2017). In Malaysia, two cross-sectional studies conducted among Malay women indicated that, obesity was associated with vitamin D status (Moy, 2011; Shafinaz & Moy, 2016). However, this relationship has not been extensively studied among the Asian population, including Malaysian women, and data on the vitamin D status among women with breast cancer in Malaysia is still lacking. Therefore, the major aim of this thesis was to measure serum vitamin D concentrations and obesity related adipokines among women with breast cancer

and to understand further the associated risk factors of breast cancer among women in Malaysia.

Obesity may have a possible link to the risk of getting breast cancer, which is likely explained by the alteration of adipokines secretion which leads to insulin resistance (Divella et al., 2016). These alterations are related to the mechanism of carcinogenesis, progression, and tumour metastases (Nieman et al., 2013), with lower concentrations of HMW adiponectin and elevated serum visfatin potentially leading to the development of insulin resistance and ultimately contributing to breast cancer progression (Divella et al., 2016; Nicholson et al., 2018). Similarly, the role of vitamin D in the development of breast cancer is also associated with the disturbance of pancreatic beta-cell function which leads to insulin resistance and hyperinsulinemia (Danescu et al., 2009).

HMW adiponectin and visfatin are associated with the risk of breast cancer in numerous studies (Minatoya et al., 2014; Guo et al., 2015; Assiri et al., 2015). To date, studies on obesity-related adipokines in Malaysia are still limited, particularly among women with breast cancer. It was therefore anticipated that this study would provide knowledge on the relationship of obesity-related adipokines with breast cancer development. As cases of breast cancer have been increasing at an alarming rate in Malaysia, urgent attention is required to identify new prognostic biomarkers involved in breast cancer that help make an early diagnosis, monitor tumour progression, and optimise medical management. Cancer research mainly focused on the roles of adipokines and vitamin D separately, thus ignoring the possible connection between the above-mentioned vital parameters and the possible role of insulin resistance. Furthermore, few cancer studies reported on the relationship between breast cancer and novel adipokines such as

HMW adiponectin and visfatin. Since both vitamin D and adipokines are involved with adipose tissue in the endocrine system, there may be a link between these two to the development of breast cancer.

1.3 Significance of the Study

This study comprised comprehensive parameters including nutritional status, reproductive health factors, and lifestyle factors such as smoking status, to examine the risk factors among women with breast cancer. Furthermore, blood serum was also taken to evaluate the glucose and lipid profile, insulin resistance, vitamin D deficiency, and adipokine levels. It was anticipated that these fundamental data would provide information to the literature pertinent to breast cancer management in Malaysia. Essentially, these findings may dictate the potential research directions and may be of interest to other researchers for better comprehension, wider perspectives, and provide motivation for further investigation of current or new breast cancer studies.

Despite various women's awareness campaigns that were launched in Malaysia at either the national, state, or community levels, unfortunately, to date, there is still no visible impact on reducing the prevalence of cancer and obesity among women, with the incidence of breast cancer and obesity rates still increasing annually. It is therefore hoped that the information obtained from this study on vitamin D and obesity-related adipokines among women with breast cancer would improve the prevention and treatment of breast cancer. In addition, this study supports the negative impact of obesity against breast cancer, thus promoting a healthy lifestyle in terms of weight management is also one key aspect of this study.

1.4 Objectives

1.4.1 General Objective

To investigate the association between vitamin D and obesity related adipokines with breast cancer occurrence among women patients in Kelantan.

1.4.2 Specific Objectives

1.4.2 (a) Phase I: A Case-control study

i. To determine the smoking status, reproductive factors, nutritional status, vitamin D receptor gene polymorphisms (FokI, TaqI and BsmI) among breast cancer and healthy women.

ii. To compare the level of lipid profiles, insulin resistance markers, vitamin D, and adipokines (HMW adiponectin and visfatin) between breast cancer and healthy women.

iii. To investigate the associations of smoking status, obesity indicators, insulin resistance markers, vitamin D receptor gene polymorphisms (FokI, TaqI and BsmI) vitamin D and adipokines (HMW adiponectin and visfatin) with breast cancer development.

1.4.2 (b) Phase II: Follow-up study

i. To determine the weight change pattern from diagnosis to 18 months post-diagnosis among breast cancer survivors.

ii. To compare the mean of serum anthropometric characteristics, lipid profiles, insulin resistance markers, vitamin D and HMW adiponectin, from diagnosis to 18 months post-diagnosis among breast cancer survivors.

iii. To investigate the association between anthropometric characteristics, lipid profiles, and insulin resistance markers with serum vitamin D at baseline and 18 months post-diagnosis among breast cancer survivors.

iv. To investigate the association between anthropometric characteristics, lipid profiles, and insulin resistance markers with serum HMW adiponectin at baseline and 18 months post-diagnosis among breast cancer survivors.

1.5 Research Questions

There are several research questions that was addressed in this study include:

1.5.1 Phase I: A Case-control study

i. What is the smoking status, reproductive factors, nutritional status, and vitamin D receptor gene polymorphism (FokI, TaqI and BsmI) and of breast cancer and healthy women?

ii. Is the mean of lipid profiles, insulin resistance, vitamin D, and adipokines (HMW adiponectin and visfatin) differ significantly between breast cancer and healthy women group?

iii. Is there any association between smoking status, obesity indicators, insulin resistance, vitamin D receptor gene polymorphisms (FokI, TaqI and BsmI) vitamin D and adipokines (HMW adiponectin and visfatin) with breast cancer development?

1.5.2 Phase II: Follow-up Study

i. Is there any weight change from the time of diagnosis to 18 months post-diagnosis among breast cancer survivors?

ii. Is there any significant difference in the mean value of anthropometric characteristics, lipid profiles, insulin resistance, vitamin D and HMW adiponectin, from diagnosis to 18 months post-diagnosis among breast cancer survivors?

1.6 Alternative hypotheses

1.6.1 Phase I: A Case-control study

i. Hα: There are significant difference of mean vitamin D, serum adipokines, lipid profiles, and insulin resistance between breast cancer and healthy group.

ii. Hα: There is a significant association between smoking status with breast cancer development.

iii. Hα: There is a significant association between obesity indicator with breast cancer development.

iv. Hα: There is a significant association between vitamin D with breast cancer development.

v. Hα: There is a significant association between serum adipokines with breast cancer development.

vi. Hα: There is a significant association between insulin resistance with breast cancer development.

vii. Hα: There is a significant association between vitamin D receptor gene polymorphisms (FokI, TaqI and BsmI) with breast cancer development.

1.6.2 Phase II: Follow-up Study

i. Hα: There are significant difference of mean vitamin D, serum HMW adiponectin, lipid profiles, and insulin resistance from time of diagnosis to 24 months post-diagnosis.

ii. Hα: There is a significant association between weight change and insulin resistance on the serum vitamin D concentration among breast cancer survivors.

iii. Hα: There is a significant association between weight change pattern and insulin resistance markers with the HMW adiponectin among breast cancer survivors.

1.7 Conceptual Framework

The background of the study is illustrated in Figure 1.1. Obesity can lead to many comorbidities and is reported to have a potential correlation with breast cancer development. Obese people tend to have lower vitamin D concentrations and altered secretion of adipokines (low HMW adiponectin and elevated visfatin) which could lead to the development of insulin resistance and breast cancer. Several risk factors that lead to obesity, including poor diets such as high-fat diets and low physical activity, have been addressed in the previous study. Furthermore, various factors can affect an individual's vitamin D status such as sunscreen use, skin pigment, ageing, season, clothing as well as indoors more. Vitamin D deficiency and abnormal secretion of adipokines have been shown to have important connections with insulin resistance, resulting in breast cancer. The VDR gene is a member of the nuclear receptor superfamily, which is expressed in breast tissues and known to modulate the rate of cell proliferation. Several studies have indicated an association between VDR gene polymorphisms and cancer development. However, the relationship between VDR polymorphisms and breast cancer is uncertain and has not been proven in all studies.

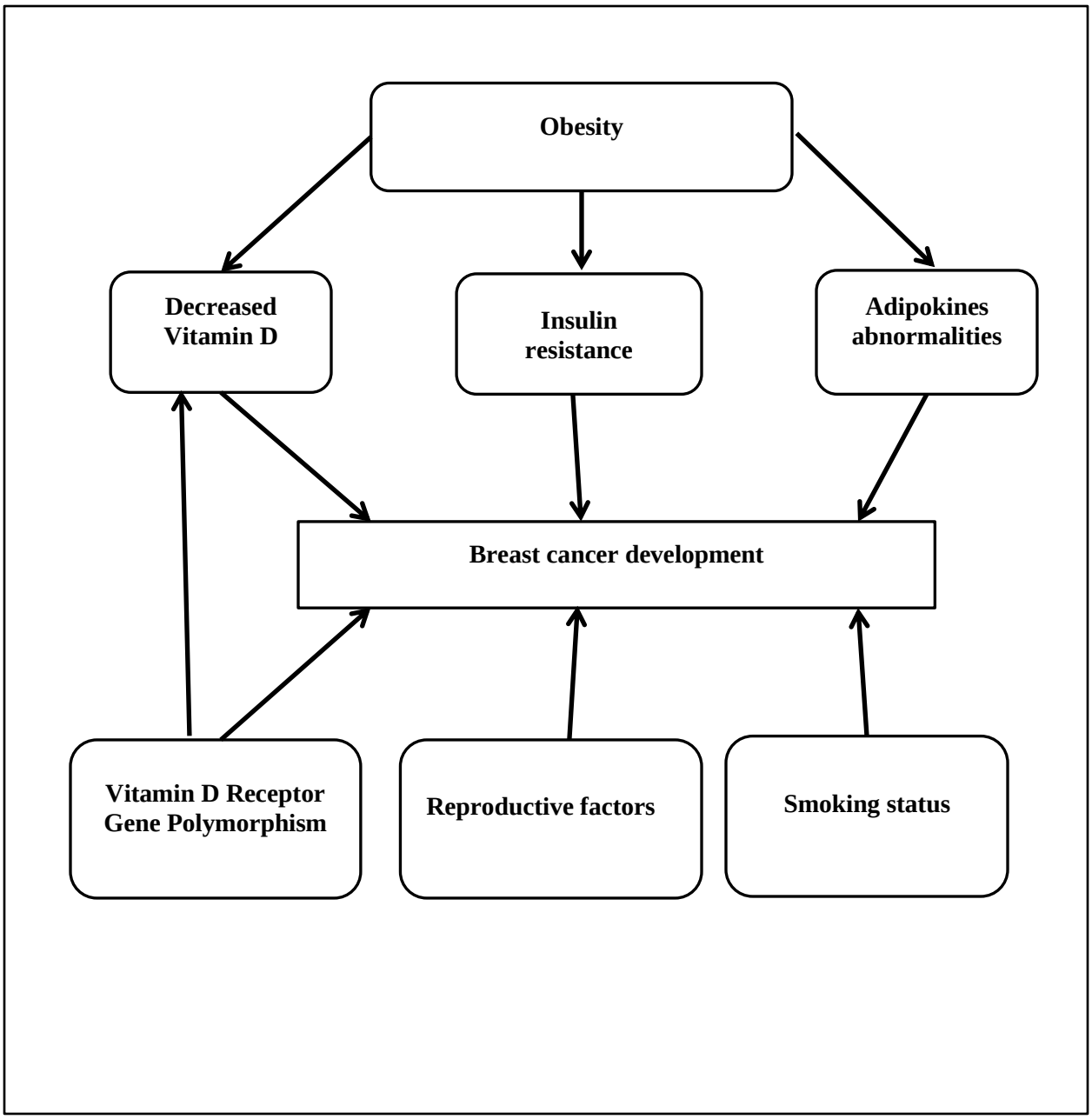


Figure 1.1 The conceptual framework of the study

1.8 Definition of Terms

Adipokines

- A protein that is secreted from (and synthesised by) adipocytes (include adiponectin, and visfatin).

Vitamin D

- A prohormone produced photochemically in the skin.

Polymorphism

- A mutation with an allele frequency of at least 1% in a given population, are subtle DNA sequence variations which occur often in the population and can have modest but real biological effects.

Insulin resistance

- A given concentration of insulin produces a less-than-expected biological effect.

Premenopausal status

- The phase of menstruation neither normal nor irregular period.

Postmenopausal status

- A permanent cessation of menstrual periods for at least 12 months.

Age at menarche

- Self-reported and defined as the chronological age (in years) when the individual had her first menses.

Age at first full-term pregnancy

- The age of the participant at the time of her first pregnancy that resulted in a birth between 39 weeks 0 days and 40 weeks 6 days gestation.

First degree relatives

- This is include the mother, sisters, and daughters.

Second degree relatives

- This is include grandmothers, aunts, cousins, and granddaughters.

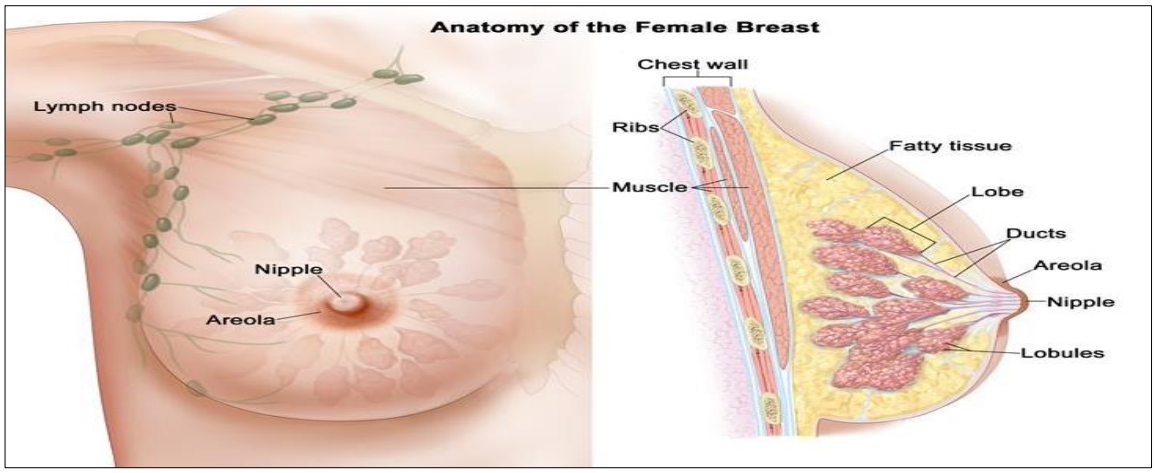
CHAPTER 2

LITERATURE REVIEW

2.1 What is a Breast?

The breast or mammary gland is a glandular organ located on the chest and is mainly made up of lobules (milk-producing glands), ducts (tiny tubes that carry the milk from the lobules to the nipple), and stroma (fatty tissues and connective tissues surrounding the ducts and lobules, blood vessels, and lymphatic vessels) as shown in Figure 2.1 (Bistoni and Farhadi, 2015; National Cancer Institute, 2017; Place et al., 2011).

Generally, the breast is used to produce milk. Breast development begins during puberty, advances by menarche and its final differentiation only occurs during pregnancy and lactation (Colditz, Baer, & Tamimi, 2006). After menopause, the hormone levels decline, and breast cells do not continue to divide. Earlier evidence suggested that breast cells are potentially more susceptible to exposure from menarche to first birth when breast tissues are undifferentiated (Colditz & Frazier, 1995; Okasha, McCarron, Gunnell, & Smith, 2003; Russo, Moral, Balogh, Mailo, & Russo, 2005) or during pregnancy when the breast tissues are growing (Kelsey & Berkowitz, 1988). Some evidence also shows that earlier life exposures might be important (Kelsey & Berkowitz, 1988; Okasha et al., 2003); thus, exposures during the critical periods of breast development are more likely to influence breast cancer development.



adopted from the National Cancer Institute (2017)

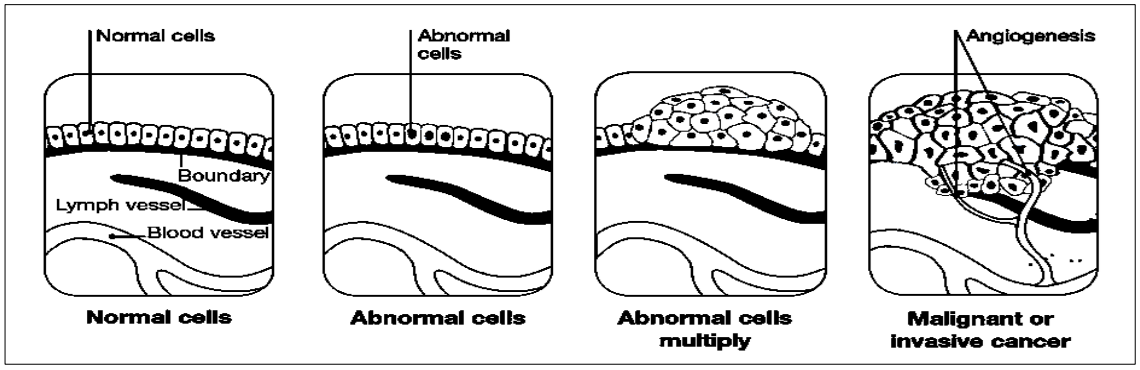
Figure 2.1 A diagram of female breast anatomy

2.2 What is Breast Cancer?

Breast cancer is a cancer that starts in breast tissues where normal cells grow uncontrolled and unregulated then become abnormal (carcinoma cells) as shown in Figure 2.2. It is adenocarcinoma (Weigelt & Reis-Filho, 2009) that occurs primarily in two forms: ductal or lobular carcinoma, in which malignancy develops in the breast ducts or lobules, respectively (American Cancer Society, 2015). However, most breast cancer incidence is ductal in origin. On top of that, breast cancer is also a heterogeneous disease with multiple tumor subtypes, where each subtype is differentially associated with various risk factors. These subtypes are also classified based on molecular subtypes, including tumors that express hormone receptors (estrogen receptor [ER] or progesterone receptor [PR]) that are classified as Luminal A or Luminal B subtypes (Lukong, 2017). Meanwhile, tumors that express human epidermal growth factor receptor 2 (HER2) and basal-like tumors are primarily classified as hormone receptor-negative. HER2 type refers to hormone receptors

[both estrogen receptor (ER) and progesterone] negative, while the triple-negative (TNBC) tumors are all negative for ER, progesterone receptor (PR), and HER2, and many of them express basal-like features (Toi et al., 2010; Anderson et al., 2014).

Receptors are proteins in or on certain cells that can attach to certain substances such as hormones that circulate in the blood. Normal breast cells and some breast cancer cells contain receptors that attach to estrogen and progesterone (Lange, 2008). These two hormones often fuel the growth of breast cancer cells. According to Kohler et al. (2015), subtype HER2- is the most common subtype diagnosed among women in the United States. Meanwhile, a study conducted in Sarawak, Malaysia on 1,034 cases of women with breast cancer showed overall findings of 48% luminal A (ER+/PR+/HER2−), 29% triple-negative (ER−/PR−/HER2−), 12% triple-positive (ER+/PR+/HER2+), and 11% HER2-overexpressing (ER−/PR−/HER2+) subtypes were observed. Additionally, it was reported that HER2-positive and triple-negative (TNBC) subtypes were more common among Malays (29% and 33%, respectively) than Chinese (22% and 23%, respectively) (Devi et al., 2012).



adopted from Cancer Council Australia (2016)

Figure 2.2 Diagram of development of carcinoma cells

2.3 Epidemiology of Breast Cancer

Breast cancer is a frequently diagnosed cancer among women worldwide (IARC, 2020). According to GLOBOCAN, new breast cancer cases have increased from about 1.67 million in 2012 to 2.09 million cases in 2018 and there were 2.26 million of new cases reported in 2020 (Torre et al., 2015; Bray et al., 2018; IARC, 2020). Of all cancer cases among women, breast cancer accounted for 25% in 2012 and 24.2% in 2018, and it has become a leading cause of cancer mortality in most countries worldwide. In 2018, the highest incidence rates were documented in Australia, New Zealand, and Western and Northern Europe with an age-standardized incidence rate (ASR) between 94.2 and 92.6 per 100,000. On the other hand, the lowest incidence rates were reported in South Central Asia with an ASR of 25.9 per 100,000 women (Bray et al., 2018).

Breast cancer incidence has been rising in most countries and regions of the world in the past few decades. Similar patterns have also been observed in Malaysia where the incidence increases year by year. Based on the report by the GLOBOCON in 2020, Malaysia had recorded 8418 new cases of breast cancer. Breast cancer also had a 20.7% mortality rate. Figure 2.3 shows breast cancer incidence in Malaysia as reported by the National Cancer Registry (NCR), the Ministry of Health Malaysia. According to the NCR, the trends of breast cancer among Malaysian women increased from 30.4% in 2002 to 34.1% in 2016. Unfortunately, the latest statistcis reported by NCR latest by 2016 cases.

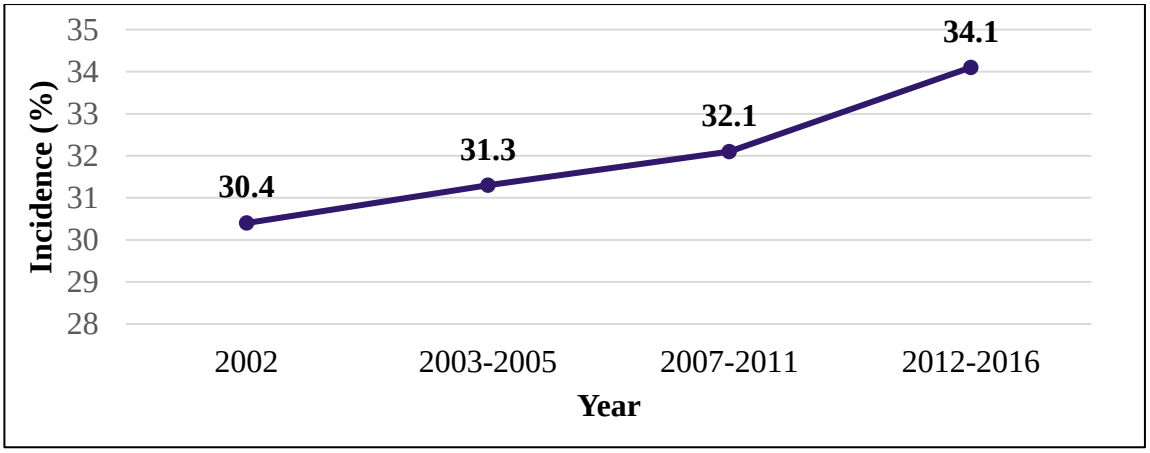


Figure 2.3 Trends of breast cancer incidence in Malaysia according to the National Cancer Registry Report

2.4 Classical Risk Factors of Developing Breast Cancer

A risk factor is anything that affects the chance of getting a disease, such as cancer. This section briefly reviews the classical breast cancer risk factors. Epidemiological studies have suggested that breast cancer risk is affected by gender (Ferlay et al., 2015, age (Shoemaker et al., 2018), history of benign breast disease, breast cancer family history (particularly in a first-degree relative, such as mother and sister) (Colditz et al., 2012), genetic and epigenetic alterations, hormonal and reproductive factors (Fortner et al., 2019), indicative of estrogen exposure (such as age at first period, age at first pregnancy, parity, age at menopause), use of hormonal drugs and high endogenous sex steroid hormone levels, and behavioral and lifestyle factors such as diet, weight, smoking status, and alcohol intake (Barukčić, 2019).

2.4.1 Gender

Being a woman is more susceptible to developing breast cancer (Ferlay et al., 2015). Breast cancer is the leading carcinoma among women worldwide as well as in Malaysia; however, men can also develop breast cancer, but this tumor is about less than 1% of all cancer types in men (Anderson et al., 2009). The United States in 2017 estimated 2,470 breast cancer cases diagnosed among men (Siegel et al., 2017), while a five-year view of surgery done in Hospital Universiti Kebangsaan Malaysia (HUKM) reported that only 1.6% of breast cancer cases involve men (Ngoo et al., 2009). Additionally, a large international population-based study reported that breast cancer among men represented only 0.6% of all breast cancer cases (Miao et al., 2011). The lifetime risk of breast cancer for a man is approximately 1:100 compared to 1:8 for a woman (Giordano, 2018). This is probably because men have fewer female hormones of estrogen and progesterone, which can promote breast cancer cell growth (Giordano, 2018).

2.4.2 Age

The risk of developing breast cancer increases as women get older, and cases are frequently reported in women around menopause age of 45 and older (Shoemaker et al., 2018). Although breast cancer is unusual among younger women, the younger age at diagnosis was found as one of the poor prognostic factors for breast cancer survival (Warner et al., 2013; Lian et al., 2017). However, breast cancer incidence in younger women has shown an increasing trend in recent years (Guo et al., 2018). Ethnic differences have also been documented in breast cancer incidence involving all ages and breast cancer mortality in younger women (Shoemaker et al., 2018). Black women are more likely to

be diagnosed at younger ages and with the more biologically aggressive triple-negative subtype (Shoemaker et al., 2018). In Malaysia, approximately more than 50% of women were diagnosed before the age of 50 years old (Nordin et al., 2018), compared to 20% in Western countries (Yip et al., 2014).

2.4.3 Family history

One of the classical risks of breast cancer is family history, such as the type of familial affected, age at which the relative developed breast cancer, and the number of relatives affected. Familial susceptibility to breast cancer accounts for <25% of all breast cancer cases. Observational studies indicated that breast cancer risk is higher among women whose close blood relatives have this disease. In a large prospective cohort study within Breast Cancer Surveillance Consortium (BCSC), first-degree family history with increased risk of invasive breast cancer in women aged 65 years and older, the hazard ratio (HR) was 1.48 (95% CI: 1.35, 1.61) for women who were 65 to 74 years old and 1.44 (95% CI: 1.28, 1.62) for women who were 75 years and older (Braithwaite et al., 2018). According to Colditz et al (2012), women with a family member diagnosed with breast cancer before the age of 50 had an increased risk of breast cancer development compared to women with family members diagnosed at older ages. A study by the BCSC also indicated that 15% of women have a first-degree family history of breast cancer (Phipps et al., 2011). This particular study examined the association between all breast cancer subtypes with first-degree family history and found a 1.56–1.73-fold increased breast cancer risk in women with a first-degree family history. Furthermore, there was a 1.47–1.63-fold increased risk of breast cancer in women with one (or more than one)

affected relative, and a 2.05–2.66-fold increased risk in women with at least two affected relatives compared to women with no family history (Phipps et al., 2011).

2.4.4 Reproductive factors

Aside from gender, age, and family history, reproductive history is the strongest known modifier factor for the risk of developing breast cancer among women. Nulliparous women are at greater risk for developing breast cancer compared to porous women (Nelson et al., 2012). A recent study has shown that porous women had a reduced risk of breast cancer with an HR of 0.82 (95% CI: 0.77, 0.88) (Fortner et al., 2019). The study on African American women also coincides with Fortner et al. (2019) that porous women had a reduced risk of developing breast cancer compared to nulliparous women (OR = 0.92; 95% CI: 0.81, 1.03), although not statistically significant (Palmer et al., 2014). A study on 158,189 Korean women with breast cancer investigated the association between parity and breast cancer subtypes. The study found that women who had more than three children were associated with a worse prognosis in triple-negative breast cancer (TNBC) (HR: 1.53; 95% CI: 1.11, 2.12) (Park et al., 2019). In addition, women with three or more childbirths had significantly reduced the risk of getting breast cancer compared to nulliparous women (RR = 0.73; 95% CI: 0.61, 0.87) (Nelson et al., 2012).

Epidemiological studies have reported a protective effect of early full-term pregnancy against breast cancer development (MacMahon et al., 1970; Albrektsen et al., 2005). According to a landmark case-control study by MacMahon et al. (1970), women who were less than 20 years old at first full-term pregnancy had a 50% lower risk of breast cancer than nulliparous women. The protective effects of parity do continue until the age of 35 years old, leading to a lower risk of developing breast cancer later in life (Meier-Abt

and Bentires-Alj, 2014; Dall, Risbridger & Britt, 2017). Interestingly, a recent study has evaluated the association between the duration of pregnancy and the long-term risk of developing breast cancer in a large Norwegian cohort (Husby et al., 2018). The study found that, on average, women who got pregnant at 33 weeks or less were not associated with a long-term breast cancer risk with 2.3% risk reduction per birth (95% CI: −10.0%, 13.2%. Meanwhile, on average, women who got pregnant at 34 weeks or longer were associated with a 12.9% risk reduction per birth (95% CI: 11.4%, 14.3%) (Husby et al., 2018).

Several mechanisms have been hypothesized for the association between parity and breast cancer development. It has been proposed that parity might induce changes in circulating hormones, especially in decreasing the levels of estrogen receptors in the breast (Dall, Risbridger, & Britt, 2017). Besides that, parity has also been suggested to increase the differentiation in the mammary gland tissues and it is further believed to permanently alter the gene expression profile and morphology of the parous mammary gland tissues to prevent the tumorigenic process (Russo, Rivera, & Russo, 1992; Russo & Russo, 2011).

Accumulative evidence from previous literature has suggested a protective role of breastfeeding with the risk of breast cancer (Faupel-Badger et al., 2013; Fortner et al., 2019). One landmark study published in 2002, which pooled approximately 50,000 breast cancer cases from 47 epidemiologic studies in 30 countries indicated that the relative risk (RR) for developing breast cancer in parous women decreased by 4.3% for every 12 months a woman breastfed and decreased by 7% from each birth independently (Collaborative Group on Hormonal Factors in Breast Cancer, 2002). However, Malaysia does not include in this reanalysis of epidemiological data. A recent prospective study