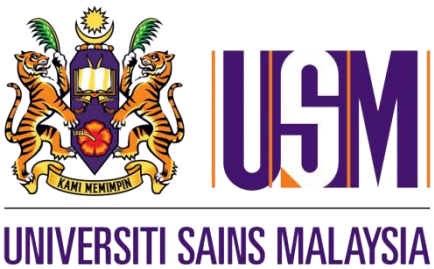
THE EFFECTS OF TUALANG HONEY ON BONE MINERAL DENSITY AS AN ADJUVANT THERAPY TO ANASTROZOLE AMONG POSTMENOPAUSAL BREAST CANCER PATIENTS

By:

DR NOR HASNINA BT MOHD HASSAN

Dissertation Submitted in Partial Fulfillment of the Requirements for Master of Medicine (Radiology)



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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

AI	Aromatase inhibitor
ATAC	Arimidex tamoxifen alone or in combination
BMD	Bone mineral density
BMI	Body mass index
DEXA	Dual energy x-ray absorptiometry
ER	Oestrogen receptor
HUSM	Hospital Universiti Sains Malaysia.
IQR	Inter-quartile range
PgR	Progesterone receptor
S.D	Standard deviation
TH	Tualang Honey

ABSTRACT

Objective: To determine the effects of Tualang Honey (TH) on bone mineral density (BMD) as assessed by dual energy X-ray absorptiometry (DEXA) scan when used as an adjunct to anastrazole among postmenopausal breast cancer patients.

Methodology: Patients (n=33) were recruited from the Oncology Clinic, Hospital USM, Kelantan, Malaysia. The inclusion criteria included postmenopausal women with breast cancer stages I, II, or III, oestrogen receptor (ER) positive and/or progesterone receptor (PgR) positive. The patients were randomized to receive either anastrozole (1 mg) alone or anastrazole (1 mg) and TH (20 g) intervention group. Bone DEXA scan was conducted twice, at baseline (during the first visit) and six months later.

Results: The T scores were significantly decreased when compared to the baseline for both femoral (−0.40 *vs* −0.50, $p = 0.013$) and the lumbar spine (−1.60 *vs* –1.70, $p = 0.034$), following six months of anastrozole alone treatment. On the other hand, there was no significant difference between the baseline and the T scores following six months of treatment for both femoral (0.35 *vs.* 0.50, $p = 0.286$) and the lumbar spine (−1.05 *vs* –0.95, $p = 0.074$) for the intervention group indicating that TH can ameliorate bone loss as a result of anastrazole use. Administration of anastrozole significantly reduced the median femoral BMD by 2.23% and median lumbar spinal BMD by 2.84% after six months.

However, in the intervention group, there was no significant change in term of both BMD and T score when compared to the baseline following six months which again indicate the potential of TH in ameliorating bone loss.

Conclusions: TH has the potential to be used as an adjunct to prevent bone loss among postmenopausal breast cancer women treated with anastrozole.

Keywords: *Bone Loss, bone mineral density, anastrozole, Tualang honey*

ABSTRAK

Objektif: Untuk menentukan kesan TH terhadap kepadatan mineral tulang (BMD) dengan menggunakan imbasan tenaga dwi x-ray absorptiometry (DEXA) dalam pesakit kanser payu dara yang telah putus haid dan dirawat dengan anastrozole.

Metodologi: 33 pesakit dari Klinik Onkologi, Hospital USM, Kubang Kerian, Kelantan, terlibat dalam kajian ini. Mereka merangkumi wanita putus haid yang menghidap penyakit kanser payu dara peringkat I, II, atau II, dengan reseptor oestrogen (ER) positif dan / atau reseptor progesteron (PgR) positif. Pesakit kemudiannya dimasukkan secara rawak ke sama ada kumpulan anastrozole sahaja (anastrozole 1mg) atau kumpulan intervensi anastrozole (1mg) dan TH (20g). Imbasan kepadatan tulang dijalankan dalam semua pesakit pada lawatan pertama dan juga semasa lawatan selepas rawatan selama enam bulan.

Keputusan: Dalam pesakit yang dirawat dengan anastrozole, skor T selepas enam bulan rawatan telah menurun dengan ketara berbanding dengan skor T pada garis dasar di tulang femur (−0.40 *vs.* −0.50, p = 0.013) dan tulang belakang lumbar (−1.60 *vs.* −1.70 , p = 0.034), manakala dalam pesakit yang dirawat dengan anastrozole dan TH, tidak ada perbezaan yang signifikan antara skor T asas dan skor T selepas enam bulan rawatan di tulang femur (0.35 *vs.* 0.50, p = 0.286) dan tulang belakang lumbar (−1.05 *vs.* −0.95, p = 0.074).

Enam bulan rawatan anastrozole mengurangkan BMD median tulang femur sebanyak 2.23% dan median BMD tulang belakang lumbar sebanyak 2.84%. Walau bagaimanapun, rawatan enam bulan dengan anastrozole dan TH tidak menunjukkan sebarang perubahan yang signifikan pada skor T dan BMD jika dibandingkan dengan nilai-nilai asas.

Kesimpulan: TH mempunyai potensi untuk digunakan sebagai rawatan pencegahan kerapuhan tulang di pesakit kanser payu dara putus haid yang dirawat dengan anastrozole.

Kata kunci*: Kerapuhan tulang, anastrozole, madu Tualang*

SECTION 1

INTRODUCTION

SECTION 1

INTRODUCTION AND LITERATURE REVIEW

Worldwide, breast cancer is the most common type of malignancy among women. Early detection of breast cancer can save lives and prolonged life expectancy. Recently, several new medications have been developed for breast cancer treatment, among which is anastrozole. Anastrozole is an aromatase inhibitor used in postmenopausal women who are hormone–positive (Buzdar *et al.*, 2006).

However, in addition to its potential benefits, anastrazole causes several some side effects including bone loss (Forbes *et al.*, 2008). Therefore, there is higher risk of osteoporosis, fall and fractures among its users. Bone loss tends to progress much more rapidly following menopause due to oestrogen deficiency. Decline serum oestradiol levels contribute to increase lifespan of the osteoclasts, leading to bone resorption while at the same time decreasing the lifespan of osteoblast. The overall effects are less bone is formed contributing to lower bone mineral density (BMD).

Bone strength and density are very closely related. For example, individuals with low BMD have increased risk for fragility fracture. Osteoporosis is the systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and risk of fracture. In 1994, with the development of the DEXA units capable of highly precise and accurate measurement of the BMD (Bontranger).the World Health Organization (WHO) recommended the use of BMD for diagnosis of the osteoporosis. Osteoporosis in the postmenopausal women is defined as having a BMD more than 2.5 standard deviation (S.D)

below the mean T score. T score were determined according to the WHO definitions as SD unit from the mean BMD of 25 years old healthy women. Based on the WHO, the BMD classifications are operationally used to define patients as the below groups:

1) Normal = T score of at least -1.0

2) Osteopenia = -1< T score < -2.5

3) Osteoporosis = T score ≤ - 2.5

4) Established osteoporosis = T ≤ - 2.5 in the presence of one or more fragile fractures

It is estimated that 80% of the worldwide population rely on the traditional medicine to meet their health care needs as alternative to the conventional medicine (Bogdanov *et al.*, 2008). Among the traditional medicine, natural products including honey, has long been used for medicinal purposes in recorded history. Honey has been proven to be of value in accelerating wound healing, as well as ulcers and skin infection (Christiansen 1991). It also has shown to be effective antioxidant and anti-inflammatory agent. Honey consists of primary sugar such as monosaccarides, dissacharides, oligosaccarides and polysaccarides as well as enzymes such as glucose oxidase, diastase, invertase, catalase and peroxidase (Bogdanov et al., 2008). The other chemical contents of honey are organic acids, vitamins, amino acid, proteins, flavonoids and phenolic acid that are beneficial for human health (Christiansen 1991).

In Malaysia, TH has been widely used by the researchers in order to discover its hidden potential value. It is known to have antimicrobial, antiparasitic, antioxidant, and anti-inflammatory effects. Its content of high

antioxidant as well as exerting anti-inflammatory effect which can act as a free radical scavenger can reduce the oxidative stress level and inhibit the cytokines. All of these can result in survival of osteoblast, reduced osteoclatogenic activity and reduced bone loss (Mohd Effendy, 2012).

The objective of this study is to determine and compare bone density of the postmenopausal breast cancer patients treated with anastrazole alone and Anastrazole + TH supplementations for six months.

SECTION 2

METHODOLOGY

General objective:

The aim of this study to determine the effect of TH on BMD (assessed by DEXA scan) in postmenopausal breast cancer patient treated with anastrozole.

Specific objectives:

1. To investigate the mean osteoporotic changes in pre and post treatment in postmenopausal breast cancer patient treated with Anastrazole (control group) and Anastrazole with TH Supplementation (intervention group).
2. To compare the mean osteoporotic changes in six months duration between these two groups.
3. To determine the effect of TH supplementations in preventing osteoporosis in postmenopausal breast cancer patient treated with anastrozole.

Methodology:

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

Population and Sample:

All postmenopausal women with breast cancer recruited during the study's duration.

Sampling technique:

Sampling using randomization based on block of four using computer generated programme will be applied.

Randomization programme from (www.randomization.com).

Inclusion Criteria:

1. Postmenopausal women with breast cancer without bone metastasis receiving anastrozole as a treatment within a year.

Exclusion Criteria:

1. History of allergy to honey
2. Receiving hormone replacement therapy
3. Known bone metabolism disorder
4. Previously diagnosed with osteoporosis, hip fracture or prosthesis
5. Liver or renal dysfunction.

Sample Size Calculation:

Sample size calculation to compare quality of life score between groups was done by comparing two means using Power and Sample size calculation software version 3.0.10**.**

Input:

$\alpha = 0.05$

power $= 0.9$

$\sigma =$ standard deviation of bone density level was 1.0 (Based on *Theresa A.Guise, The oncologist Journal*)

$\delta =$ clinically significant difference between in bone density level between groups is set at 0.8

m=ratio of control to intervention group is 1

n = 34

The calculated sample size is 34 in each group; however after considering 25% drop out, the final sample size for each group is 42. The total of subject is 84.

Research Tools

a. Physical examination (height, body weight)
b. Bone densitometry scan machine (DEXA SCAN , Hologic, Dynamic 2 at Radiology Department, HUSM)

Subjects

- Subject will be screened against the inclusion/exclusion criteria. Subjects who agreed to participate will be asked to sign written

informed consent forms. They will be informed to come again in the 2 weeks for the bone scan appointment.

- Patient will be randomized based on blocks of 4 for two groups (Anastrozole group/ control group) and Anastrozole with TH (intervention group) using a computer generated programme.

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

- 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 serum biochemical marker and bone densitometry scan (DEXA) will be performed. Bone densitometry scan will be reported by radiologist, Dr. Nik Munirah Nik Mahdi or Prof Mohd Shafie bin Abdullah and radiology medical officer, Dr Nor Hasnina Mohd Hassan.

At visit 2 (month 6), repeated serum biochemical and bone densitometry scan (DEXA) will be performed. Patient will be 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 will be made every month to ensure compliance. TH was supplied every two months.

Methodology of Bone Densitometry

Patients would undergo a bone densitometer examination using a standard imaging protocol and Hologic, Dynastic 2 bone scan at the Department of Radiology USM Kubang Kerian, Kelantan. The bone densitometry assessment has very low radiation dose, about 1/10 of normal CXR exposure.

Patients will be asked to lie on their back, on a padded table, in a comfortable position. The lumbar spine (lower back) and the hip are the skeletal sites and are usually examined by using the scan. The densitometer normally performs spine and femur scans in about 6 minutes and 3 minutes, respectively, with adequate spatial resolution (1.2 x 1.2 mm). Total body scan can between 10 to 20 minutes.

After a routine scan, the T and Z scores will be measured for the total body, dual femur and AP spine. Densitometer graph will be plotted based on the BMD and patient's age will be compared against the T and Z scores. The Z-scores can be used to compare a measurement to a reference value. The Z-score is the number of standard deviations away from the average value of the reference group. This reference group usually consists of people of the similar age and gender and sometimes race and weight are also included.

The T-score is the relevant measure when screening for osteoporosis. It is the bone mineral density (BMD) at the site when compared to the young normal reference mean. It is a comparison of a patient's BMD to that of a healthy thirty-year-old. The US standard is to use data for a thirty-year-old of the same sex and ethnicity, but the WHO recommends using data for a thirty-year-old white female in general. Values for thirty-year-olds are used in post-menopausal women and men over age 50 because they better predict risk of future fracture. The criteria of the WHO 2005 are:

- Normal is a T-score of -1.0 or higher
- Osteopenia is defined as between -1.0 and -2.5
- Osteoporosis is defined as -2.5 or lower, (BD that is 2.5 standard deviations below the mean of a thirty-year-old man/woman)
- Established osteoporosis = T ≤ -2.5 in the presence of one or more fragile fractures

The bone densitometer images and data will be analysed by a radiologist. Data will be entered and analyzed using SPSS version 20 and $p < 0.05$ will be considered as statistically significant. The confidence interval will be set at 95%.

MANUSCRIPT

INTRODUCTION

Breast cancer is the most common malignancy affecting women in both developed and the less developing world. In 2011, it is estimated that more than 508 000 women died due to breast cancer worldwide (World Health Organization, 2013). In Malaysia, the National Cancer Registry (2003-2005) reported an age-standardized rate of 47.3 per 100 000 women for breast cancer (Lim *et al.*, 2008). Since advanced breast cancer in postmenopausal women remains an incurable disease its treatment is aimed at palliation and improved quality of life, inhibition of disease progression as well as improvement in survival time where possible.

Endocrine therapy for breast cancer is now a widely accepted treatment modality and is primarily directed at reducing the synthesis of oestrogen or alternatively blocking oestrogen receptors in tumours that are hormone-sensitive. Anastrozole is a nonsteroidal oral aromatase inhibiting drug approved for use in postmenopausal woman with localized breast cancer (Howell *et al.*, 2005, Buzdar *et al.*, 2006). It has been reported that five years of anastrozole administration is superior to tamoxifen if administered for the same duration and therefore, anastrozole has become the most commonly prescribed adjuvant endocrine therapy for hormone-dependent early breast cancer in postmenopausal women (Baum *et al.*, 2003, Dowsett *et al.*, 2010, Thurlimann *et al.*, 2005). Nevertheless, in addition to its potential benefits, anastrozole known to cause bone loss (Forbes *et al.*, 2008).

In women, bone loss progresses much more rapid following menopause due to oestrogen deficiency. Oestrogen is known to play a central role in the maintenance of normal BMD in women (Leslie *et al.*, 1995). In postmenopausal

women, low oestradiol levels are associated with increased bone turnover, low BMD, and an increased risk of fracture (Chapurlat *et al.*, 2000, Chapurlat *et al.*, 2001, Cummings *et al.*, 1998, Ettinger *et al.*, 1998, Garnero *et al.*, 2000, Rogers *et al.*, 2002). In the 10 years following the menopause, there is a reduction in BMD averaging 2% per annum (World Health Organization, 1994) with osteoporosis being the major cause of morbidity in postmenopausal women. Anastrozole markedly suppresses oestrogen levels and due to its mode of action, has the potential to have a deleterious effect on the skeletal health of postmenopausal women receiving the drug as adjuvant treatment for early breast cancer.

BMD measurements are important in evaluating patients at risk of osteoporosis (Kanis and Gluer, 2000). In general, the preferred method of testing is to use DEXA scans of the central skeleton to measure BMD of the lumbar spine and hip. In fact, the WHO working group recommended that the diagnosis of osteoporosis should be based only on the T-score obtained at the hip measured by DEXA. Therefore, BMD measurements in postmenopausal women should be interpreted using the WHO T-score definitions of osteoporosis and osteopenia (Table 1) (Kanis and Gluer, 2000).

One of the strategy used for osteoporosis treatment is calcium supplementation (Mackerras and Lumley, 1997). Other alternative treatments use natural products. For example, polyphenols in fruits and vegetables have demonstrated beneficial effects on bone in rats (Muhlbauer *et al.*, 2002, Devareddy *et al.*, 2008). Natural antioxidants such as palm tocotrienols, have been shown to prevent bone loss in many osteoporosis-induced rat models (Ahmad *et al.*, 2005).

Honey is a natural product that has been widely used for its therapeutic effects due to its antioxidant effect. Honey is composed primarily of fructose and glucose but also contains fructo-oligosaccharides (Chow, 2002) and many amino acids, vitamins, minerals and enzymes. The composition of honey varies depending on the plants on which the bee feeds. However, almost all natural honey contains flavonoids, phenolic acids, ascorbic acid, tocopherols, catalase, superoxide dismutase, reduced glutathione, some Millard reaction products and peptides. Most of the compounds act together synergistically (Johnston *et al.*, 2005, Rakha *et al.*, 2008).

TH (*Koompasia excelsea*) is a multifloral honey collected in the jungles of Malaysia and in many other Asian countries. It is traditionally used as a health and anti-ageing supplement. A study on its antioxidative compounds reported that TH had the highest total phenolic, protein and ascorbic acid content when compared to other honey types in Malaysia (Kishore *et al.*, 2011). In addition, TH had also shown the highest radical scavenging activity, suggesting that it is a good source of antioxidant able of free radical scavenging which can help reduce bone resorption activity by osteoclasts and subsequently maintaining overall bone health.

Therefore, the objective of this study is to determine and compare BMD of postmenopausal breast cancer patients, treated with anastrozole alone or anastrozole plus TH supplementation for six months.

METHODOLOGY

The aim of this study was to determine the effect of TH on BMD (assessed by DEXA scan) in postmenopausal breast cancer patient treated with anastrozole. Specifically, it was to investigate the mean osteoporotic changes in pre and post treatment in postmenopausal breast cancer patient treated with anastrozole (control group) and anastrozole with TH Supplementation (intervention group). Besides, the mean osteoporotic change in six months duration between was also compared between the two groups.

This study was a randomized, prospective controlled trial for six months conducted in Radiology Clinic, Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan between March 2015 and April 2016. The ethical approval was obtained from Human Research Ethics Committee of Universiti Sains Malaysia Research Ethics Committee of Universiti Sains Malaysia (JEPem USM Code USMKK/PPP/JEPeM/[260.3(210)] which complies with the Declaration of Helsinki.

By using the two means formula, sample size with power of 90%, alpha 0.05, difference of interest of 0.8, and S.D of 1.0, a total of 34 patients was recruited for both groups. The inclusion criteria were postmenopausal women with breast cancer without bone metastasis receiving anastrozole as a treatment within a year. Patient with history of allergy to honey, receiving hormone replacement therapy, known bone metabolism disorder, previously diagnosed with osteoporosis, hip fracture or prosthesis and liver or renal dysfunction were excluded from this study.

Recruited patients were then assigned into either control group or intervention group using randomization based on block of 4 by a computer

generated programme. The patient in control group received anastrozole (1 mg) daily, while the patient in group 2 received Anastrozole (1 mg) daily and TH supplement (20 g) daily. The choice of the dose of TH in this study is based on the study conducted by Nik Hazlina *et al*.(2012).

After obtaining written informed consents from the patients, bone densitometry scan (DEXA) was performed at the first visit. Patients underwent a bone densitometer examination using a standard imaging protocol and Hologic, Dynastic 2 bone scan at the Department of Radiology USM Kubang Kerian, Kelantan. The bone densitometry assessment has very low radiation dose, about 1/10 of normal CXR exposure.

Patients were asked to lie on their backs on a padded table, in a comfortable position. The lumbar spine (lower back) and the hip are the skeletal sites and are usually examined by using the scan. The densitometer performed spine and femur scans for approximately 6 and 3 minutes respectively, with adequate spatial resolution (1.2 x 1.2 mm). For the total body scan, the time was approximately 10 to 20 minutes. Following a routine scan, the T and Z scores were measured for the total body, dual femur and AP spine. Densitometer graph was plotted based on the BMD and patient's age was compared against the T and Z scores.

After six months of treatment at second visit, repeated measurements of serum biochemical and bone densitometry scan (DEXA) were performed. Patient was informed about study completion and advised to continue normal routine follow up. Patients were also allowed to withdraw from the study at any time during the study duration.

Quantitative data were analyzed using Microsoft® Office Excel and Statistical Product and Service Solutions (SPSS) for Windows, SPSS Inc.© (version 18, SPSS Inc., Chicago, IL,USA). Continuous variables are expressed as mean ± S.D or median (interquartile range, IQR) and categorical variables as percentages. The student's and paired-sample t-tests were performed if normality was demonstrated by histogram. Otherwise, non-parametric tests (Mann-Whitney U or Wilcoxon signed rank tests) were used. Pearson chi-square test and Fisher's exact test were used to compare the categorical variables. All statistical tests were considered significant when the two-sided *p* value was < 0.05.

RESULTS

Patients

In this randomized control trial study, 40 postmenopausal breast cancer patients were screened and were found to be eligible for the study. They were then randomized into two treatment groups, i.e. anastrozole and anastrozole + TH. However seven patients withdrew from the study (two due to noncompliance to honey, three refused to continue with the study, a patient was found unsuitable due to osteoporotic fracture which occurred during the study and a patient unfortunately passed away) (Table 2).

Finally, a total of 33 patients was recruited and among them 13 patients received anastrozole treatment and the remaining 20 patients received anastrozole together with TH supplementations during the six months study period. At the time baseline DEXA scan was done, the patient's age ranged between 41 and 76 with a mean age of 58 years. The postmenopausal breast

cancer patients had a mean menopause age of 49 years. They were diagnosed with breast cancer stages I to III and with the majority (54%) suffering from on stage II disease. The mean body mass index (BMI) of the patients was 28.0 kg/m^2 which is considered to be in the overweight range.

The effects of Treatment on T-score and BMD

Table 3 demonstrated the results of the T score at baseline and six months after treatment for both anastrozole and anastrozole + TH treatment groups. In the patients treated with anastrozole, the baseline median T score of femoral and lumbar spine were −0.40 and −0.60 respectively. The Wilcoxon Signed-ranks test indicated that the T scores after six months of treatment were significantly decreased compared to the baseline scores at both femoral (−0.40 *vs.* − 0.50, p = 0.013) and lumbar spine (−1.60 *vs.* –1.70, p = 0.034). TH supplement significantly demonstrated protective effect against bone loss following anastrozole treatment. In patients treated with anastrozole and TH supplement, there was no significant difference between the baseline T scores and the T scores after six months of treatment at both femoral (0.35 *vs.* 0.50, p = 0.286) and lumbar spine (− 1.05 *vs.* – 0.95, p = 0.074).

Table 4 showed the result of the BMD at baseline and six months after treatment for both groups. The baseline median BMD of the study patients in anastrozole group was 0.808 g/cm^2 at femur and the mean BMD at the lumbar spine was 0.811 g/cm^2. In comparing the BMD of femur before and after anastrozole treatment, there was significant decrease of BMD at six months when compared with the baseline value after analysis with Wilcoxon Signed Rank test (0.808 g/cm^2 *vs.* 0.70 g/cm^2, p = 0.001). For measurement at lumbar

spine, the BMD also significantly decreased after six months of anastrozole treatment, analyzed with paired t test (0.811 g/cm^2 *vs.* 0.788 g/cm^2, p = 0.006). For patients in anastrozole + TH treatment group, the baseline median BMD was 0.901 g/cm^2 at femur and the mean BMD at the lumbar spine was 0.893 g/cm^2. There were no significant changes seen in the bone density between the baseline and after six months of treatment at both femoral (0.901 g/cm^2 *vs.* 0.914 g/cm^2, p = 0.100) and lumbar spine (0.893 g/cm^2 vs. 0.897 g/cm^2, p = 0.346).

DISCUSSION

The evolution of adjuvant endocrine treatment increased the survival rates of postmenopausal hormone-related early breast cancer patients. The emergence of anastrozole has changed the initial treatment strategy with the previous gold standard of Tamoxifen. Anastrozole has been demonstrated to have higher efficacy than Tamoxifen in ER-dependent breast tumors' pharmacological strategies. However, as accelerated bone loss is associated with oestrogen deficiency, the most common complication of anastrozole treatment is aromatase inhibitor-associated bone loss. Osteoporosis can be developed and is amplified by age-related lack of oestrogens, which increases the risk of vertebral and hip fractures. There is a study found that anastrozole was associated with a greater fracture incidence than Tamoxifen (11% versus 7.7%, respectively) (Baum *et al.*, 2003).

This randomized controlled trial was conducted to evaluate the protective effect of TH on bone loss caused by anastrozole treatment in postmenopausal breast cancer women. The mean BMD measurement at baseline for the study

patients was 0.85 g/cm^2 at the femoral and the BMD at the spine was 0.86 g/cm^2, while the measured mean femoral T score was −0.048 and the mean T score at the lumbar was −1.19. Most of the women were with normal BMD and T score at the baseline before treatment and only some of them were osteopenic.

The result at six months of treatment with anastrozole showed significant change in term of both T score and BMD when compared to the baseline levels. Six months of anastrozole significantly reduced median femoral BMD by 2.23% and median lumbar spinal BMD by 2.84%. Our findings are in accordance with the findings from previous studies on the effect of anastrozole to bone loss. Eastell *et al.* (2008) demonstrated in their study that anastrozole is associated with accelerated bone loss over the 5-year treatment period in 57 postmenopausal breast cancer women. Inoue *et al.* (2015) also found in their study that in patients treated with anastrozole, the BMD of lumbar spine decreased by 4.3% from baseline at month 24 ($p < 0.0001$). Anastrozole treatment-related bone loss did not continue into the off-treatment follow-up period. Eastell *et al.*(2010) found that the recovery in lumbar spine BMD and absence of further loss at the hip is consistent with the reduction in the annual rate of fracture observed after treatment cessation in the main ATAC trial.

The mechanism of action of the anastrozole is to block the peripheral conversion (aromatization) of oestrogen from androgen precursors, effectively lowering tissue and circulating oestrogen levels (Winer *et al.*, 2005, Osborne and Tripathy, 2005). Oestrogen has been shown to regulate bone remodeling by stimulating the expression of anti-resorptive factors such as osteoprotegerin. Oestrogen deprivation increases bone turnover and osteoclast activity, causing

bone resorption and formation to become unbalanced (Riggs *et al.*, 2002). This imbalance results in net bone loss as well as decreased bone quality. Such bone loss can lead to osteoporosis, which is associated with an increased fracture risk, decreased bone strength, diminished quality of life, and increased mortality (Higano, 2003, Hoff and Gagel, 2005, Higano, 2004). Therefore, the prevention of bone loss is important for postmenopausal women breast cancer women treated with anastrozole in order to preclude a fracture.

Over the past few years, TH has been shown to exhibit protective effects on bone. Study performed by Zaid et al. (2010) has shown that daily consumption of TH for two weeks in female ovariectomized rats was able to promote an increase in bone density. Our study investigated the protective effect of TH on bone in postmenopausal breast cancer women treated with anastrozole and the result at six months of combined anastrozole and TH treatment showed no significant change in term of both BMD and T score when compared to baseline values. Our data demonstrated that the TH could control BMD at both femur and lumbar spine from decreasing due to effect of anastrozole treatment.

The protective effect on bone is probably due to the antioxidants found in honey such as flavonoids and phenolic acids (Jaganathan and Mandal, 2009, Buratti *et al.*, 2007). The main phenolic and flavonoid compounds in TH include kaempferol, quercetin, ellagic acid, gallic acid, hesperetin, and catechin (Hussein *et al.*, 2011). Previous studies reported that flavonoids mainly quercetin and kaempferol exert a potent inhibitory effect on osteoclastic bone resorption and apoptosis in a rabbit long bone osteoclast model (Wattel *et al.*, 2003). They are also involved in inhibition of NF-κB and activator protein-1, a

transcription factor highly related to osteoclastic differentiation (Wattel *et al.*, 2004). Flavonoids may also inhibit RANKL-induced formation of multinucleated osteoclasts and expression of osteoclastic differentiation markers; RANK and osteocalcin receptor (Pang *et al.*, 2006). Flavonoids have been shown to inhibit production of nitric oxide and expression of inducible nitric oxide synthase (iNOS) (Gonzalez-Gallego *et al.*, 2007) which will result in inhibition of osteoclast activity. These protective mechanisms of flavonoids on bone strongly indicate that it can be considered as protective agent against bone loss. The positive effects of TH on bone are probably due to its anti-oxidative property. Apart from that, it may due to its anti-inflammatory (Al-Waili and Boni, 2003, Owoyele *et al.*, 2011) and anti-osteoporotic properties (Farid, 2009).

Our study demonstrated that combined treatment of anastrozole with TH did not significantly decrease BMD and T scores at both femur and lumbar spine after six months. Thus, TH has the potential to be used as a preventive agent against bone loss in postmenopausal breast cancer women treated with anastrozole.

The main limitation of this study is the difficulty in recruiting expected sample size due to time, non probability sample of convenient, financial constraints and longevity of the study. Even though the recruited sample size was smaller than the expected size, this study still managed to produce significant outcomes from the statistical analysis. However, due to limited small sample size, the study was under-powered (power of study is 0.58), so the result cannot be applied to the general population. Another limitation was that the measurements and intervention were made without blinding of the researcher to the experimental group, which has potential for bias. However, potential bias was minimized by

random assignment of participant and following standardized protocol by investigator.

Thus, a larger and multi-centered randomized controlled trial should be conducted in future to further explore the mechanism of TH on overall bone metabolism and its side effects.

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