EFFECT OF KACIP FATIMAH (LABISIA PUMILA) WATER EXTRACT ON MAMMOGRAPHIC DENSITY – A PILOT STUDY

By

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List of Abbreviations.

AEC	Automatic exposure control
BIRADS	Breast Imaging Reporting and Data System
BMI	Body mass index
CC	Cranio-caudal
CEE	Conjugated equine estrogens
DHEA	Dehydroepiandrosterone
HRT	Hormone replacement therapy
HUSM	Hospital Universiti Sains Malaysia
IMR	Institute for Medical Research Malaysia
KF	Kacip Fatimah
LP	Labisia pumila
MLO	Medial-lateral oblique
MPA	Medroxyprogesterone acetate
TDLU	Terminal ductal lobular units

Abstracts

Abstract (Malay Version)

Pendahuluan.

Kacip Fatimah ialah sejenis herba tradisional yang mengandungi phytoestrogen. Ia sering digunakan oleh orang melayu untuk mengubati penyakit gynekogikal dan sebagai rawatan alternatif kepada rawatan hormon. Kegunaan hormon selepas putus haid adalah disabitkan dengan peningkatan kepadatan tisu buah dada pada mammogram. Kepadatan tisu buah dada pada mammogram merupakan salah satu faktor yang mengakibatkan kanser buah dada.

Tujuan

Tujuan kajian ini ialah untuk menguji kesan Kacip Fatimah terhadap kepadatan tisu buah dada pada mammogram.

Metodologi

Satu kajian perspektif telah dijalankan kepada 69 pesakit yang telah dibahagikan kepada empat kumpulan iaitu kumpulan 140 mg/hari, 280 mg/hari, 560 mg/hari dan plasebo. Mammogram telah dikaji dan diselidik dengan menggunakan cara skala peratusan, klasifikasi BIRAD dan pengukuran berpandukan komputer.

Keputusan

Tiada perubahan yang ketara telah direkodkan dengan kajian cara kategorikal seperti cara skala peratusan dan klasifikasi BIRAD. Pengukuran berpandukan komputer telah menunjukkan penambahan kepadatan tisu buah dada pada mammogram yang kecil antara mammogram sebelum rawatan dan selepas rawatan. Penambahan ini adalah tidak ketara dari segi statistik. Penambahan kepadatan tisu buah dada pada mammogram selepas rawatan berbanding dengan kepadatan tisu buah dada pada mammogram sebelum

rawatan adalah 0.2 %, 0.1 %, 1.5 % and 0.6 % untuk kumpulan placebo, 140mg , 280mg dan 560mg masing-masing. Ini mungkin kerana phytoestrogen adalah sejenis estrogen yang lemah.

Kesimpulan

Kajian ini menunjukkan bahawa Kacip Fatimah tiada kesan terhadap kepadatan tisu buah dada pada mammogram.

Abstract (English Version)

Introduction

Kacip Fatimah is a traditional herb that contains phytoestrogen and is commonly used by the Malay population in Malaysia to treat various gynecological illnesses. It is also used as an alternative to hormone replacement therapy due to its estrogenic effect. Postmenopausal hormone use is associated with increase in mammographic density and mammographic density is an independent risk factor for breast cancer.

Objective

Our purpose was to evaluate the effect of Kacip Fatimah (Labisia pumila) water extract on mammographic density in postmenopausal women.

Material and Method

A prospective, randomized, double-blind placebo-controlled pilot study was conducted. A total of 69 postmenopausal women were equally randomized to receive Kacip Fatimah water extract 140 mg/day, 280 mg/day, 560 mg/day or placebo. Mammograms were performed at baseline and after 6 months of treatment. Mammographic density was evaluated according to percentage scale, BIRAD classification and computer assisted measurement of breast density.

Result

The categorical assessments showed that there was no significant shift in categorical classification as assessed by BIRAD and percentage categories in either control or treatment groups. There was slight increase in breast density as assessed by computer assisted method although the increases were not statistically significant. The increases in breast density over pretreatment baseline were 0.2 %, 0.1 %, 1.5 % and 0.6 % for placebo,

140 mg group, 280 mg group and 560 mg group, respectively. These values were not significantly different from one another. This small increase in breast density might be due to the fact that phytoestrogen is a weak estrogen.

Conclusion

Kacip Fatimah extract given over a period of 6 months did not significantly affect mammographic density.

Introduction

1.0 Introduction

Labisia pumila (Kacip Fatimah) is a traditional herb commonly used by Malay women during peripartum period to make the process of childbirth easier. It is also used as traditional herbal preparation during the postpartum period to improve the general condition of mothers. Earlier research on Kacip Fatimah in Institute Medical Research, Malaysia showed that it has estrogenic-like activity and behaves like phytoestrogen.

Menopause is defined as permanent cessation of menstruation resulting from loss of ovarian follicular activity. Natural menopause is recognized to have occurred after 12 consecutive months of amenorrhea for which there is no other obvious pathological or physiological cause. Menopausal and perimenopausal issues are important topics worldwide and throughout the twentieth century. The life span of women has improved significantly in the last two decades. Malaysian women life expectancy now has reached 76.4 years and for male it was 70.6 years. (Department of statistic Malaysia 2005). However, the age of onset of menopause has not changed and remained at 50 to 52 years old despite improvement in women's life expectancy.

Following menopause, there are major changes in androgen, estrogen, and progesterone and gonadotrophin secretion due to cessation of ovarian follicular activity. There is reduction of circulating androstenedione to approximately 50% of the concentration among young women. The Luteinising hormone (LH) and follicular stimulating hormone (FSH) levels rise substantially with FSH usually higher than LH. As a result of these hormonal changes, postmenopausal women will experience the sequelae of menopause like vasomotor symptoms, mood changes, urogenital and sexual changes.

Hormone replacement therapy (HRT) is commonly prescribed to postmenopausal women to improve their postmenopausal symptoms. Kacip Fatimah was claimed to be able to alleviate these symptoms as well due to its estrogenic effect. However, prolonged administration of hormone replacement therapy has been associated with a slight but statistically significant increase in the risk of breast cancer. HRT slows normal breast involution and causes an increase in mammographic density. Many studies have confirmed that HRT affects mammographic density. Mammographic changes vary with different HRT regimens.

Mammographic density is one of the parameters evaluated in the mammogram and is determined by the relative amounts of epithelial tissue, connective tissue and fat in the breast. All non fatty breast tissue e.g. epithelial tissue and connective tissue will appear as white on mammogram and referred to as mammographic densities. The extent of breast density varies widely among women. In general, the breast density decreases with age especially after menopause.

The amount of dense tissue in the breast can be assessed either categorically or continuously. Wolfe was the first to describe four categorical patterns of mammographic density. BIRAD also divides mammograms into four categories of increasing density.

Continuous estimation of the proportion of the breast area that is made up of dense breast tissue can be assessed by percent-density methods of evaluating mammographic density.

Mammographic density is an independent risk factor for breast cancer. The degree of risk associated with mammographic density is greater than the degree of risk associated with almost all other known breast cancer risk factors. Knowledge of the variables associated with increased mammographic density allows greater understanding of the nature and causes of breast cancer. As a result, measurement of mammographic density is useful as a means of investigating the etiology of breast cancer and of testing hypotheses about potential preventive strategies.

Literature Review

2.0 Literature Review

2.1 Labisia Pumila

Labisia pumila is a plant found in Malaysia, Thailand and Indochina (Stone, 1988). It is synonymous with Labisia pumila Benth or Labisia pathoina Lindl and comes from plant genus Mysinaceae. There are 3 varieties of this plant e.g. Labisia pumila var alata, Labisia pumila var pumila and Labisia pumila var lanceolata. This herbaceous shrub has creeping rhizomes and thrives on shady areas in the tropical rain forest of Malaysia about 80 – 100 meters above sea level (Indu Bala jaganath, 1997). This plant grows relatively slowly and open sunlight or even partial shade can be harmful to the growth of the plant. Their leaves are few about 4-14 in numbers and more or less upright. The whole leaf is about 5-35cm long and 2-8cm wide. It flowers are very small, pink or white in colour (A. Prof Dr Zhari Ismail, 1999). Figure 1 - 3 illustrate the plant in Malaysia.

Herbal therapies are popular alternative to conventional therapies. Some herbs contain phytoestrogen which is a natural Dehydroepiandrosterone (DHEA) precursor. Examples of such herbs are 'Black cohosh', 'Wild Mexican Yam Root' and 'Dong quai'. These herbs are claimed to be effective in treating post menopausal symptoms. These herbs are increasingly being used because there is uncertainty and lack of consensus of standard estrogen replacement therapy. Women Health Initiative Study had added to the above confusion. In this trial, there is an increased risk of cardiovascular disease, breast cancer, stroke and thromboembolic events (Writing Group for the Women's Health Initiative Investigators, 2002).

Labisia pumila is also popularly known as 'Kacip Fatimah' or 'Selusoh Fatimah' by the Malay population. It is one of the most popular and potent ingredients used in traditional herbal therapy in the Malay population especially for postnatal health care, tonics for women and in the treatment of various gynecological disorders (Norhayati Ismail, 2005). It is commonly prescribed by midwives and traditional healers for various indications. It is given to pregnant mothers between one to two months before delivery as it is believed to induce and expedite labour. It is also used in postnatal care in a mixed preparation to help contract the birth canal, to delay fertility and to regain body strength. It is also commonly used for treating dysmenorrheal, venereal disease, flatulence, dysentery and rheumatism. The plant is often mixed with other herbs, boiled and the concoction taken orally.

Many studies have been done on this herb. Two benzoquinoid compounds were isolated as major components from Labisia pumila alata leave and roots (P. J Houghton, 1999). The use of Labisia pumila in parturition suggests that it contains phytoestrogens (plant estrogens). The studies by Jamia et al using in vitro estrogen bioassay has shown that ethanol extract of L. pumila var alata was weakly estrogenic (Jamia A. Jamal, 1999). Studies done at Institute of Medical Research (IMR) showed that the water extract of Kacip Fatimah was able to displace the 17b estradiol from binding to anti-estradiol. This proves that the water extracts of Kacip Fatimah contain a substrate that recognizes the estrogen-binding moiety of the immunoglobulin raised against estradiol. Tissue culture experiments carried out by IMR also showed that water extract of Kacip Fatimah exhibited a weak but specific estrogenic effect on the human endometrial adenocarcinoma cells. Therefore, it not only binds to estradiol antiserum, it also has estrogenic effects.

In vitro toxicity studies have shown that Kacip Fatimah has no toxic effect on human cells. Julai et al exposed various cell lines including breast cancer cell lines MCF-7 and liver cell line WRL – 68 to various concentrations of Kacip Fatimah. In all concentrations tested, Kacip Fatimah has no toxic effect on the cells lines (Julai Timor, 2002). Animal studies on pregnant Sprague – Dawley rats has also shown that Kacip Fatimah has no teratogenic potential and no significant abnormal finding on the biochemistry, hematology and mortality of treated rats. Human studies on Kacip Fatimah have also been carried out by the Institute of Medical Research. One study has shown that it is safe for human consumption with no significant change in hematological and biochemical parameters (Wan Nazaimoon WM, 2002).

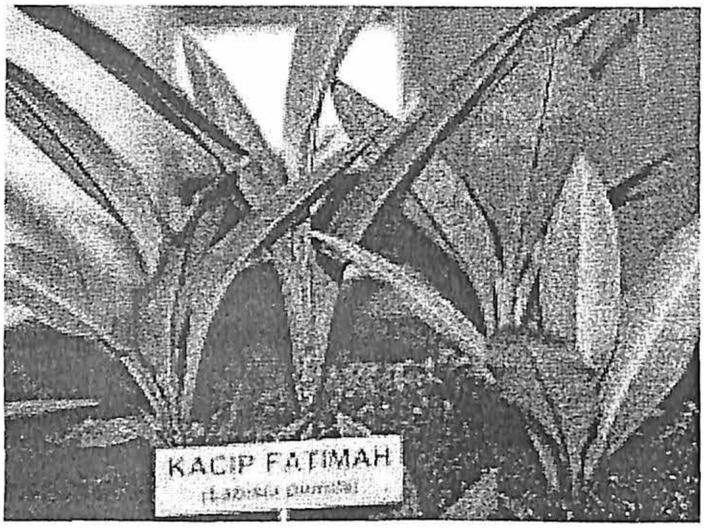

Figure 1. Kacip Fatimah Plant

These are leafy plants with leaves ranging from 4-14 arranged in an upright position.

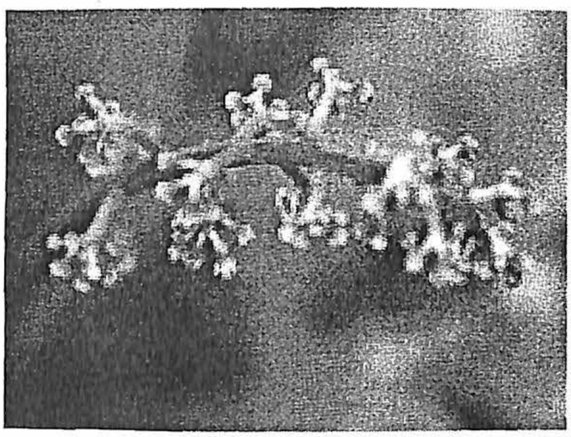

Figure 2 Kacip Fatimah Flower.

The purple-pinkish blooms of the highland Kacip Fatimah found in the Gunung Beremban jungle in Tanah Rata, Pahang

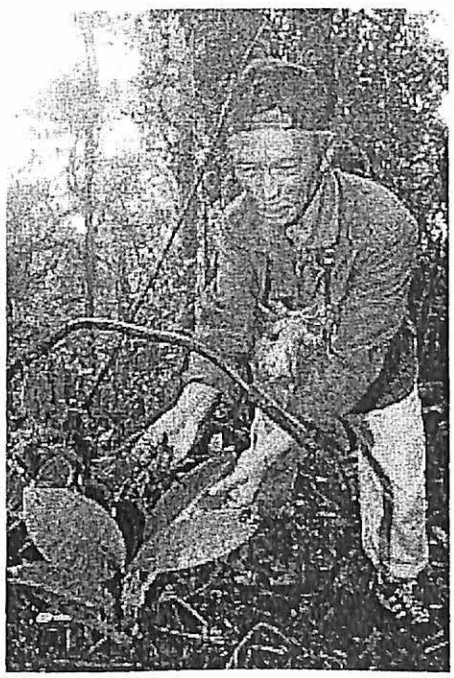

Figure 3 Kacip Fatimah in Jungle.

It is commonly found in shady areas of the jungles at elevations of 80 m to 100 m. It can also be found in higher elevations.

2.2 Phytoestrogen

Phytoestrogen is a plant substance that has varying degrees of estrogenic activity. These plant substances may or may not be structurally similar to gonadal estradiol 17β. There are three main groups of phytoestrogen e.g. isoflavones and lignans, coumestans, and resorcyclic acid lactones. Active isoflavones include daidzein and genistein. Phytoestrogens are metabolized to active forms after consumption so there may be wide variations in the available blood levels of any given individual.

Major sources of phytoestrogens are soy and red clover. Labisia pumila or Kacip Fatimah has been shown to contain phytoestrogens. Other plants that contain phytoestrogens include whole grain cereals, seeds, berries and nuts (mainly lignans). The interest on plant estrogens has increased considerably since there is controversy about the safety of hormone replacement therapy. The Women's Health Initiative, a large randomized trial evaluating the use of HRT, demonstrated that the use of combined estrogen/progestagen for more than 5 years is associated with an increased risk of breast cancer, stroke and venous thromboembolism. As a result, phytoestrogens are becoming popular in the treatment of menopause. However, there is lack of scientific evidence as to whether phytoestrogen is effective in relieving menopausal symptoms. In the randomized placebo controlled trial conducted by Nikander et al to study the effect of phytoestrogens in treatment of menopause in breast cancer patients, pure isoflavones did not alleviate subjective menopausal symptoms (Nikander et al., 2003).

Asian countries like Japan, China and Korea have a lower incidence of breast cancer compared to western countries. This was attributed to the fact that Asian population consumed traditional diets rich in plant food especially soy product which have a higher dietary intake of phytoestrogens (Mishra et al., 2003). Vegetarian and traditional Japanese diets have approximately 4.5 times the amount of phytoestrogens than the average American diet. It has been observed that the risk of breast cancer increased after immigration from Asia to United States (Adlercreutz, 2002). This may be due to dietary changes after migration. Phytoestrogens may play a significant inhibitory role during the initiation and promotional phases of cancer development. It has hormone-like compounds that exhibit ability to bind with low affinity estrogen receptors but fail to elicit a full estrogenic response. A case control study was conducted by Ingram et al to investigate the association between phytoestrogens and human breast cancer. The result showed that a statistically significant reduction in breast cancer risk was associated with women who had a high urinary excretion over 72 hours of phytoestrogens equol and enterolactone (Ingram D, 2000).

Phytoestrogens has been used and marketed as 'bust enhancing' herbal products (Fugh-Berman, 2003). However, no clinical trials have been published so far on this matter. Several of these herbs contain phytoestrogen which may have effects on the breast. Atkinson et al showed that isoflavone supplement for a year did not increase mammographic breast density (Atkinson et al., 2004).

2.3 Mammary gland

The mammary glands are specialized and modified accessory glands of the skin capable of secreting milk. The nipple is surrounded by a coloured skin area called the areola. Breast tissue consists of a system of ducts embedded in connective tissue.

Mammary gland develops on the chest wall between the clavicle and the sixth to eighth ribs. The base of breast extends from the second to the sixth rib and from the lateral margin of the sternum to the midaxillary line. It gradually enlarges during puberty and will attain a hemispherical shape under the influence of the ovarian hormones. The ducts elongate and the deposition of fat increases the size of breasts. Most of the breast tissue lies in the superficial fascia and a small part called axillary tail extends upward and laterally to the axillary region by piercing the deep fascia at the lower border of the pectoralis major muscle.

There are fifteen to twenty lobes in each breast. The main duct from each lobe converges toward the nipple and opens separately on the summit of the nipple. The base of the nipple is surrounded by the areola which has tiny tubercles produced by underlying areolar glands. The lobes of the gland are separated by fibrous septa. The breast is separated from the deep fascia covering the underlying muscles by an area of loose connective tissue known as the retromammary space.

Mammary glands are supplied by perforating branches of the internal thoracic artery and the intercostal arteries. The axillary artery also supplies the gland through the lateral thoracic and thoracoacromial branches. The veins correspond to the arteries. The lymphatic system of the breast is of clinical importance in term of cancerous spread. The lateral quadrants of the breast drain into the anterior axillary or pectoral nodes. The medial quadrants drain into the nodes along the internal thoracic artery. A few lymph vessels follow the posterior intercostal arteries and drain posteriorly into the posterior intercostal nodes. Some lymphatic vessels communicate with the lymphatic vessels of the opposite breast.

2.4 Mammography.

2.4.1 Mammogram

A mammogram is an x ray of the breast. It is an essential part of the modern multidisciplinary approach to effective investigation and management of breast disease. The standard technique used for breast imaging is screen film mammography. It requires consistent high quality images with optimum film density and contrast, high resolution and low radiation dose. This is particularly important for the detection of small cancers due to subtle radiological signs. The acceptable physical criteria for optimal mammogram are mean optical density of 1.4 to 1.8, high contrast spatial resolution of 10 line pairs per mm and mean glandular dose of 2 mGy per view.

Dedicated mammography x ray equipment is necessary for producing high quality images. A high voltage generator should produce a near constant potential output. The most commonly used target filter combination is a molybdenum target with 0.03 mm Mo filter. The peak kilovoltage is normally in the range of 26 - 30 kV and typically 28 kV. The resultant X ray spectrum exhibits characteristic x rays at 17.4 and 19.4 keV. A small focal spot of 0.3 – 0.35 mm is necessary for high resolution images. A fine focal spot of 0.1 mm is used for magnification techniques. The focus to film distance is in the range of 60-65 mm.

Automatic exposure control (AEC) is used in mammography to automatically control the exposure duration so that the optimum optical density of the mammogram is maintained over a wide range of different breast sizes and densities. The AEC device is either a photo timer or ionization chamber. It is normally positioned 3-5 cm posterior to the nipple because most dense glandular tissue is likely to be retroareolar. Moving grid system is used in mammography to improve resolution and contrast by decreasing scattered radiation. It is not required in magnification mammography because the scatter radiation is reduced by an air gap.

Breast compression is essential to ensure high quality mammograms and is applied using a powered system operated by a foot control. Film compression should be applied to the entire breast. The purposes of even and firm compression are to reduce dose, scatter radiation, geometric unsharpness, movement and tissue overlap. Single emulsion film is used with single intensifying screen in film screen mammography to ensure optimum resolution. Good film screen contact in cassettes is also important. The speed of the film screen combination is increased with the use of rare earth materials e.g. gadolinium oxysulphide. This will allow a decrease in the radiation dose while maximizing contrast.

Processing the mammogram is a critical part of the mammographic procedure because sharp, high contrast, artifact-free images are important for optimal diagnostic value. A dedicated processing facility is essential to ensure image quality. The chosen processing conditions and chemistry need to be matched to the film screen combination in use. Extended processing is used in mammography to obtain better contrast. The processing time is extended up to 3.5 minutes together with a lower developer temperature (34 degree Celsius) to achieve optimal film density and contrast.

2.4.2 Mammography projections.

The standard mammography consists of a medial-lateral oblique (MLO) and cranio-caudal (CC) view of each breast. Careful positioning is essential for optimal demonstration of breast tissue.

The standard CC view is obtained with a vertical x ray beam and the breast being compressed between the two plates horizontally. The CC view demonstrates the subareolar, medial and lateral portion of the breast. However, the posterolateral aspect of the breast may be incompletely demonstrated.

The medial-lateral oblique view is usually obtained with the x ray tube angled between 30 - 60 degree to the horizontal depending on the built and pectoralis muscle of the women. More breast tissue is demonstrated on the MLO view than any other view. The nipple should be in profile in MLO view. The pectoralis muscle should be visible down to the level of nipple and the inframammary fold should be visible without sagging. The glandular tissue should be evenly compressed and adequately penetrated. There should be no skin folds superimposed on the breast. Patients need to be relaxed to achieve optimal positioning.

Supplemental views are occasionally obtained to further evaluate breast tissue. Magnification views are obtained by increasing the object-film distance to product an air gap and a fine focal spot is used to increase resolution. A magnification factor of 1.5 is usual and the increased resolution obtained is particularly helpful for detailed analysis of microcalcification and margin of the small mass lesions. Localized compression views are obtained by using a small paddle compression device. This is to displace the overlying breast tissue so that better demonstration of the features of the lesion can be obtained.

2.5 Mammographic Density

The breast has two major components which are the fibroglandular tissue and fat on the basis of mammographic appearance. Fibroglandular tissue is a mixture of fibrous connective tissue and glandular tissue. Glandular tissue includes the epithelial cells that line the ducts and fibrous connective tissue includes the stroma of the breast. Fat appears darker on mammogram because it is more radiolucent than fibroglandular tissue. Fibroglandular tissue appears as radio-opaque areas and often referred as mammographic density or breast density (Byng et al., 1998).

There is a wide range of breast appearances on mammogram due to variation in breast compositions. The breast compositions of fat and fibroglandular tissue will have major impact on mammographic density. The fibroglandular tissue is influenced by estrogen level in the blood. Estrogen increases cell proliferation and progesterone enhances the effect. Breasts of premenopausal women will undergo cyclic changes during the menstrual cycle. During the follicular phase, cell proliferation increases and is further enhanced during the luteal phase. As a result, the breast will appear denser on mammogram during the luteal phase compared to the follicular phase.

With the reduction of estrogen and progesterone levels after menopause, the lobular tissue regresses. As a result, the breasts will appear more radiolucent on mammogram. The breast will continue to become less dense in about 50% of women in their 40s and 65% of women in their 20s have 50% or greater breast density (Stomper et al., 1996).

2.6 Methods of measuring breast density

2.6.1 Wolfe Classification.

In 1976, Wolfe classified breast parenchyma on mammogram into four categories based on radiographic appearance (Wolfe, 1976). Wolfe parenchymal pattern is based not only on the extent of the densities but also on the characteristics of the densities seen (prominent ducts and dysplasia). The following are the description provided by Wolfe. N1 category refers to the parenchyma composed primarily of fat with at most small amounts of dysplasia and no ducts are visible. P1 category refers to parenchyma composed chiefly of fat with prominent ducts in the anterior portion up to the one–fourth the volume of the breast. P2 category indicates severe involvement of the breast with prominent ductal pattern occupying more than one-fourth the volume of breast. DY category refers to severe involvement with dysplasia which often obscures an underlying prominent ductal pattern. Nulliparous women and women with family history of breast cancer are those most likely to fall into the P2 and DY categories (Ernster et al., 1980). This classification has been applied inconsistently and has wide range of interobserver agreement.

Breast density can be assessed from the mammogram film or digitized film. Jeffreys et al reported that density assessment from the original film and from a digitized image are reasonably similar but there is some evidence to suggest that the digitized image is assigned a higher Wolfe category and agreement using percentage categories system was moderately good (Jeffreys et al., 2003).

2.6.2 Laszlo Tabar Classification

Laszlo Tabar developed a method of classification based on anatomic-mammographic correlation. There are five patterns in this classification. Pattern I involve scalloped contours from Cooper's ligaments and evenly scattered terminal ductal lobular units (TDLU) as 1-2 mm nodular densities on the mammogram. There are also oval shaped lucent areas corresponding to fatty replacement in this pattern. Patterns II represents complete fatty replacement of breast. Pattern III is the combination of retroareolar prominent duct pattern due to periductal elastosis and fatty involution. Pattern IV demonstrates extensive nodular and linear densities throughout the breast. The linear densities are due to periductal elastic tissue proliferation with fibrosis called fibroadenosis. The scattered nodular densities of 3-7 mm are due to proliferating glandular structures and enlarged TDLU referred as adenosis. Pattern V consists of homogeneous, uniformly dense parenchyma with smooth contour due to extensive fibrosis. In terms of risk of developing breast cancer, patterns I to III are considered as low risk groups and patterns IV and V are categorized into high risk group.

Gram et al reported that the overall agreement on high risk versus low risk for Tabar and Wolfe classification was 55% with k value of 0.23. This indicates a poor agreement between these two classifications (Gram et al., 1997). The study also showed that the strength of association between high risk mammographic patterns and the three selected breast cancer risk factors e.g. parity, number of children and age at first birth, was of greater magnitude when the Tabar instead of the Wolfe classification was applied. Tabar classification also appears to be more closely related to breast cancer risk factors among perimenopausal women compared to Wolfe classification.

2.6.3 Percentage Categories

Visual estimation of the percentage of the breast occupied by fibroglandular tissue has been used frequently. According to Stomper et al, parenchymal density on mammogram was assessed visually and was categorized as less than 10%, 10-49%, 50-89% and 90% or greater dense tissue. The subcutaneous and retromammary fat regions in each breast were excluded during the determination of dense tissue. In patient with asymmetric tissue, the breast with greater density was categorized. (Stomper et al., 1996)

Boyd et al proposed a five categories systems which are <10%, 10-25%, 25-50%, 50-75% and >=75%. In this study, the reliability of this classification was assessed by rereading 100 images. Boyd reported intraclass correlation coefficient of 0.94 for repeated reading. This indicates high degree of reliability (Boyd et al., 1995).

Wolfe's parenchymal pattern and the percentage of breast densities were each related to the breast cancer risk. These two classifications are not independent but instead substantially overlap and are highly correlated. However, according to Brisson et al, parenchymal pattern added little to the prediction of breast cancer risk once percent density was taken into account (Brisson et al., 2003).

2.6.4 BIRADS Density Categories

In the United States, Breast Imaging Reporting and Data System (BIRADS) is used in mammographic reporting to standardize mammography reporting terminology and recommendation categories. Density classification is also included in the BIRADS reporting to inform referring physicians of the decline of sensitivity of mammography with increasing breast density. The four categories used in the BIRADS are almost entirely fatty, scattered fibroglandular densities, heterogeneous density and extremely dense. This is based on qualitative assessment and there is only moderate interobserver agreement seen with the use of BIRADS density categories. Berg et al reported an overall kappa value of 0.43 among observers in describing breast density (Berg et al., 2000).

2.6.5 Computer assisted method

Computer assisted measurement of breast density has been used recently. The mammographic density can be assessed quantitatively with the use of a continuous scale. Mammogram film is digitized and a simple observer assisted technique called interactive thresholding was used to measure mammographic density. This technique is easily applied to a digital representation of the mammogram with a personal computer workstation. The brightness of each pixel in the digitized image is represented by a grey level value in a histogram. An observer will manipulate a colour overlay to select threshold grey levels that helped identify the edges of density regions on a digital display of the image. The first threshold helps distinguish the edge of the breast from the image background. Then a second threshold is selected that best outlines regions of mammographic density on the image. Pixels above this threshold are interpreted as mammographic density. The total breast and density area is calculated by summing pixels within each region. The ratio of the total breast areas and area of mammographic density is the percentage of breast density. It is more consistent with less interobserver variability. The interobserver agreement is reported to be 90% or greater (Byng et al., 1998).

2.6.6 Radiographic method.

Breast glandularity could also be determined from radiographic data e.g. tube potential (kV), tube loading (mAs) and compressed breast thickness. Radiographic data was collected by exposing different thickness of phantom material of varying glandular and adipose compositions at 27 kV. Then a fitted equation was derived and applied to calculate breast glandularity. In the study conducted by Jamal et al using this method, the average breast glandularity of Malaysia women was 48.9% (Jamal et al., 2004).

2.7 Relationship between mammographic density and breast cancer

Glandular tissue of the breast is the common site for breast cancer and contributes to the mammographic density. It is the most vulnerable tissue among the tissues that makes up the breast e.g. adipose, skin and areolar tissue. The amount of glandular tissue is linked to breast cancer risk. So an objective analysis of glandular tissue can aid risk estimation.

Wolfe et al already demonstrated a relationship between breast density and breast cancer risk in 1976 (Wolfe, 1976). Wolfe revealed that there was 37 times greater incidence of breast cancer for those at highest risk group compared to the low risk group. Some studies that have made use of Wolf grades confirmed that a relative risk between 2 and 4 exists.

Quantitative methods have been used by many studies to report association between breast densities with breast cancer risk. Studies that used quantitative estimates of the area of density show a substantially higher odds ratio than studies using Wolfe's classification. In the study conducted by Brisson et al, women with >85% of the breast density have a >5 fold increase in risk compared with women with no density of the same age and body weight (Brisson et al., 2003). Using computer assisted interactive thresholding technique; Byng et al reported a 2% increase in breast cancer risk with each 1% increase in breast density (Byng et al., 1998).

Boyd et al reported a 43% increase in the relative risk between the lower and the next higher category of density as determined by radiologists and there was a 32% increase as determined by the computer assisted method (Boyd et al., 1995). He concluded that increases in the level of breast tissue density as assessed by mammography are associated with increases in risk for breast cancer. The elevated risk associated with breast densities persisted for at least 5 years after the mammogram used in classification had been taken. In this study, statistically significant increases in breast cancer risk associated with increasing mammographic density were higher for older than for younger women.

The biological basis for the association of mammographic density with risk of breast cancer is not completely clear. There are published articles suggesting that mammographic density is associated with epithelial and stromal proliferation. Li et al analyzed breast tissue obtained at forensic autopsy and not selected from the presence of abnormality. Breast tissue obtained by surgical biopsy or mastectomy may give biased result because the histological features of breast tissue from subjects known or suspected of having breast disease may not be representative of all women. In the study conducted by Li et al, the percentage of mammographic density was associated with the proportion of the biopsy area occupied by nuclei, both epithelial and nonepithelial and by collagen and the area of glandular structures. Several breast cancer risk factors that are also associated with variations in mammographic density, notably body weight, parity and menopausal status were also associated with differences in one or more of these tissue features. All risk factors for breast cancer must ultimately exert their influence by an effect on the breast and some of these factors exert their effect through their influence on the number of cells and the quantity of collagen which will be reflected in mammographic density (Li et al., 2005).

There is a direct relationship between mammographic density and cytological abnormality. Lee et al found that highly dense mammograms were associated with cytological atypia (Lee et al., 1994). In this study, the odds ratio of high density mammogram (over 50%) with nipple aspirate fluid cytological atypia was 4.4. Women with nipple aspirate cytological diagnoses of atypia had three times greater risk of breast cancer than women with normal cytology. Boyd et al reported that the risk of developing the epithelial lesions of atypical hyperplasia and in situ cancer, and of hyperplasia without atypia was greater in women aged less 50 with extensive mammographic densities than women with little density (Boyd et al., 2000). This finding supported the postulate that mammographic dense tissue indicates proliferation of breast epithelium and stroma. This mechanism will lead to increased risk of those types of benign breast disease that confer an increased risk of breast cancer.

If breast density is a moderate risk factor and the risk factor is fairly common, breast cancers attributable to increased breast density could potentially account for an important percentage of total breast cancer cases. In Canadian breast cancer screening study, Boyd et al reported that 18.6% of cancers occurred in those subjects with the most extensive category of density of radiologists' classification and 44.1% of the cancers occurred in the two most extensive categories. The attributable risk associated with dense breast is therefore much higher than for most risk factors for breast cancer. (Boyd et al., 1995) For example, family history of breast cancer is present in about 1% of breast cancer cases but mammographic density is much more common in women with breast cancer. Therefore family history (which typically has an attributable risk of 7%) has lower attributable risk than breast density of more than 50% (as assessed radiologically) which has a greater population attributable risk of approximately 30% (Cuzick et al., 2004).

Increased parenchymal density is of greater clinical significance as a cause of false negative mammograms (Stomper et al., 1996). This is because it is difficult to achieve good contrast throughout a mammogram in women with extensive mammographically dense tissue. This will obscure some of the subtle signs of breast abnormalities. As a result, it is more difficult to diagnose breast cancer in such women (Byng et al., 1998). Barlow et al showed that sensitivity and specificity generally declined as breast density increased in diagnostic mammograms (Barlow et al., 2002).

Family history is one of the most well established breast cancer risk factor. A women with a mother or sister with breast cancer has an approximately two to threefold increase in risk of developing breast cancer. It has been established that approximately 7% of all breast cancer cases are related to inherited genes (Hulka and Moorman, 2001). The first breast cancer susceptibility gene BRCA1 gene linked to both breast and ovarian cancer was identified in 1990. This gene has been associated primarily with early onset disease before age 50. Family history of breast cancer is also associated with breast density. Ziv et al reported that women with higher breast density were more likely to have first degree relatives with breast cancer. Thus, the genetic factors that determine breast cancer risk may determine breast density as well (Ziv et al., 2003).

Other risk factors for breast cancer are parity, age, menopause status, age at menarche and HRT. Obesity is also a risk factor for development of breast cancer in postmenopausal women. This may be due to delayed detection secondary to greater difficulty in performing clinical examination of the larger breast of obese women. An increased body mass index was also associated with greater compressed breast thickness, resulting in increased geometric unsharpness, decreased image contrast and greater potential for motion unsharpness (Guest et al., 2000). This resulted in detrimental effect on mammographic image quality and lead to false negative screening mammogram. Hunt et al showed that increasing adiposity correlates with progressive increases in the rates of recall, biopsy and cancer detection for women undergoing screening mammography. Increasing adiposity also correlated with increased cancer size and stage (Hunt and Sickles, 2000). Mammographic densities are also associated with some other risk factors