MAGNETIC RESONANCE IMAGING EVALUATION OF THE EFFECT OF TUALANG HONEY IN BREAST TISSUE AMONG POSTMENOPAUSAL BREAST CANCER PATIENT TREATED WITH ANASTRAZOLE IN HUSM

By:

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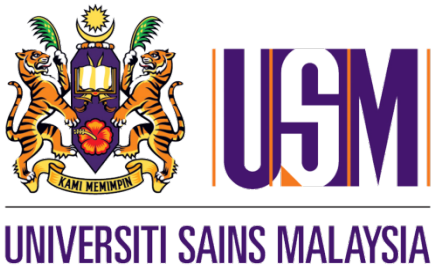

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

AI	Aromatase inhibitor
ATAC	Arimidex, Tamoxifen, Alone or in combination
BPE	Background parenchymal enhancement
ER	Oestrogen receptor
FGT	Fibroglandular tissue
HUSM	Hospital Universiti Sains Malaysia.
IQR	Inter-quartile range
MRI	Magnetic resonance imaging
PgR	Progesterone receptor
S.D	Standard deviation
TH	Tualang Honey
PACS	Picture archiving and communication system
BIRADS	Breast Imaging Reporting and Data System

ABSTRAK

Objektif: Untuk menyiasat sama ada rawatan kombinasi dengan menggunakan madu Tualang dapat mempengaruhi *background parenchymal enhancement* (BPE) payudara dalam pengimejan resonans magnet di kalangan wanita putus haid yang menghidapi penyakit kanser payudara yang menerima rawatan anastrozole.

Metodologi: Seramai 22 pesakit dari Klinik Onkologi, Hospital USM, Kubang Kerian, Kelantan terlibat dalam kajian ini. Mereka dibahagikan dalam dua kumpulan, iaitu kumpulan kawalan (1mg anastrozole sekali sehari) dan kumpulan intervensi (1 mg anastrozole sekali sehari + 20 mg TH sekali sehari). Mereka termasuk wanita putus haid yang menghidapi penyakit kanser payudara unilateral pada peringkat I, II dan III dengan reseptor estrogen (ER) atau reseptor progesteron (PgR) positif dan menerima anastrazole sebagai rawatan kurang atau tidak lebih daripada satu tahun. BPE payudara contralateral sebelum dan 6 bulan selepas rawatan telah dibandingkan sebelah menyebelah menggunakan ujian statistik.

Keputusan: 10% daripada wanita yang menerima rawatan anastrozole telah menunjukkan penurunan BPE payudara dari kategori sederhana ke kategori ringan (p = 0.317). 42% daripada wanita yang menerima rawatan kombinasi anastrozole dan TH telah menunjukkan pengurangan BPE (p = 0.034). Penurunan BPE ke satu kategori berlaku dalam empat wanita, manakala penurunan sebanyak dua kategori berlaku dalam seorang wanita.

Kesimpulan: Rawatan kombinasi TH dan anastrozole adalah lebih berkesan berbanding dengan penggunaan anastrazole sahaja dalam mengurangkan BPE dikalangan pesakit kanser payudara menopaus dengan ER atau PgR positif. dan dengan itu mengurangkan risiko berulangnya kanser..Penemuan kajian ini meningkatkan lagi nilai TH dalam bidang perubatan.

ABSTRACT

Objective: To investigate whether the combination treatment with Tualang honey (TH) influences the background parenchymal enhancement (BPE) at breast magnetic resonance imaging (MRI) in postmenopausal women with history of breast cancer treated with anastrozole.

Methodology: A total of 22 patients were recruited for both control (1 mg anastrozole daily) and intervention (1 mg anastrozole daily + 20 mg TH daily) groups from Oncology Clinic, Hospital USM, Kubang Kerian, Kelantan. Inclusive criteria included postmenopausal women with unilateral breast cancer stages I, II, or III, oestrogen receptor (ER) positive and/or progesterone receptor (PgR) positive and receiving anastrazole as treatment for or less a year were included. The BPE of contralateral breast before and 6 months after treatment was compared side by side using sign test.

Results: A decrease in BPE was noted in 10% of women (p = 0.317) on anastrozole treatment, resulted in a category change of BPE from moderate to mild. The combination of anastrozole and TH treatment showed a decrease in BPE in 42% (p = 0.034) of postmenopausal breast cancer women. BPE decreased by one category of assessment in four women and by two categories of assessment in one woman.

Conclusions: The combination of TH and anastrozole is more efficacious than anastrozole alone in decreasing breast BPE of ER-positive postmenopausal breast cancer women and thus reducing the risk of cancer recurrence. These findings further support the medicinal value of TH.

1.0 INTRODUCTION& LITERATURE REVIEW

Globally an estimated 1.6 million new cases of breast cancer are diagnosed every year with an estimated 560,000 death in 2015 (World Health Organization, 2015). Breast cancer is the leading cause of death among Malaysian women (National Cancer Registry of Malaysia 2005-2007). Approximately 5,000 Malaysian women are diagnosed with breast cancer every year, most of whom aged between 30 and 60 years, while approximately 50% of those affected are under 50-years of age (Lim *et al.*, 2008).

Annual breast MRI is often considered in many of these women for high-risk screening and/or surveillance. Approximately three-quarters of all invasive breast cancers are hormone receptor positive. The main stay of treatment for these oestrogen and/or progesterone receptor–positive breast cancers is endocrine therapy. In postmenopausal women with hormone receptor–positive disease, aromatase inhibitors (AIs) (*eg,* anastrozole) have become a standard of care as an adjuvant treatment aimed in preventing tumor relapse.

Treatment with these agents has been associated with decreased recurrence rates and greater disease-free survival than treatment with tamoxifen, a selective oestrogen receptor modulator with a different mechanism of action (Howell *et al.*, 2005). The adjuvant treatment with anastrozole or letrozole is associated with visible decreases in BPE in postmenopausal breast cancer survivors. These results provide support for the hormone sensitivity of BPE and suggest it has potential to be investigated as a marker of hormonal treatment response (King *et al.*, 2012).

However, several adverse events are reported in patients treated with anastrozole including increases the risk of osteoporosis, falls, and fractures in women affected (Chen *et al.*, 2005a, Chen *et al.*, 2005b, Kanis *et al.*, 1999). Many authors suggested that honey, which is produced from complex enzymatic process of nectar and saccharine exudates collected from various kinds of floral sources (Ball, 2007) has high antioxidant activity (Yao, 2003, Iurlina, 2009, Pyrzynska, 2009) and potential to prevent development of cancer (Valko *et al.*, 2007).

Phytochemical available in honey can be narrowed down into phenolic acids and polyphenols. Variants of polyphenols in honey are reported to have antiproliferative property against several types of cancer (Jaganathan and Mandal, 2009). TH is a wild jungle honey produced by Apis dorsata bees which make hives on tall Kompassiaexcelsa (Tualang) trees of tropical rainforests (Tan *et al.*, 2009, Nasir *et al.*, 2010). TH is reported to have positive modulation effects on carcinogen 7,12-dimethylbenz(α)anthracene in rats-induced breast cancers in rats in preliminary study (Kadir *et al.*, 2013).

Therefore, this study is aimed to investigate whether combination treatment with TH influences BPE at breast MRI in postmenopausal women with prior history of breast cancer treated with anastrozole.

1.1 LITERATURE REVIEW

1.1.1 Background Parenchymal Enhancement (BPE)

Magnetic resonance imaging (MRI) of the breast is a useful tool for the detection and characterization of breast disease, assessment of local extent of the disease, evaluation of treatment response, and guidance for biopsy and localization. MRI findings should be correlated with clinical history, physical examination results, and the results of mammography and any other prior breast imaging.

Lesion detection at contrast material–enhanced breast MRI is primarily based on a lesion's vascularity relative to normal breast tissue. Diagnostic criteria for lesion assessment and management include both morphologic features and enhancement kinetics. Normal breast tissue can also enhance on MR imaging, and this enhancement of normal breast tissue is termed BPE. Normal BPE can be classified as minimal (<25% of glandular tissue demonstrating enhancement), mild (25%–50% enhancement), moderate (50%–75% enhancement), or marked (>75% enhancement) (Morris, 2007).

Kuhl (2007) listed a number of general factors that affect the degree and amount of tissue enhancement: the amount or concentration of the contrast agent (although the effects may not be linear), T1-weighted contrast of the pulse sequence used, baseline T1-weighted relaxation times of different breast tissues, inherent T1-weighted relaxivity of the contrast agent, and diffusion rate of the contrast agent. Specifically within the breast, the anatomy of the mammary vascular system and hormonal influences on the mammary tissue also affect the pattern and degree of BPE.

Endocrine therapy with anti-hormonal treatment that includes selective oestrogen receptor modulators or aromatase inhibitors is an important part of treating oestrogen/progesterone receptor-positive tumors. Because these agents act as anti-oestrogenic agents, they can decrease hormonal stimulation of the normal background breast tissue. King *et al.* (2012) have evaluated the effects of aromatase inhibitors on BPE, and their study demonstrated a decrease in BPE in 33.9% of postmenopausal patients with cancer that was treated with aromatase inhibitors. Investigators in a separate pilot study (Mousa *et al.*, 2012) evaluated the effect of high-dosage aromatase inhibitors on BPE in healthy postmenopausal women. They performed breast MR imaging examinations before and after 3 days of high-dosage aromatase inhibitor therapy and reported a statistically significant decline in the degree of BPE after treatment.

1.1.2 Anastrozole

In the late 1970's, after a discovery that aminoglutethimide, an anti-convulsant, leads to adrenal insufficiency and suppresses estrogen synthesis via aromatase inhibition (Santen *et al.*, 1978), it was developed for the treatment of metastatic breast cancer (Smith *et al.*, 1978). Aromatase inhibitors suppress plasma estradiol levels in postmenopausal women by inhibiting the enzyme aromatase responsible for the conversion of andronstenedione to oestrone and testosterone to estradiol. Efforts to improve the efficacy and therapeutic index of this class of drugs lead to the development of the current significantly more specific third generation aromatase inhibitors: anastrozole, letrozole, and exemestane. The third generation aromatase inhibitors are divided into two major types, namely type I and type II inhibitors. The type I

inhibitors *e.g.* exemestane, are steroidal analogues of androstenedione and bind irreversibly to the same site on the aromatase molecule resulting in enzyme inactivation. Conversely, the type II inhibitors *e.g.* anastrozole and letrozole are non-steroidal in nature and bind reversibly to the heme group of the enzyme (Smith and Dowsett, 2003). Because of this reversible interaction with the enzyme, anastrozole must be constantly present to result in aromatase inhibition (Buzdar, 2003).

To reduce the early risk of relapse that often occurs in breast cancer patients after surgery, it is important to identify more effective adjuvant treatments. The Arimidex, Tamoxifen, Alone or in combination is the most significant multicenter prospective double-blinded randomized clinical trial undertaken to compare the up-front use of anastrozole, tamoxifen, or a combination of the two drugs, in postmenopausal women with early localized operable breast cancer with an intention to treat analysis. Initial analysis of the trial, performed at 33 months of median follow-up, exhibited no differences between the groups that received the combination tamoxifen and anastrozole or tamoxifen alone (Baum *et al.*, 2002). Further analysis was conducted at 47 months of median follow-up, after completion of the five years' adjuvant treatment (Howell *et al.*, 2005) and at 100 months of median follow-up (Forbes *et al.*, 2008). Since the earliest time points, it appeared clear that anastrozole was more efficacious than tamoxifen in prolonging disease free survival, time to relapse and in reducing the incidence of contralateral breast cancer.

Tamoxifen has the advantage of protecting postmenopausal women from bone loss, but increases the risk of developing thromboembolic disorders or gynecologic complications such as hot flushes, vaginal bleeding and discharge or endometrial cancer. These major side effects associated with tamoxifen were

significantly lower in the patients treated with anastrozole. The major disadvantage of anastrozole therapy lies in the increased incidence of fracture episodes, especially of the spine (Baum *et al.*, 2002). Interestingly, the increase in the number of yearly fractures was observed only during the actual anastrozole treatment, whereas in the post treatment follow-up period there were no significant differences between the anastrozole and tamoxifen treatment groups (Forbes *et al.*, 2008).

1.1.3 Honey

Honey is the substance made when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees. Honey is composed of a complex mixture of water, carbohydrates and a myriad of other minor compounds (White, 1978). The chemical composition of honey is variable and depends on regional and climatic conditions and on the type of flowers visited by the bees, thus, its classification as unifloral or polyfloral. However, on average, natural honey is composed of 17.1% water, 82.4% carbohydrates, approximately 38.5% fructose, 31% glucose and 12.9 % other sugars and 0.5% proteins, amino acids, vitamins, phenolic compounds, organic acids and multiple minerals, among other minority constituents (White, 1978, García, 1986, Moreira, 2001, Fredes, 2006, Khan *et al.*, 2007, Montenegro, 2008).

In natural honey, as well as in propolis and royal jelly, most of the phenolic compounds are present in the form of flavonoids (Viuda-Martos, 2008), a vast family of phytochemicals comprising chalcones, flavandiols, flavonols, anthocyanins and proanthocyanidins. Flavonoid biosynthesis derived from the phenylpropanoid pathway, one of the most widely studied secondary metabolic

routes in plant systems. Several phenolic compounds, especially flavonoids, are associated with multiple benefits on human health, including anti-inflammatory, anti-oxidant, anti-allergic, anti-thrombotic, anti-diabetogenic, hypoglycemic, normolipidemic, hepato-protective, anti-viral and anti-carcinogenic activities (Havsteen, 2002, Middleton *et al.*, 2000, Pérez-Trueba, 2003).

Breast cancer is the major cause of cancer deaths among women globally. Besides several other factors, the circulating levels of estrogens and dysregulated estrogen signaling pathways play a predominant role in the development and progression of breast cancer (Germain, 2011). As a result, breast cancer therapy often targets the estrogen receptor (ER)-signaling pathway. There have been some attempts to investigate if honey could modulate this important pathway. Tsiapara and colleagues evaluated the potential of Greek thyme, pine and fir honey extracts to modulate the estrogenic activity and cell viability of breast cancer cells (MCF-7) (Tsiapara, 2009). The authors found that the honey samples exhibited a biphasic activity in MCF-7 cells depending on the concentration an anti-oestrogenic effect at low concentrations and an estrogenic effect at high concentrations. In the presence of estradiol, thyme and pine honey extracts were found to antagonize oestrogen activity, while fir honey extract enhanced estrogen activity in MCF-7 cells. The study also reported variations on the effects of the three honey extracts on cell viability. While the study found no effect of thyme and pine honey on MCF-7 cells, fir honey enhanced the viability of MCF-7 cells. These dual effects of honey extracts are mostly likely due to their high contents of phenolic compounds such as kaempferol and quercetin. Phenolic compounds are phytoestrogens which exert dual action both inhibitory and stimulatory effects (Kyselova, 2011). Phytoestrogens are phytochemicals which are structurally

similar to mammalian oestrogens and therefore can bind to oestrogen receptors (Patisaul and Jefferson, 2010). They can elicit oestrogenic or anti-oestrogenic effect depending on certain factors such as its concentration (Patisaul and Jefferson, 2010, Ziegler, 2004).

2.0 STUDY PROTOCOL

2.1 Document Submitted for Ethical Approval

Title:

Magnetic Resonance Imaging Evaluation of the Effect of Tualang Honey (TH) in Breast Tissue among Breast Cancer Patient Treated with anastrozole in HUSM

General objective:

The aim of this study is to investigate if treatment with TH influences BPE at breast MRI in postmenopausal women with prior history of breast cancer treated with anastrozole.

Specific objectives:

1. To investigate change of BPE in pre and post therapy in control group (on anastrozole)
2. To investigate changes of BPE in intervention group (anastrozole with TH).
3. To compare changes of BPE between controlled and intervention group

Study Hypothesis:

TH decreases the BPE in postmenopausal breast cancer survivors with administration of anastrazole.

Methodology:

This is a randomized controlled trial for six months duration which will be conducted in Oncology Clinic, Hospital USM, Kubang Kerian, Kelantan.

Study population:

All volunteered breast cancer patient receiving adjunct chemo-adjuvant hormonal therapy

Inclusion criteria:

1. Postmenopausal women with unilateral breast cancer patients; stages I to III
2. Have HPE result available to confirm the diagnosis
3. Having ER positive and/or PgR positive
4. Receiving Anastrazole treatment with maximum of 1 years

Exclusion criteria:

1. History of allergy to TH
2. Receiving hormone replacement therapy
3. On over the counter supplement - *e.g* honey, herbs,
4. Liver or renal impairment

Sampling technique:

Randomization method based on block of four using computer generated program will be applied for sampling.

Sample size:

Based on study by King *et.al.*,2012

Sample size calculation done by dichotomous variable using G*Power Software Calculation version 3.1.3 (Germany),

Input:

α = 0.05

power = 0.9

Effect size = 0.3 (% of decrease BPE in anastrozole (control group)

Constant proportion = 0.5 (% of expected result decreased BPE in TH (intervention group)

n= 28

The highest calculated sample size was 28. However after considering a 20% drop-out, the final sample size for each group is 31

Research tool:

MRI Philips Achieva 3.0T X-Series

Study protocol:

1. Subjects were screened against the inclusion/exclusion criteria. Subjects who agreed to participate will be asked to sign written informed consent forms. They were informed to come again in the 2 weeks time for the MRI appointment.
2. Patient were randomised based on blocks of 4 for two groups (anastrozole group/ control group) and anastrozole with TH (intervention group) using a computer generated program.

i. Group 1 (control group: Subject receiving anastrozole 1 mg daily (po)

ii. Group 2 (intervention group) : Subject receiving anastrozole 1 mg daily and TH supplement of 20 gram daily. The choice of the dose of TH in this study is based on the study conducted by Nik Hazlina *et.al.* (2012).

Data Collection:

During visit 1 (baseline) for the postmenopausal breast cancer patient on anastrozole the baseline of contrast enhanced MRI breast was performed using MRI Philips Achieva 3.0T X-Series. All patients must immediately report if adverse event occurs. Each patient was given opportunity to ask any queries regarding the study. MRI breast were reported by a consultant breast radiologist with 11 year experience.

At visit 2 (month six), repeat contrast enhanced MRI breast was performed and any adverse event were assessed. Patient were informed about study completion and advised to continue normal routine follow-up. Patients are allowed to withdraw from the study at any point of the study.

Compliance:

In order to ensure compliance of honey and anastrozole, subject will be educated regarding the importance of compliance to the supplement and anastrozole. Subjects will be given a diary and phone calls made every month to ensure compliance.

TH was supplied every 2 monthly.

3.0 MANUSCRIPT

3.1 Abstract

Objective: To investigate whether the combination of anastrozole and Tualang honey (TH) influences background parenchymal enhancement (BPE) in breast magnetic resonance imaging (MRI) of postmenopausal women with a history of breast cancer who were treated with anastrozole.

Methodology: A total of 22 patients were recruited from the Oncology Clinic, Hospital Universiti Sains Malaysia, Kelantan. The patients were divided into the control (anastrozole 1 mg daily) and intervention (anastrozole 1 mg daily + TH 20 mg daily) groups. The inclusion criteria were postmenopausal women with stage I, II, or III unilateral breast cancer; with estrogen receptor (ER)-positive and/or progesterone receptor (PgR)-positive disease; and who received anastrozole treatment for one year or less. The BPE of the contralateral breast before and six months following treatment was compared using the sign test.

Results: There was a decrease in BPE in 10% of the women (p=0.317) who received only anastrozole, which resulted in a change of BPE category from moderate to mild. However, the combination of anastrozole and TH evoked a decrease in BPE in 42% of the patients (p=0.034), among which BPE decreased by one category of assessment in four women and by two categories of assessment in one woman.

Conclusions: The combination of TH and anastrozole is more efficacious than anastrozole alone in decreasing breast BPE of postmenopausal women with ER-positive breast cancer and thus reducing the risk of cancer recurrence. These findings support the medicinal value of TH as an adjuvant treatment to anastrozole.

3.2 Introduction

Globally, it is estimated that 1.6 million new cases of breast cancer are diagnosed every year, with 560,000 fatalities (World Health Organization, 2015). Breast cancer is the leading cause of death among Malaysian women (National Cancer Registry of Malaysia 2005-2007). Approximately 5,000 Malaysian women aged between 30 and 60 years are diagnosed with breast cancer every year, and approximately 50% of these women are relatively young and below 50 years of age (Lim *et al.*, 2008).

Unlike advanced disease, breast cancer is potentially curable if detected early (Wolff, 2000). Treatment of early stage breast cancer following surgery may involve adjuvant therapy consisting of systemic endocrine therapy and/or chemotherapy to prevent or delay tumor recurrence. The ultimate goal of adjuvant endocrine therapy is to treat early breast cancer successfully with minimal adverse effects.

The introduction of aromatase inhibitors (AIs) as an adjuvant treatment for postmenopausal women with hormone receptor-positive breast cancer has significantly changed the management of this disease. These agents are commonly used in place of or together with tamoxifen because of the demonstrated improvement in disease-free survival compared to the use of tamoxifen alone (Burstein *et al.*, 2010). In postmenopausal women with hormone receptor–positive disease, AIs (such as anastrozole, letrozole and exemestane) have become standard of care as an adjuvant treatment aimed at preventing tumor relapse. Anastrozole is the only third-generation AI with available data as an adjuvant treatment for early breast cancer in postmenopausal women. Initial and updated results from the Arimidex, Tamoxifen alone or in combination (ATAC) trial confirmed that anastrozole was

more effective than tamoxifen for postmenopausal women with hormone-responsive tumors (Tobias, 2004).

Magnetic resonance imaging (MRI) of the breast is often used following breast cancer surgery to monitor disease recurrence (Brennan *et al.*, 2010). Recent studies have suggested that background parenchymal enhancement (BPE) of normal breast parenchyma is useful in breast cancer risk prediction (King *et al.*, 2012a) and in treatment response and outcome assessments (King *et al.*, 2012b). Although it has been postulated that BPE on MRI is related to vascular supply and permeability, the precise causes of differences in enhancement between and within individuals during their lifetime are not well understood.

The risk of breast cancer for women with increased breast density is reported to be four to six times higher than that in women with less dense breast tissue (Harvey and Bovbjerg, 2004). Treatment with anastrozole decreases recurrence rates and is associated with a higher chance of disease-free survival for breast cancer patients following a reduction in BPE. Thus, BPE is used as an indicator of the response to hormonal treatment in breast cancer patients. Evidence suggests that hormonally sensitive physiological changes that occur in the breast may be reflected by variations in BPE during the menstrual cycle (Kuhl *et al.*, 1997). Another study indicated an increased BPE in women on hormone replacement therapy (Hussain *et al.*, 1999) but a decreased BPE among patients taking anti-estrogen medications, including tamoxifen (King *et al.*, 2012b), raloxifene (Eng-Wong *et al.*, 2008) or toremifene (Oksa *et al.*, 2009), indicating that BPE is a good indicator of the treatment response to hormones and may be used to aid breast cancer diagnosis.

Although anastrozole is extensively used for breast cancer treatment, several adverse effects have been reported with its use, including the risk of osteoporosis, falls and fractures (Chen *et al.*, 2009, Chen *et al.*, 2005a, Chen *et al.*, 2005b, Kanis *et al.*, 1999). Many authors have suggested that honey, which is produced via complex enzymatic processes from nectar and saccharine exudates from various floral sources, has high antioxidant activity (Lurlina, 2009, Pyrzynska, 2009, Yao, 2003) with good potential to prevent the development of cancer (Valko *et al.*, 2007). However, to the best of our knowledge, its use as an adjuvant to modern therapy has not been investigated.

Several types of polyphenols in honey have been reported to exert antiproliferative activity against different types of cancer (Jaganathan and Mandal, 2009). An example of a honey with such properties is Tualang Honey (TH), a wild jungle honey produced by *Apis dorsata* bees, which make their hives on tall *Koompassia excelsa* (Tualang) trees in tropical rainforests (Nasir *et al.*, 2010, Tan *et al.*, 2009). The main active constituents in TH from Malaysia are phenolic acids and polyphenols (MI Khalil *et al*., 2011). In a preliminary study, TH was reported to confer positive modulatory effects on carcinogen (7,12-dimethylbenz(α)anthracene)-induced breast cancer in rats (Kadir *et al.*, 2013), indicating its potential as an anticancer therapy. The aim of this study was to investigate whether TH is a useful adjuvant to anastrozole in postmenopausal women.

3.3 Methodology

The main objective of this study is to investigate if treatment with TH influences BPE in postmenopausal women with prior history of breast cancer treated with anastrozole. Comparison of BPE of non-affected breast between control and intervention group were made pre and post treatment.

3.3.1 Patient and Study Design

Ethical approval was obtained from the Human Research Ethics Committee of Universiti Sains Malaysia (USM), USMKK/PPP/JEPeM/[260.3(21)], which complies with the Declaration of Helsinki. This randomized controlled trial was conducted for 1.5 years (from October 2014 until April 2016) in the Oncology Clinic, Hospital USM, Kubang Kerian, Kelantan, Malaysia. A total of 22 patients were recruited and then divided into the control (n=10) and intervention (n=12) groups. Postmenopausal women with stage I-III unilateral estrogen receptor (ER)- and/or progesterone receptor (PgR)-positive breast cancer who received anastrozole treatment for less than a year were included in the study. Patients with a history of allergy to TH, who took over-the-counter supplements such as honey or herbs, who received hormone replacement therapy or who presented with liver or renal impairments were excluded.

Patients who consented to participate in the study were asked to provide written informed consent. Patients were randomized based on a block size of four using a computer-generated program. The patients were randomly assigned into either the control or intervention group. Patients in the control group received anastrozole (1 mg) daily, while patients in the intervention group

received anastrozole (1 mg) and TH (20 g) daily. The TH supplements were individually packaged in aluminum foil containing a fixed amount of honey in order to standardize the administered amount. The dose of TH in this study was based on a previous study conducted by Nik Hazlina *et al.* (2012). In addition, supplement packages were counted at the end of the study to ensure that the patients complied with the treatment regimen. The BPE of the non-affected breast in the control and intervention groups was compared before (at baseline) and after treatment (after six months). Patients were allowed to withdraw from the study at any time.The dose of TH in this study is based on a previous study conducted by Nik Hazlina *et.al*. (2012).

3.3.2 MRI Protocol

Breast MRI with intravenous gadopentetate dimeglumine (Magnevist®) was performed using a Philips Achieva 3.0T X-Series MRI at the Department of Radiology, USM, on the first (0 months) and second visits (six months). The patient was placed in a prone position, and a breast coil was applied to the breasts. The standard imaging protocol included a localizing sequence, followed by a sagittal T1-weighted fat-saturated sequence performed before and after intravenous injection of Magnevist® (15 ml) via an in-dwelling intravenous catheter and a subsequent saline flush (10 ml). The slice thickness was 3 mm. Following image acquisition, the pre-contrast images were subtracted from the contrast images on a pixel-by-pixel basis to generate a maximum intensity projection (MIP). All MRI images were reviewed on a high resolution PACS monitor (Centricity, GE Healthcare).

3.3.3 MRI Interpretation

All images were independently reviewed by a breast imaging radiologist who was blinded to the study group. A combination of pre- and post-contrast T1 fat-saturated images and MIP images was used to determine the BPE. For each MRI examination, BPE was prospectively assigned into one of four categories in accordance with the anticipated BIRAD MRI lexicon classification system: “minimal” (<25% of glandular tissue demonstrating enhancement), “mild” (25%–50% enhancement), “moderate” (50%–75% enhancement), or “marked” (>75% enhancement) (Figure 1). The pre- and post-treatment breast MRI results were compared side-by-side to evaluate the changes in BPE, which increased the sensitivity since images could be compared simultaneously (King *et al.* (2012a).

3.3.4 Statistical Analysis

Statistical analysis was performed using SPSS (version 18, SPSS Inc., Chicago, IL, USA). The data are presented as the mean ± S.D. However, if the data were not normally distributed, the median and inter-quartile range were calculated. Data normality was verified using the Shapiro-Wilks test. A side-by-side comparison of BPE before and six months following treatment was utilized to determine the changes in BPE. The sign test was used to identify significant differences in the number of women with BPE changes. Categorical data were compared using chi-square and Fisher’s exact tests. A p value <0.05 was considered statistically significant.

3.4 Results

3.4.1 Patient Characteristics

A total of 40 postmenopausal women with unilateral breast cancer (stage I to III) were assessed for eligibility for this study. Ten subjects have to be excluded [claustrophobia (n=7), inconsistent history of bilateral breast cancer (n=1), hip implant (n=1) and refused MRI (n=1)]. From the 30 patients recruited, seven dropped out due to refusal of MRI follow up (n=4), while three patients were non-compliant with the honey regimen. A patient passed away due to disease. As a result, a total of 22 patients aged 50 to 76 years were recruited. Among these patients, 10 were randomly allocated to the control group (anastrozole only), while 12 were placed in the intervention group (honey supplement and anastrozole); all patients received treatment and were followed for six months.

Patient characteristics (n=22) are shown in Table 1. The majority of the patients were Malays (90.9%), which reflects the demography of the local population; the remaining patients were Chinese. The mean patient age was 59 ± 5.9 years at the time the baseline MRI was taken, and the mean menopausal age was 50 ± 3.0 years; all patients experienced natural menopause.

3.4.2 Background Parenchyma Enhancement Changes

Based on the MRI findings, the patient's BPE was assigned one of several different categories. In the control group, the majority (90.0%) of the women had a stable BPE, but 10% showed a decrease in BPE (Table 2). The difference between the number of patients with decreased and stable BPE was not statistically significant (p=0.317). Approximately 25% of the patients with a moderate BPE at baseline showed a reduction in BPE to mild (Figure 2). None

of the women with minimal (n=1), mild (n=2) or marked (n=3) BPE showed any change in BPE.

In the intervention group, 41.7% had a decrease in BPE, while 58.3% did not show any BPE change (p=0.034) following treatment (Table 3). TH improved the BPE by a single category in four women and by two categories in one woman (Figure 3). Among the women who had a moderate BPE prior to treatment, 29% experienced a decrease in BPE to mild after treatment.