ASSOCIATION BETWEEN TYPES OF MAMMOGRAPHIC BREAST DENSITY AND HISTOLOGICAL SUBTYPES OF INVASIVE BREAST CARCINOMA

DR. NUR HANEEFA BINTI AHMAD

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR MASTER OF MEDICINE (RADIOLOGY)

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1. Associate Professor Dr. Juhara Haron as my supervisor, whom had assisted and provided guidance in carrying out this study until its completion.
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4. Radiographers, colleagues, and all the other staff in the Department of Radiology in Hospital USM.

DISCLAIMER

I declare that this dissertation records the results of the study performed by me and that it is of my own composition.

…….

(NUR HANEEFA BINTI AHMAD)

Date:

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

BestARi	Breast Cancer Awareness and Research Unit
BIRADS	Breast Imaging Reporting and Data System
BMI	Body mass index
DCIS	Ductal carcinoma in-situ
DFS	Disease-free survival
HPE	Histopathological examination
H&E	Hematoxylin and eosin
HT	Hormonal therapy
IDC-NOS	Invasive ductal carcinoma, not otherwise specified
LIS	Lab Information System
MD	Mammographic density
NST	No special type
OS	Overall survival
ST	Special type carcinoma
PACS	Picture archiving and communication system
PMD	Percent mammographic density
TNM	Tumor, node, metastasis
WHO	World health organization

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ABSTRAK

Latar belakang: Ketumpatan payudara mamografi merupakan salah satu faktor risiko yang dikenal pasti untuk kanser payudara. Ketumpatan payudara mammografi menunjukkan komponen lemak, tisu epithelial dan tisu penghubung yang mempunyai ciri-ciri yang tertentu di imej mammografi. Wanita yang mempunyai lebih ketumpatan payudara adalah lebih berisiko untuk mendapat kanser payudara. Klasifikasi kanser payudara berdasarkan histologi boleh dibahagikan kepada kanser invasif tanpa jenis khas (NST) dan jenis khas (ST) yang merangkumi *lobular, tubular*, *cribiform, metaplastic, apocrine, mucinous*, *papillary,* dan beberapa lagi. Berdasarkan sorotan literatur, kebanyakan kanser payudara jenis khas mempunyai prognosis yang lebih baik berbanding kanser invasif tanpa jenis khas. Oleh itu, klasifikasi yang tepat boleh memberikan anggaran prognosis kepada pesakit dan membantu memberikan rawatan optimum kepada pesakit. Tujuan kajian ini adalah untuk mengkaji hubungan korelasi antara ketumpatan payudara mamografi dengan histologi kanser payudara jenis invasif.

Metod: Kajian keratan lintang dijalankan di Hospital Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia ke atas 125 pesakit yang telah didiagnosis dengan kanser payudara dan telah menjalani ujian mamografi sebelum pembedahan. Ketumpatan payudara ditentukan dengan menggunakan klasifikasi *Breast Imaging Reporting and Data System (*BI-RADS) dan Tabar. Ketumpatan payudara dibahagikan kepada

kategori tumpat (BI-RADS C dan D, Tabar I, IV dan V) dan tidak tumpat (BI-RADS A dan B, Tabar II dan III). Kaedah korelasi Pearson digunakan untuk menilai korelasi antara ketumpatan payudara dan jenis histologi kanser payudara yang dikategorikan sebagai kanser invasif tanpa jenis khas (NST) dan jenis khas (ST). Statistik cohen Kappa digunakan untuk menilai kebolehpercayaan *inter-rater* untuk persetujuan antara klasifikasi BIRADS dan Tabar.

Keputusan: Tidak ada korelasi yang signifikan di antara ketumpatan payudara mamografi menggunakan klasifikasi BI-RADS dan Tabar dengan jenis histologi kanser payudara (p=-.833 dan p=0.336). Statistik cohen Kappa menunjukkan persetujuan yang baik antara klasifikasi BIRADS dan Tabar, dengan pekali Kappa Cohen 0.8 ($p < 0.001$).

Kesimpulan: Secara keseluruhan, kajian ini tidak menunjukkan korelasi yang signifikan antara ketumpatan payudara mamografi dengan histologi kanser payudara jenis invasif. Walaubagaimanapun, disebabkan oleh sorotan literatur yang terhad, kajian yang lebih lanjut berkemungkinan akan menunjukkan hubungan korelasi antara ketumpatan payudara dengan dengan jenis histologi kanser payudara.

Kata kunci: kanser payudara, ketumpatan payudara mamografi, jenis histopatologi, mammogram

ABSTRACT

Background: Mammographic density is an established risk factor for breast cancer. It reflects the relative component of fat, epithelial tissue, and connective tissues that has a specific appearance due to different tissue attenuation on the mammogram. Women with dense breast have more epithelial and connective tissue and less fat tissue, and are at higher risk to get breast cancer. Histological subclassification of breast cancer can be divided into invasive carcinoma of no special type (NST) and special types (ST) which include lobular, tubular, cribiform, metaplastic, apocrine, mucinous, papillary, and many others. From various works of literature, the majority of ST breast carcinoma carries a better prognosis than NST. Thus, appropriate classification can allow accurate estimation of a patient's prognostication and facilitate the identification of optimal treatment strategies. The purpose of this study is to evaluate the association between mammographic breast density and histological subtypes of invasive breast carcinoma.

Methods: A cross-sectional study was conducted in Hospital Universiti Sains Malaysia (USM), Kota Bharu, Kelantan, Malaysia on 125 patients who had been diagnosed with breast carcinoma and had undergone mammogram prior to surgery. Breast density was determined using the Breast Imaging Reporting and Data System (BI-RADS) and Tabar classification. It was divided into dense (BI-RADS C and D, Tabar type I, IV, and V) and non-dense (BI-RADS A and B, Tabar II, and III) categories. Histological subtypes of breast carcinoma are categorized into invasive carcinoma of no-special type and special type breast carcinoma. The association between mammographic breast density and histological subtypes of breast carcinoma

had been carried out using Pearson chi square test. Cohen's kappa analysis was done to determine the agreement between BI-RADS and Tabar classification.

Results: There is no significant association between mammographic breast density using BI-RADS and Tabar classification with histological subtype (p=0.833 and p=0.336 respectively). However, Cohen's kappa analysis shows good agreement between BI-RADS and Tabar classification, with a Cohen's kappa coefficient of 0.8 ($p < 0.001$)

Conclusion: Our study shows no significant association between mammographic breast density and histological subtypes of invasive breast carcinoma. However, with limited literature, further research might be plausible to show the association between breast density and histopathological subtypes of breast carcinoma.

Keywords: invasive breast carcinoma, mammographic breast density, histopathological subtypes, mammogram

CHAPTER 1: BACKGROUND

1.1 Introduction

Breast cancer is the most common cancer for women in Malaysia and most part of the world. In Malaysia, one in nineteen women is at risk with breast carcinoma and almost fifty percent of women diagnosed with breast carcinoma were under fifty years old (Lee *et al.*, 2019). The overall 5-year survival rate in Malaysia was 49% with median survival time of 68.1 months (Abdullah *et al.*, 2013). Early diagnosis and treatment of breast cancer can be achieved through screening with mammography, which has been shown to reduce mortality from breast cancer.

The well-known risk factors for breast cancer include age, sex, lifestyle and environmental factors, reproductive factors, and radiation exposure. Mammographic breast density is another well-established risk factor for breast cancer and is a strong predictor for breast cancer (Yaghjyan *et al.*, 2015). The pattern of breast density can be assessed from mammogram. The increased risk of breast cancer persists for 10 years or more after density assessment in both pre-and post-menopausal women and is independent of other breast cancer risks (Yaghjyan *et al.*, 2015).

Some epidemiological risk factors for breast cancer such as age, menopausal status, body mass index, age at first child-birth, past use of hormonal therapy, and alcohol consumption have shown an association with only certain tumour subtypes, suggestive of etiologic heterogeneity (Momenimovahed and Salehiniya, 2019)

Breast cancer is a heterogeneous disease in its presentation, pathological classification, and clinical course (Yerushalmi, Hayes and Gelmon, 2009). Most tumours are derived from mammary ductal epithelium, principally the terminal duct-lobular unit.

The classification of breast tumour is a dynamic process and continues to evolve. The latest 5th edition of the World Health Organization (WHO) classification of tumours of the breast was published in 2019 with the introduction of new entities which included mucinous cystadenocarcinoma and tall cell carcinoma with reversed polarity (Tan *et al.*, 2020).

The histological type of breast tumours refers to the growth patterns of the tumours. The commonest type of breast carcinoma is invasive carcinoma of no special type (NST) which was previously known as invasive ductal carcinoma, not otherwise specified (IDC-NOS). It is a diagnosis of exclusion and includes adenocarcinomas that fail to exhibit sufficient characteristics to warrant their classification in one of the special types. Special type breast carcinoma (ST) comprises 25% of all breast carcinoma (Weigelt, Geyer and Reis-Filho, 2010). Special types breast carcinoma are defined in terms of specific histological criteria that recognize a clustering of features (Page, 2003). The specific subtypes include invasive lobular carcinoma, tubular carcinoma, cribiform carcinoma, metaplastic carcinoma, carcinoma with apocrine differentiation, salivary gland/skin adnexal type tumours, mucinous carcinoma, invasive papillary carcinoma, invasive micropapillary carcinoma, inflammatory carcinoma, and other exceptional rare types and variants (Tan *et al.*, 2020).

It is well known that certain histological types of breast carcinoma carry a favourable prognosis. Mucinous carcinoma, tubular carcinoma, invasive cribiform carcinoma, medullary carcinoma, infiltrating lobular carcinoma, and tubule-lobular

carcinoma have all show better prognosis than invasive carcinoma of no special type (Pinder *et al.*, 1994). These specific tumour types are defined by their morphology, and are also linked to particular clinical, epidemiological, and molecular features.

Classification for breast density was first described by Leborgne in 1953 and was further described by Wolfe in 1976 (Freer, 2015). The fifth edition of the American College of Radiology Breast Imaging Reporting and Data System (BI-RADS) uses a more subjective four-category overall assessment of breast composition to convey the potential that a dense area may obscure a cancer. There are four BI-RADS categories for breast density. Category A – the breasts are almost entirely fatty, Category B - there are scattered areas of fibroglandular density, Category C - the breasts are heterogeneously dense, which may obscure small masses, and Category D - the breasts are extremely dense, which lowers the sensitivity of mammography (Winkler *et al.*, 2015).

Another classification for radiological breast density is Tabar classification. Tabar classification is based on anatomic-mammographic correlation using three-dimensional subgross (thick slice) technique and on the relative proportion of four "building blocks" (nodular densities, linear densities, homogeneous fibrous tissue, radiolucent fat tissue) (Gram *et al.*, 2005). Low risk types include Type I : Balanced proportion of all components of breast tissue with a slight predominance of fibrous tissue, Type II : Predominance of fat tissue, and Type III : Predominance of fat tissue with retroareolar residual fibrous tissue. High risk types include Type IV: Predominant nodular densities and Type V : Predominantly fibrous tissue.

Based on a previous study by Winkel et al (2016), BI-RADS and Tabar classifications show a moderate association. From this study, women categorized into Tabar's fatty PII and PIII were seen in the two low density BI-RADS categories.

Likewise, Tabar's PIV and PV were mainly seen in the two high-density BI-RADS categories.

This study aims to determine whether there is an association between types of mammographic breast density and histological subtypes of breast carcinoma, which for this study focusing on invasive carcinoma of NST which is the most common type and ST which include all invasive carcinoma, other than NST. By identifying the subtype-specific breast cancer risk factors, we would be able to understand more about breast cancer aetiology, develop subtype-predictive models, and eventually possible preventive strategies.

There is increasing evidence stating that the biological heterogeneity of breast cancer has clinical and epidemiological implications. Reports from the available epidemiology studies had stated that histologic subtypes of breast carcinoma differ in their association with established breast cancer risk factors.

However, to date, the studies are more focused on comparing primarily the association between hormonal exposures and histologic subtypes. Limited studies comparing the association between mammographic breast density and histological subtypes of breast carcinoma. Thus, it is plausible that association with breast density could vary across histologic subtypes.

1.2 Objectives

1.2.1 General Objective

To study the association between types of mammographic breast density and histological subtypes of invasive breast carcinoma.

1.2.2 Specific Objectives

1. To determine the types of mammographic breast density using BI-RADS and Tabar classification in patient with invasive breast carcinoma.
2. To look for the association between types of mammographic breast density and histological subtypes of invasive breast carcinoma which are NST and ST carcinoma.
3. To determine the agreement between the two classifications of mammographic breast density (BI-RADS and Tabar classification).

1.3 Hypothesis

There is an increased likelihood of a patient with higher mammographic breast density to develop invasive breast carcinoma of NST.

1.4 Research Question

1. What is the distribution of mammographic breast density among breast cancer patients in Hospital USM?
2. What is the association between types of mammographic breast density and histological subtypes of invasive breast carcinoma?
3. Is there agreement between the two classifications of mammographic breast density (BI-RADS and Tabar classification)?

CHAPTER 2: LITERATURE REVIEW

2.1 Human breast

The human breast is a modified cutaneous exocrine gland composed of skin and subcutaneous tissue, breast parenchyma (ducts and lobules), and supporting stroma, including fat interposed in a complex network of ligaments, nerves, arteries, veins and lymphatics (Jesinger, 2014)

Age, the composition of breast tissue, and hormonal environment (both past and present) contribute to the development of breast tissue. The most proliferative phase is during the teenage period after puberty which the lobules are developing. The lobules are fully developed by the age of 25 (Reid *et al.*, 1996)

Breast changes throughout a woman's life. These changes are due to cyclical changes during menses, altered physiology and anatomy during pregnancy and lactation and the progressive factor of ageing. Childbirth with its post-lactational involution tends to change the lobular structure to a more differentiated structure. Menopause plays an important role in the major involution which includes a preclimacteric phase and postmenopausal phase. During the latter phase, the ductal and lobular epithelium as well as the adjacent fibrous connective tissue, regress and eventually are placed by adipose tissue.

2.2 Breast cancer

2.2.1 Epidemiology of breast cancer

Breast cancer is the commonest cancer in women in Malaysia, with one in nineteen women is at risk with breast cancer (Lee *et al.*, 2019). Based on the latest data from Malaysia National Cancer Registry Report, a total of 21,634 cases of female breast cancer were diagnosed from 2012 to 2016 compared with 18,206 cases in 2007-2011 report. The overall 5-year survival rate in Malaysia was 49% with median survival time of 68.1 months (Abdullah *et al.*, 2013)

Breast cancer is a heterogeneous disease; different molecular subtypes of breast cancer have distinct clinical-morphological features and biomarkers that influence patient survival (Phipps *et al.*, 2011). Most tumours are derived from mammary ductal epithelium, principally the terminal duct-lobular unit.

2.2.2 Risk factors for breast cancer

Mammographic breast density is a well-established and strong predictor of breast cancer risk (Yaghjyan *et al.*, 2015)

The effects of age, pregnancy, and menopause on mammographic density resemble a theoretical model of susceptibility to breast cancer developed by Pike *et al* (1983)(Pike *et al.*, 1983). The model is based on the concept that breast tissue age or breast tissue exposure rate, rather than chronological age, is the relevant measure for describing susceptibility to breast cancer. Breast tissue exposure is closely associated with exposure to hormones that affect breast cell division and susceptibility to carcinogens. It is greatest at the time of menarche, decreases with the first pregnancy,

is further reduced in the perimenopausal period, and is lowest after menopause (Boyd *et al.*, 2009).

In the literature, increasing breast density was similarly positively associated with breast cancer risk across the histologic subtypes (Phipps *et al.*, 2011). However, because of limited literature review regarding the association between types of breast density and histological subtypes of breast carcinoma, it is plausible that association with breast density could vary across the histologic subtypes.

Other risk factors for breast carcinoma include current age, alcohol intake, BMI, hormone replacement therapy with oestrogen and progesterone, radiation exposure, early menarche, late menopause, age at first childbirth, history of breast cancer, family history, and germline mutation (Singletary, 2003).

There are significant positive associations with family history and breast density across both the histological subtypes (invasive lobular carcinoma and invasive ductal carcinoma) (Phipps *et al.*, 2011). Hormone therapy usage is associated with increased risk for all subtypes, however showed a stronger association with invasive lobular carcinoma. Comparing with nulliparous women, parous women have a lower risk for ductal carcinoma, however not lobular cancers. Late age at first birth was associated with increased risk in both the histologic subtypes (Phipps *et al.*, 2011).

2.2.3 Classification of breast tumour

The classification of breast tumour is a dynamic process and continues to evolve. The latest 5th edition of the WHO classification of tumours of the breast was published in 2019 with the introduction of new entities (Tan *et al.*, 2020).

The histological type of breast tumours refers to the growth patterns of the tumours. The commonest type of breast carcinoma is invasive carcinoma of NST which was previously known as invasive ductal carcinoma, not otherwise specified (IDC-NOS). It is a diagnosis of exclusion and includes adenocarcinomas that fail to exhibit sufficient characteristics to warrant their classification in one of the special types. ST breast carcinoma comprises 25% of all breast carcinoma (Weigelt, Geyer and Reis-Filho, 2010). Special types breast carcinoma are defined in terms of specific histologic criteria that recognize a clustering of features (Page, 2003). The specific subtypes include invasive lobular carcinoma, tubular carcinoma, cribiform carcinoma, metaplastic carcinoma, carcinoma with apocrine differentiation, salivary gland/skin adnexal type tumours, mucinous carcinoma, invasive papillary carcinoma, invasive micropapillary carcinoma, inflammatory carcinoma, and other exceptional rare types and variants (Tan *et al.*, 2020).

It is well known that certain histological types of breast carcinoma carry a favourable prognosis. Mucinous carcinoma, tubular carcinoma, invasive cribiform carcinoma, medullary carcinoma, infiltrating lobular carcinoma and tubule-lobular carcinoma have all show better prognosis than invasive carcinoma of no special type (Pinder *et al.*, 1994). These specific tumour types are defined by their morphology, but are also linked to particular clinical, epidemiological, and molecular features.

2.3 Mammographic breast density

Mammographic breast density is a well-established and strong predictor of breast cancer risk (Yaghjyan *et al.*, 2015). Breast density on mammogram can significantly vary between individuals. The appearance of the breast on the mammogram is a reflection of the amount of fat, connective tissue, and epithelial

tissue in the breast. Light (non-radiolucent) areas on the mammogram represent the fibrous and glandular tissues (mammographically dense), whereas the dark (radiolucent) areas on the mammogram are primarily fat. Women with 75% or greater percent density (proportion of the total breast area that appears dense on the mammogram) are at four to six-fold greater risk of breast cancer compared to the women with more fat tissue in the breast.

2.3.1 Classifications of breast density

Classification of breast density was first described by Leborgne in 1953 and was further described by Wolfe in 1976 (Freer, 2015). Wolfe classified the breast density into 4 categories based on the relative amount of fat, epithelial and connective tissue density as well as prominent ducts. N1 (lowest risk) : Parenchyma mainly composed primarily of fat, P1 (low risk) : Parenchyma chiefly of fat with prominent ducts in anterior portion up to one fourth of volume of the breast, P2 (high risk) : Severe involvement with prominent duct occupying more than one fourth of the breast volume and DY (highest risk) : Severe involvement with dysplasia.

The fifth edition of the American College of Radiology Breast Imaging Reporting and Data System (BI-RADS) lexicon has modified the previous density categorization system by eliminating the methods described in earlier editions that were based on estimated density percentage quartiles (Winkel *et al.*, 2016). Rather than using an overall estimate of the percentage of fibroglandular density, the current BI-RADS revision suggested using a more subjective four-category overall assessment of breast composition to convey the potential that a dense area may obscure cancer. There are four BI-RADS categories for breast density.

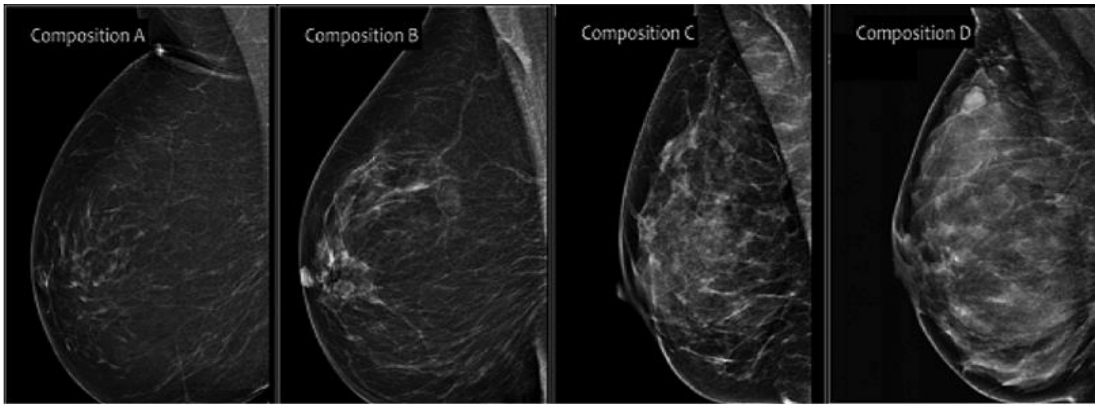

Figure 1 Breast imaging reporting and data system (5th edition categories
Composition A – the breasts are almost entirely fatty, Composition B - there are scattered areas of fibroglandular density, Composition C - the breasts are heterogeneously dense, which may obscure small masses, and Composition D - the breasts are extremely dense, which lowers the sensitivity of mammography

Adapted from E.U Ekpo *et al*.

Another classification for breast density is Tabar classification. Tabar classification is based on anatomic-mammographic correlation using three-dimensional sub gross (thick slice) technique and on the relative proportion of four "building blocks" (nodular densities, linear densities, homogeneous fibrous tissue, radiolucent fat tissue) (Tabar et al, 1997).

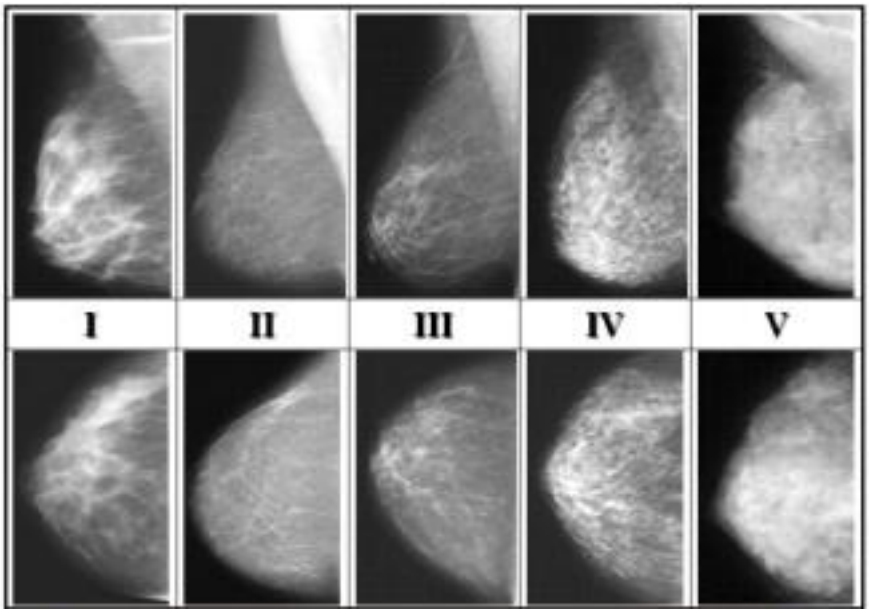

Figure 2 Tabar Classification of types of breast parenchymal patterns Low risk types include Type I : Balanced proportion of all components of breast tissue with a slight predominance of fibrous tissue, Type II : Predominance of fat tissue, and Type III : Predominance of fat tissue with retroareolar residual fibrous tissue. High risk types include Type IV: Predominant nodular densities and Type V : Predominantly fibrous tissue.

Adapted from Zulfiqar *et al.*

Based on a previous study by Winkel *et al*. (2016), BI-RADS and Tabar classifications show a moderate association. From this study, women categorized into Tabar's fatty PII and PIII were seen in the two low density BI-RADS categories. Likewise, Tabar's PIV and PV were mainly seen in the two high-density BI-RADS categories.

2.4 Conceptual framework

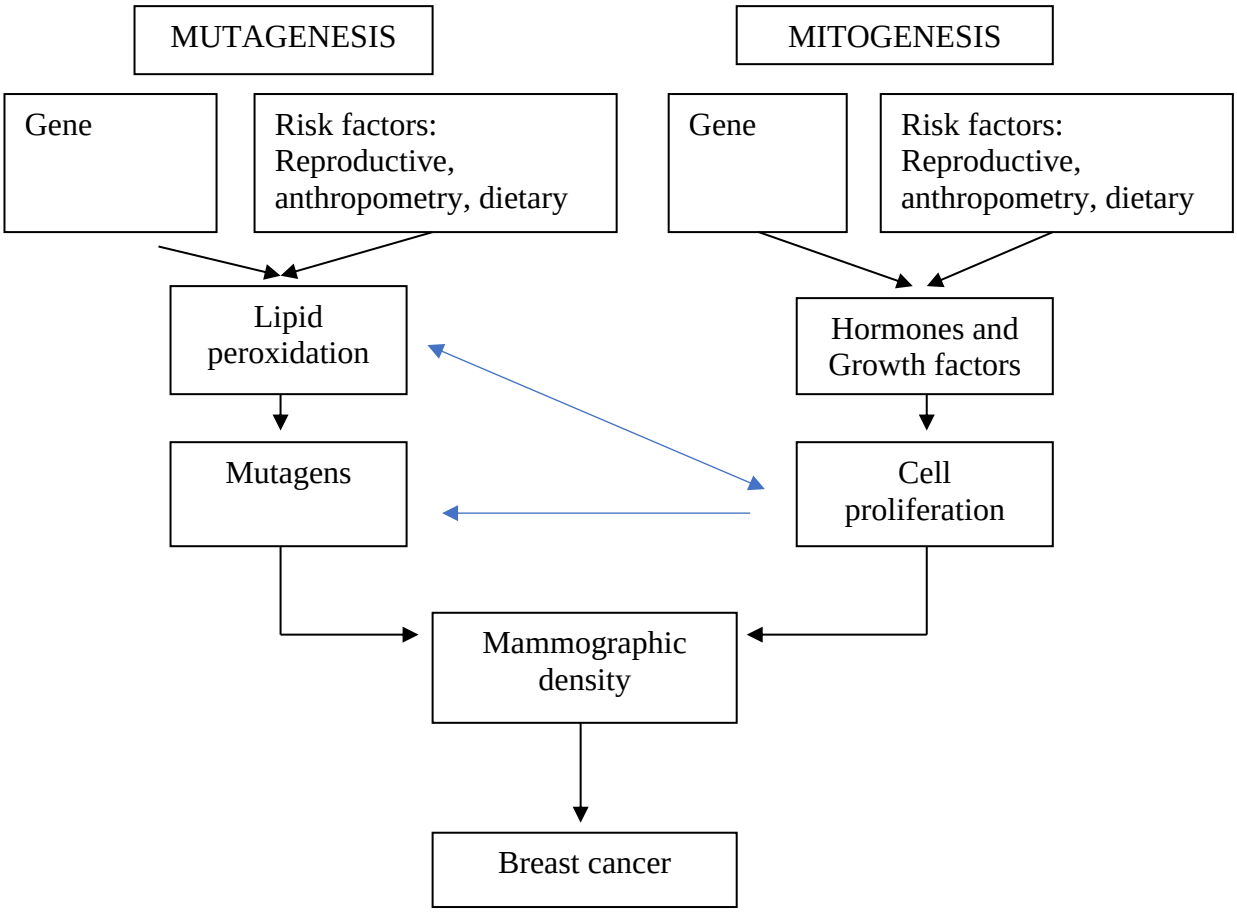

Figure 3: Conceptual framework

2.5 Rationale of Study

This study aims to determine whether there is an association between types of mammographic breast density and histological subtypes of breast carcinoma, which for this study focusing on invasive carcinoma of NST which is the most common type and ST carcinoma which include all invasive carcinoma, other than NST. By identifying subtype-specific breast cancer risk factors, we would be able to understand more about risk factors of breast cancer, develop subtype-predictive models and eventually possible preventive strategies.

CHAPTER 3: METHODOLOGY

3.1 Study Design

This was a cross-sectional study which was conducted in Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia for a period of 6 months from May 2020 to November 2020. Data collected was from January 2014 till January 2020.

3.2 Sample Population

i. Reference population – Women diagnosed with breast carcinoma who underwent mammogram in Kelantan.

ii. Source population – Women diagnosed with breast carcinoma who underwent mammogram in Hospital USM.

iii. Target population – Women diagnosed with invasive breast carcinoma of NST and ST invasive breast carcinoma who underwent mammogram in Hospital USM.

iv. Sampling frame – Eligible patients according to inclusion and exclusion criteria from the target population

3.3 Sample Size Calculation

1. For specific objective 1 and 2, sample size estimation was calculated using Sample Size Calculator v2.0, prepared by Dr Wan Nor Ariffin, Unit of Biostatistics and Research Methodology, School of Medical Sciences, Health Campus, University Sains Malaysia. Based on the previous study in 2013 by Bertrand *et al.*, (2013) for breast density and risk of breast cancer by age and tumour characteristic, with probability (power) 0.8, significance level of 0.05, the minimum sample size is 86. With an additional 10% dropout rate, the corrected sample size is 96.
2. For specific objective 3, sample size estimation for agreement between BI-RADS and Tabar classification were calculated based on the previous study by Muhimmah *et al.* (2006). From this study, the correlation between BI-RADS and Tabar measures, with k=0.408. Hence, the sample size calculated is 31 with probability (power) 0.8, significance level of 0.05. With an additional 10% dropout rate, the corrected sample size is 35.

3.4 Sampling Method

No sampling method was applied. All eligible patients that fulfilled the inclusion criteria were enrolled in the study.

3.5 Inclusion Criteria

1. Female patients.
2. HPE proven invasive breast carcinoma of NST and ST
3. The mammogram prior to surgery should be available.

3.6 Exclusion Criteria

1. Patient diagnosed with mixed NST and ST.
2. Patient with concomitant carcinoma (i.e: cervical carcinoma, uterine carcinoma, colon carcinoma, lung carcinoma).
3. Patient which has prior invasive or in situ breast cancer.
4. Patient on hormonal replacement therapy

3.7 Research Tools

1. Picture Archive Communication System (PACS) in Hospital USM (PACS Universal Viewer Version 5.0 SP6)
2. Hologic Selenia Dimensions mammogram machine.
3. Hologic Women's Health Solution diagnostic workstation.

3.8 Operational Definition

1. Dense breast:

- BI-RADS composition C and D.
- Tabar classification type I, IV and V

2. Non-dense breast:

- BI-RADS composition A and B.
- Tabar classification type II and III

3. Invasive breast carcinoma - cells that break through the duct and lobular wall and invade the surrounding fatty and connective tissues of the breast (Sharma *et al.*, 2010).

4. Breast carcinoma in situ – cells that are confined to the ducts and do not penetrate the basement membrane into the surrounding fatty and connective tissues of the breast (Sharma *et al.*, 2010).

5. Invasive breast carcinoma of no special type (NST) - This group of breast cancers comprises all tumours without the specific differentiating features that characterize the other categories of breast cancers (Sinn and Kreipe, 2013).

6. Special type carcinoma (ST) – Include all invasive carcinoma, other than NST as listed in the table below (Tan *et al.*, 2020)

Special type carcinoma (ST)
Invasive lobular carcinoma
Tubular carcinoma and cribiform carcinoma
Metaplastic carcinoma

Carcinoma with apocrine differentiation
Salivary gland/skin adnexal type tumours
Mucinous carcinoma and carcinoma with signet-ring-cell differentiation
Invasive papillary carcinoma
Invasive micropapillary carcinoma
Inflammatory carcinoma
Exceptional rare types and variants

3.9 Data Collection

Patient Cohort

This is a cross-sectional study that was conducted in Hospital USM, Kota Bharu, Kelantan, Malaysia for 6 months from May 2020 to November 2020. Data collected was from January 2014 till January 2020. This study had obtained approval from the Human Research Ethics Committee of USM (USM/JEPeM/20020103). We included 125 female patients who were diagnosed with HPE proven breast carcinoma and has done mammogram prior to surgery. We excluded patient who was diagnosed with mixed NST and special type carcinoma ST, concomitant carcinoma (i.e cervical carcinoma, uterine carcinoma, colon carcinoma, lung carcinoma), prior invasive or in situ breast cancer and patient who was on hormonal replacement therapy. The patients

were identified through a census established by Breast Cancer Awareness and Research Unit (BestARi).

Mammogram

The mammogram performed at the time of diagnosis were selected for assessment. All mammograms were performed on Hologic Selenia Dimensions mammogram machine, utilizing the standard mammogram protocol. Density resolution was set at 14-bit, spatial resolution 3.5 lp/mm and pixel size 70 microns. The size of the images was 3328 x 4096 pixels. The monitor used for assessment was 3MP BARCO diagnostic displays, 21 inches. To avoid errors in classifying the breast density due to distortion of the breast parenchyma by tumours, we measured the contralateral side of the breast in mediolateral oblique view.

Breast density assessment

The breast density was subjectively categorized using qualitative assessment of fibroglandular breast tissue according to pattern recognition. The breast density was classified according to two radiological methods: The 5th edition of the American College of Radiology (ACR)'s Breast Imaging Reporting and Data System (BIRADS) and the Tabar classification on parenchymal patterns. Both classifications were explained in details in Winkel *et al.* (2016). BIRADS classified mammogram density into four categories: A: entirely fatty, B: scattered areas of fibroglandular density, C: heterogeneously dense and D: extremely dense. The Tabar classification classified mammograms in five patterns (I to V) based on histologic-mammographic correlation

and on the relative proportion of four “building blocks” (nodular densities, linear densities, homogeneous fibrous tissue, radiolucent fat tissue) : I: balanced proportion of all components of breast tissue, II: predominance of fat tissue, III: predominance of fat tissue with retro areolar residual fibrous tissue, IV: predominantly nodular densities and V: predominantly fibrous tissue. BIRADS composition C and D and Tabar classification type I, IV and V were categorized as dense breast. BIRADS composition A and B and Tabar classification type II and III were categorized as non-dense breast. Both observers classified all the mammograms using both methods.

Pathology assessment

The pathological assessment was carried out at the Department of Pathology, Hospital USM. For breast core biopsy specimen, specimens will be placed in 10% neutral buffered formalin once obtained and processing was performed by transferring all tissue directly into cassettes. Routine examination of multiple levels is required. For breast tumour resection, the specimen will be sent immediately to the laboratory, ideally in a fresh state, and transferred to fixative. External descriptions will be done and followed by dissection. Internal inspection will be done when the tissue is fixed, samplings of areas of interest will be performed. The sample tissue will be submitted for further processing such as overnight fixation, paraffinization, sectioning and H&E staining.

Data collection

Data taken included age at diagnosis, parity, family history, clinical data (mass, nipple discharge, and calcifications). These data were collected from the patient's medical record. Family history includes the history of breast cancer in both first- and second-degree relatives. Age, parity, and clinical data were taken at the time of diagnosis/referral. Age at diagnosis was categorized into 5 groups. Parity at diagnosis was taken as continuous variable. Family history and clinical data were categorized as yes or no.

The histopathological reports of the tumour were obtained from the Lab Information System (LIS) database. The histopathological subtypes were defined using the 5th edition of the World Health Organization's classification system.

Validation

A senior breast radiologist with 5 years' experience in breast radiology and researcher independently classified a random sample of 15% of the mammograms according to the two radiological methods and the inter-rater reliability were assessed using Kappa test.

3.10 Statistical Analysis

All data were analysed using Statistical Product and Service Solutions (SPSS) for Windows, SPSS Inc.© (Version 24, SPSS Inc., Chicago, IL, USA). The descriptive statistics for discrete variables (age, parity, mass, nipple discharge, calcifications, breast density, and histopathology subtypes) were presented as n=frequency (%). Statistical analyses were presented on tables. Pearson chi square test was used to examine the association between the mammographic breast density and histopathological subtypes of breast carcinoma. Kappa test was used to compare rating between BI-RADS and Tabar classification.

3.11 Confidentiality and Privacy

The subjects were identified by a serial number. No identifiable data were shared publicly. Upon completion of the study, all data were stored in CDs, and the database on the computer was erased. The data were retained by the researchers for knowledge purposes only. Neither the name nor any identifying information was used in any publication or presentation resulting from this study.

3.12 Ethical Consideration

The study was approved by Human Research Ethics Committee of Universiti Sains Malaysia (JEPeM code: USM/JEPeM/20020103).

3.13 Study Flow Chart

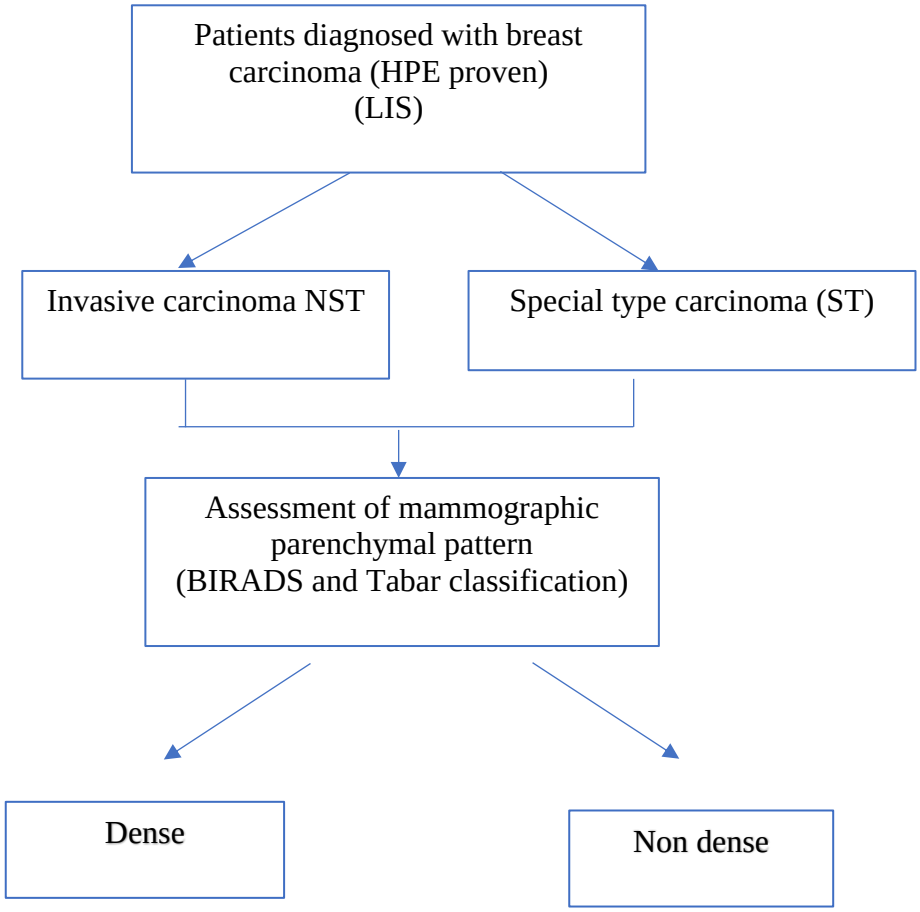

Figure 4 : Study flowchart