SAFETY PROFILES AND EFFECTS OF TUALANG HONEY ON THE LEVELS OF INFLAMMATION, BONE MARKERS AND OESTRADIOL IN BREAST CANCER PATIENTS

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by

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LIST OF ABBREVIATIONS

%	percent
µL	microliter
µm	micrometre
AIs	aromatase inhibitors
$AlCl_3$	aluminium chloride
ANCOVA	one-way analysis of covariance
BMI	body mass index
BRCA	breast cancer susceptibility gene
BRCA1	breast cancer susceptibility gene 1
BRCA2	breast cancer susceptibility gene 2
BSE	breast self-examination
CBE	clinical breast examination
CN	core needle
COOH	carboxyl
CRP	C-reactive protein
CTX	carboxy-terminal crosslinked telopeptide of type 1 collagen
DNA	deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
eq	equivalent
ER	oestrogen receptor

FAK	focal adhesion kinase
FBG	fasting blood glucose
Fe^{2+}	ferrous ion
$FeCl_3.6H_2O$	Ferric chloride hexahydrate
$FeSO_4.7H_2O$	ferrous sulphate heptahydrate
FRAP	ferric reducing antioxidant power
H_2O_2	hydrogen peroxide
Hb	haemoglobin
HCL	hydrochloride acid
HMF	5-hydroxymethyl-2-furfural
hsCRP	high sensitivity C-reactive protein
IL-1α	interleukin-1 alpha
IL-1β	interleukin 1 beta
IL-6	interleukin 6
IL-8	interleukin 8
kg	kilogram
LCIS	lobular carcinoma in situ
mAU	mili-absorbance unit
mg	milligram
min	minute
mL	millilitre
MPO	myeloperoxidase
MRI	magnetic resonance imaging
n	sample size
Na_2CO_3	sodium bicarbonate

NF-κB	Nuclear factor kappa B
NH_2-	amide
nm	nanometre
^oC	celcius
P1NP	procollagen type 1 amino-terminal propeptide
RANK	receptor activator of nuclear factor-B
RANKL	nuclear factor kappa-B ligand
RBC	red blood cell
SEM	standard error of the mean
TNF-α	tumour necrosis factor alpha
TPTZ	2,4,6-tripyridyl-s-triazine
TRANCE	TNF-related activation induced cytokine
USA	United State of America
USM	Universiti Sains Malaysia
WBC	white blood cell

PROFIL KESELAMATAN DAN KESAN MADU TUALANG PADA ARAS KERADANGAN, PENANDA TULANG DAN ESTRADIOL DALAM PESAKIT KANSER PAYUDARA

ABSTRAK

Madu dilaporkan mempunyai sifat-sifat biologi seperti sifat antimikrobial, antioksidan dan anti-radang. Ia secara tradisinya digunakan dalam kalangan pesakit kanser payudara di Malaysia dengan kepercayaan ia dapat meningkatkan kesihatan keseluruhan. Walau bagaimanapun, untuk pengetahuan kita, tiada kajian melaporkan tentang kesan suplemen madu Tualang pada profil keselamatan, penanda radang, penanda tulang dan estradiol dalam kalangan pesakit kanser payudara. Oleh itu, tujuan kajian ini untuk menentukan profil keselamatan, dan kesan madu Tualang pada penanda keradangan, penanda tulang, dan oestradiol dalam kalangan pesakit kanser payudara. Percubaan terkawal secara rawak dilakukan pada pesakit kanser payudara yang dirawat dengan anastrozole dan direkrut dari Klinik Onkologi, Universiti Sains Malaysia. Pesakit dirawak ke dalam kumpulan kawalan dan madu. Kumpulan madu telah disuplemen dengan madu Tualang sebanyak 20 g/sehari selama 12 minggu. Darah dikumpulkan di peringkat pra-intervensi dan di peringkat pos-intervensi untuk menilai profil keselamatan, tahap penanda keradangan, penanda tulang dan estradiol antara dan dalam kumpulan. Pada peringkat pra-intervensi, kadar hemoglobin, interleukin-6 dan carboxy-terminal crosslinked telopeptide kolagen jenis 1 (CTX) bertambah tinggi dengan ketara, manakala paras procollagen amino-terminal propeptide jenis 1 (P1NP) adalah jauh lebih rendah di dalam kumpulan kawalan berbanding kumpulan madu. Walau bagaimanapun, tiada perbezaan

signifikan bagi semua parameter antara kedua-dua kumpulan di peringkat pos-intervensi selepas penyesuaian untuk faktor-faktor yang mengelirukan. Dalam kumpulan kawalan, tahap albumin, alanine aminotransferase, kreatinin, interleukin-1 beta, faktor tumor nekrosis alfa dan CTX di peringkat pos-intervensi (selepas 12 minggu), adalah jauh lebih tinggi berbanding paras yang sama di peringkat pre-intervensi. Dalam kumpulan madu, di peringkat pos-intervensi (selepas 12 minggu) jumlah kiraan sel darah putih, kiraan platelet, kreatinin dan tahap P1NP adalah jauh lebih tinggi manakala tahap CTX adalah jauh lebih rendah berbanding dengan paras yang sama di peringkat pre-intervensi. Walau bagaimanapun, tiada perbezaan yang signifikan didapati untuk tahap estradiol antara dan dalam kumpulan. Kesimpulannya, intervensi 20 g suplemen madu harian untuk pesakit kanser payudara selama 12 minggu adalah selamat, menghalang peningkatan keradangan, mengurangkan penyerapan tulang dan meningkatkan pembentukan tulang tanpa perubahan pada kadar estradiol. Kesan-kesan yang menguntungkan ini adalah mungkin melalui tindakan sebatian fenolik yang terdapat dalam madu Tualang yang mempunyai ciri-ciri antioksidan, anti-radang, dan antikanser. Penemuan ini boleh mencadangkan kesan-kesan berfaedah suplemen madu pada keradangan dan tulang dalam kalangan pesakit kanser payudara yang dirawat dengan anastrozole dan potensi penggunaan madu sebagai terapi pembantu. Walau bagaimanapun, kajian lanjut diperlukan untuk menjelaskan mekanisme tindakan yang tepat terhadap kesan-kesan yang bermanfaat bagi madu Tualang dan pemantauan parameter tersebut adalah disyorkan untuk pengambilan madu yang lebih lama.

SAFETY PROFILES AND EFFECTS OF TUALANG HONEY ON THE LEVELS OF INFLAMMATION, BONE MARKERS AND OESTRADIOL IN BREAST CANCER PATIENTS

ABSTRACT

Honey is traditionally used among breast cancer patients in Malaysia with the belief that it can improve the overall health. However, to our knowledge, no study has reported on the effects of Tualang honey supplementation on safety profiles, inflammatory markers, bone markers and estradiol among breast cancer patients. The aim of this study was therefore, to determine safety profile, and effects of Tualang honey on inflammatory markers, bone markers, and on oestradiol among breast cancer patients. A randomised controlled-trial was conducted on breast cancer patients who were treated with anastrozole and recruited from Oncology Clinic, Universiti Sains Malaysia. The patients were randomised into control and honey groups. Honey group was supplemented with 20 g/day of Tualang honey for 12 weeks. Blood was collected at pre- and post-interventions to evaluate the safety profiles, and levels of inflammatory markers, bone markers and oestradiol between and within the groups. At pre-intervention, haemoglobin, interleukin-6 and carboxy-terminal crosslinked telopeptide of type 1 collagen (CTX) levels were significantly higher, whereas procollagen type 1 amino-terminal propeptide (P1NP) level was significantly lower, in control group compared to honey group. There were no significant differences found for all parameters between two groups at post-intervention after adjustment for confounding factors. In control group, levels of albumin, alanine aminotransferase, creatinine, interleukin-1 beta, tumour necrosis factor alpha and CTX at post-intervention (after 12 weeks), were significantly higher

compared to their corresponding levels at pre-intervention. In honey group, at post-intervention (after 12 weeks) total white blood cell count, platelet count, creatinine and P1NP levels were significantly higher whereas CTX level was significantly lower compared to their corresponding levels at pre-intervention. However, no significant differences were found for oestradiol levels between and within groups. In conclusion, the intervention which consists of 20 g daily honey supplementation to breast cancer patients for 12 weeks was significantly safe, prevented the increased inflammation, reduced bone resorption and increased bone formation without changes in estradiol level. These beneficial effects are probably through the action of phenolic compounds present in Tualang honey which had antioxidant, anti-inflammatory and anticancer properties. These findings may suggest the beneficial effects of honey supplementation on inflammation and bone among breast cancer patients treated with anastrozole and the potential use of honey as an adjuvant therapy. However, further studies are required to elucidate the exact mechanism of action on these beneficial effects of Tualang honey and monitoring of those parameters is recommended for longer honey intake.

CHAPTER 1

INTRODUCTION

Breast cancer is highlighted to be the most common cancer in women which is attributed to 29% of all newly diagnosed cancers. It is at the second place of the most common cause of cancer death after lung cancer (Siegel *et al.*, 2016).

There are many risk factors that may contribute to breast cancer, for examples the lifestyle factors such as lack of regular exercise, the consumption of a high-fat diet and breastfeeding habits (Kamarudin *et al.*, 2006). Gajalakshmi *et al.* (2009) have shown that breastfeeding becomes one of the risk factors for breast cancer when the lifetime duration of breastfeeding is less than 1 year. Other factors are family history (Sattin *et al.*, 1985), hormone replacement therapy (Narod, 2011), smoking, tobacco use and obesity (Othman, 2012).

Inflammation has a vital role in tumour development which includes providing an environment for the growth of the tumour, facilitating genomic instability, angiogenesis, metastasis, and resistant to therapy (Coussens and Werb, 2002). Previous study has shown that poor response to treatment and high cancer risk are caused by the inflammation (McMillan, 2009). Interleukin 1 beta (IL-1β) is present in human breast carcinoma and its high level is often associated with tumour invasiveness (Jin *et al.*, 1997). Most of the patients with breast cancer have a high level of C-reactive protein (CRP), suggesting that inflammation is a prevalent problem for breast cancer patients (Mikirova *et al.*, 2012).

The proliferation of breast cancer epithelium is majorly contributed by oestrogen (Russo and Russo, 2006). Therefore, aromatase inhibitors (AIs) such as anastrozole and letrozole are increasingly being used for the treatment after primary therapy among postmenopausal women by blocking the conversion of adrenal androgens into oestrogen (Osborne and Schiff, 2011). However, osteoporosis, bone loss and high fracture risk are mainly contributed by oestrogen deficiency caused by these AIs compared to tamoxifen or placebo (Pandya and Morris, 2006).

Tualang Honey is a multi-floral Malaysian wild honey produced by bees (*Apis dors*ata) has high phenolic content (Kishore *et al.*, 2011), and has antioxidant (Mohamed *et al.*, 2010), anticancer (Jaganathan and Mandal, 2009), and anti-inflammatory properties (Bashkaran *et al.*, 2011).

A study on safety profiles among healthy postmenopausal women who consume Tualang honey at 20 g/day for 4 months has shown no significant change in haematological, liver and renal profiles but significant increases in total cholesterol, low density lipoprotein cholesterol and fasting blood glucose (FBG) (Husniati *et al.*, 2013). A study done by Tartibian and Maleki (2012) has reported that the seminal plasma inflammatory markers such as interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α) and interleukin-8 (IL-8) are significantly reduced after honey intake at 70 g daily for 8 weeks among cyclist.

The trabecular bone structure in rats supplemented with Tualang honey has shown more improvement compared to rats that received calcium only (Zaid *et al.*, 2012). An earlier study has reported that the level of oestradiol in postmenopausal women has not changed after 4 months of Tualang honey consumption orally at 20 g/day (Hussain *et al.*, 2012).

Honey is traditionally used among breast cancer women in Malaysia to improve general health. However, to date, the safety profile and the effects of Tualang honey on the levels of inflammation, bone markers and oestradiol in breast cancer patients are yet to be reported. Therefore, the general objective of this study was to determine safety profile, levels of inflammation, bone markers and oestradiol in breast cancer patients.

The specific objectives of the study were:

1) To characterise and assess the quality of Tualang honey such as physicochemical properties and antioxidant properties.
2) To determine safety profile (full blood count, fasting blood glucose, liver function test and renal function test) of Tualang honey supplementation in breast cancer patients.
3) To determine the effects of Tualang honey supplementation on inflammatory markers (high sensitivity C - reactive protein [hsCRP], interleukin-6 [IL-6], interleukin-1 beta [IL-1β] and tumour necrosis factor alpha [TNF-α]) in breast cancer patients.
4) To determine the effects of Tualang honey supplementation on bone markers (carboxy-terminal crosslinked telopeptide of type 1 collagen [CTX], and procollagen type 1 amino-terminal propeptide [P1NP]) in breast cancer patients.
5) To determine the effect of Tualang honey supplementation on oestradiol level in breast cancer patients.

The hypotheses of this study were:

1) Honey supplementation is significantly safe in breast cancer patients.
2) Honey supplementation significantly reduces inflammation in breast cancer patients.
3) Honey supplementation significantly reduces bone resorption marker and increases bone formation marker in breast cancer patients.
4) Honey supplementation caused no change in oestradiol level in breast cancer patients.

CHAPTER 2

LITERATURE REVIEW

2.1. An overview of breast cancer

Breast cancer continues to become the most common type of cancer in women and the second most common cause of cancer death worldwide. In 2016, breast cancer was accounted for 29 % of all newly diagnosed cancers and 14 % of estimated cancer death in women (Siegel *et al.*, 2016). There are three important stages that are responsible for the development of cancer which include initiation, promotion, and progression (Barrett, 1993). When the cell is altered, it is named as an initiation of carcinogenesis and it is often associated with a mutational event. When the initiated cell grows into an observable tumour frequently into a benign lesion, it is called promotion. The progression step of carcinogenesis is the conversion of a benign tumour into a malignant neoplasm (Barrett and Wiseman, 1987). Benign tumours are usually not harmful, rarely invade tissues around them, and don't usually spread away to other parts of the body. Moreover, they can be removed and don't grow back. Malignant tumours, on the other hand, are a threat to a person and have the ability to invade other organs and tissues, can spread to other body parts and can recur after being removed. Cancer cells do not follow the normal signals that regulate the cell cycle, divide abnormally and excessively as well as invade other tissues. The spread of cancer cells away from the initial site is called metastasis (Campbell and Reece, 2008).

2.2 Risks factors for breast cancer

Breast cancer risk has been associated with two types of major factors which include reproductive factors such as age at menarche, parity, menopause, first pregnancy and breastfeeding, and non-reproductive factors such as family history, menopausal hormone therapy, body mass index, alcohol intake and others. However, the reproductive factors are largely linked with development of breast cancer which characterises exposure to sex hormone (Key *et al.*, 2001).

Previous studies have shown that increased parity and breastfeeding duration (Cancer, 2002b), late of menarche, and lower body mass index (Reeves *et al.*, 2007) can lower the risk of breast cancer. However, several factors are found to be linked with the increased risk breast cancer such as the presence of family history (Cancer, 2001), late of menopause (Cancer, 2002b), high alcohol consumption (Cancer, 2002a) and menopausal hormone therapy usage (Cancer, 1997; Collaborators, 2003).

Women with family history of breast cancer are 1.5-3 times higher risk to get breast cancer compared to non-family history women (Martin *et al.*, 2010). Few studies indicated that women with first-degree relative (eg., mothers, sisters, and daughters) are at highest risk of getting breast cancer compared to women who have second-degree relative (grandmothers, aunts, and grand-daughter) and non-relative with breast cancer (Sattin *et al.*, 1985; McPherson *et al.*, 2000). Clustering of cancer and increased cancer risk in families can be due to genetic predisposition (Key *et al.*, 2001). Breast cancer can be inherited as an autosomal dominant with limited penetrance. The mutated gene is a dominant gene located on one of the nonsex chromosomes (autosomes) and can be transmitted without developing cancer themselves. Breast cancer genes such as breast cancer susceptibility gene (*BRCA*), *BRCA1* and *BRCA2* located on the long arms of chromosomes 17 and 13,

respectively, have been identified to account for breast cancer in the high-risk family (McPherson *et al.*, 2000).

2.3 Pathogenesis of breast cancer and inflammation

Breast is made up of ducts (tubes that carry milk to the nipple) and lobules (glands that produce milk). The ducts and lobules are lined by cells known as epithelial cells, thus, when these cells increase in number (normally lined by two layers of cells) either in ducts and/or lobules, this condition is named hyperplasia. When the growths look abnormal under the microscope, it is called as atypical hyperplasia. There are three distinguishable etiological causes of cancer which include chemical carcinogens (eg., benzpyrene, asbestos and over 800 chemicals), physical carcinogens (eg., ionizing radiation, ultraviolet radiation, etc.), and biological carcinogens (eg., viruses, bacteria, fungi) (Pitot and Dragan, 1991). When the presents of carcinogens weaken cell walls, invasion by microbes will take place and disturbs the genetic apparatus of cell. The primary cause of cancer has been reported to have correlation by the damage of the genetic apparatus of the cell, and the pathogenesis of cancer is recognised as a process of transformation of a normal cell into a tumour cell. The damage of genetic apparatus of cells is reported to be caused by mutation, disturbance of gene expression, activation of tumour promoter gene, inactivation of tumour suppressor genes, and etc. (Halazonetis *et al.*, 2008; Collisson *et al.*, 2012). Next, inflammation will take place at the site of infection (Philip *et al.*, 2004), disturbance of the immune system (Prendergast and Jaffee, 2007), immune avoidance by tumour cells (Poggi and Zocchi, 2006; Burkholder *et al.*, 2014), permanent reproduction of tumour cells influenced by autocrine and paracrine stimulation of cell division (Vona-Davis and Rose, 2007), angiogenesis

(Ferretti *et al.*, 2007), and oxidative-nitrosative stress (production of reactive oxygen and nitrogen species) (Toyokuni *et al.*, 1995; Ferretti *et al.*, 2007; Reuter *et al.*, 2010).

During the early process of inflammation, the regulation of molecules produced by rapidly responded macrophages and mast cells in the tissues will promote the migration of neutrophils into the inflammatory sites (Coussens and Werb, 2002; Nathan, 2002). As the inflammation progresses, numerous numbers of growth factors, cytokines, and chemokines created signalling network which resulted in activation and attraction of various types of leukocytes, lymphocytes, and other inflammatory cells to the inflammatory sites (Coussens and Werb, 2002; Nathan, 2002). All the inflammatory cells at the inflamed site are vital to defence against tissue injury (Coussens and Werb, 2002). The inflammatory responses are needed for enabling an immune reaction, however, may also set the stage for promoting neoplastic disease.

The relationship between cancers and inflammation has been found since more than 150 years ago which indicated that the sites of chronic inflammation tended to be the beginning points of cancer (Balkwill and Mantovani, 2001). Chronic inflammation will take place if the inflammation resolution is dysregulated and the inflamed sites are controlled and predominated by lymphocytes, plasma cells and macrophages (Nathan, 2002; Philip *et al.*, 2004). Eventually, deoxyribonucleic acid (DNA) damage will occur as the generation of massive numbers of cytokines, growth factors, and reactive oxygen and nitrogen species from macrophages and other inflammatory cells to fight infection (Maeda and Akaike, 1998; Coussens and Werb, 2002). Continuous activation of macrophages will subsequently result in continuous

tissue damage (Macarthur *et al.*, 2004). A microenvironment with presence of all the above mentioned elements together with persistent cell proliferation induced by continued tissue damage, will eventually turns chronic inflammation to neoplasia (Balkwill and Mantovani, 2001).

The inflammatory status may also be one of the prognostic factors for breast cancer (Pierce *et al.*, 2009). Clinical and experimental data have shown that the development of the mammary tumour is promoted by chronic inflammation via chronic activation of humoral immunity and infiltration of Th2 cells and polarised innate inflammatory cells mechanisms (DeNardo and Coussens, 2007). For example, studies have shown that elevation of CRP concentration in breast cancer patients before undergoing surgery and also in women with advanced disease (O'Hanlon *et al.*, 2001; Blann *et al.*, 2002). Thus, suggesting that CRP plays a role in determining the tumour progression and prognosis.

2.3.1 Inflammatory cells and tumour development

The breakage of the cell basement membrane is required for the invasion and migration of tumour cells which is facilitated by the tumour inflammatory microenvironment (Coussens and Werb, 2002). The important components in the inflammatory microenvironment consists of leukocytes, macrophages, neutrophils, eosinophils, dendritic cells, mast cells and lymphocytes (Coussens and Werb, 2001; Macarthur *et al.*, 2004; Yang *et al.*, 2005).

Normally, the presence of immune cells into tumour area may be helpful to supress the development of tumour itself (Clark *et al.*, 1989; Clemente *et al.*, 1996; Brigati *et al.*, 2002; Dunn *et al.*, 2002). However, the inflammatory cells now

become the supporter of the tumour in inflammation-associated cancers (Coussens and Werb, 2002; Khong and Restifo, 2002; Smyth *et al.*, 2004).

2.3.2 Inflammatory markers

Breast cancer among women is found to be influenced by the elevation of the inflammatory markers such as CRP and IL-6. Increased level of IL-6 will stimulate liver and other organs to respond and generate CRP thus increase the level of CRP (Ravishankaran and Karunanithi, 2011). IL-1β is present in breast carcinoma and its high level is often associated with tumour invasiveness (Jin *et al.*, 1997). In addition, TNF-α is also a major inflammatory cytokine and is highly expressed in breast carcinoma (Leek *et al.*, 1998).

2.3.2(a) C-reactive protein (CRP)

CRP was discovered in 1930 and named as CRP because of its strong binding affinity to C-polysaccharide of *Streprococcus pneumoniae* (Tillett and Francis, 1930). It is a classical acute phase, non-specific, hepatic protein (synthesised by hepatocytes in liver) from pentraxin family. CRP is secreted in response to cytokines produced during inflammation, trauma and tissue damage (Pepys and Hirschfield, 2003; Black *et al.*, 2004). The main inducer of CRP gene is IL-6 which will promote the secretion of CRP in response to elevated IL-6 levels (Hirschfield and Pepys, 2003) and also other proinflammatory cytokines such as TNF-α and IL-1 (Kolb-Bachofen, 1991; Jupe, 1996). It is suggested that CRP plays a major role in opsonisation the damaged cells and infectious agents (Kolb-Bachofen, 1991; Ballou and Kushner, 1992).

Many studies have indicated the use of CRP as a marker of systemic inflammation. It has an association with progression of disease and can act as a monitor towards infection (Vigushin *et al.*, 1993; Vogt *et al.*, 2007; Marsik *et al.*, 2008; McMillan, 2009). Usually, plasma CRP is measured by using standards CRP test with detection limits of 3 to 8 mg/L. However, a lower detection limit of the assay procedure has been developed named as high-sensitivity CRP (hsCRP) which has detection limit of <0.3 mg/L which means that the assay can accurately detect lower protein concentrations in comparison with standard CRP assay (Allin and Nordestgaard, 2011).

2.3.2(b) Interleukin-6 (IL-6)

IL-6 is a pleiotropic cytokine which acts as a very important mediator of the inflammatory response and has a major role in the physiology of cancer (Hodge *et al.*, 2005; Rose-John *et al.*, 2006). It is produced by macrophages, B-cells, T lymphocytes, fibroblasts or monocytes and tumour cells (Takenawa *et al.*, 1991; Salgado *et al.*, 2003). A negative prognosis and shorter breast cancer patients survival are majorly correlated to high levels of IL-6 and not depending on the status of oestrogen receptor (ER) (Matsumoto *et al.*, 2005; Knüpfer and Preiß, 2007; Sasser *et al.*, 2007). The effect of cytokine IL-6 is carried out through transcriptional regulation of genes taking part in cell proliferation, survival and differentiation which results from glycoprotein 130-mediated activation of signalling pathways (including the JAK/STAT and MAP kinase pathways). The tumour growth is supported by IL-6 through up-regulation of proangiogenic and anti-apoptotic proteins in tumour cells (Heinrich *et al.*, 1998; Banks *et al.*, 2000; Kovacs, 2001; Trikha *et al.*, 2003). For example, the production of vascular endothelial cell growth factor in breast cancer cells is promoted by IL-6 which contributes to angiogenesis, thus leads

to tumour survival and contributing to metastatic potential (Cohen *et al.*, 1996; Benoy *et al.*, 2002).

Furthermore, an increase in the level of oestradiol-17β hydroxysteroid dehydrogenase type I, which converts estrone to oestradiol (the biologically active oestrogen) has been found to be caused by an increase level of IL-6 (Purohit *et al.*, 2002). Therefore, it is believed that the proliferation of breast cancer cells is stimulated by Il-6 and IL-1β through production of oestrogen by activating steroid-catalysing enzymes in the tissue (Honma *et al.*, 2002). The concentration of IL-6 in breast cancer patients is higher than in healthy women suggesting the correlation between the disease progressions with the level of IL-6 (Kozłowski *et al.*, 2002). The level of IL-6 in breast cancer patients with metastatic disease is higher than its level in those without metastases (Zhang and Adachi, 1999; Benoy *et al.*, 2002). In addition, breast cancer cells promote the increase level of IL-6 in osteoblasts which subsequently results in an increased level of osteoclast activation and thus bone resorption (Dhurjati *et al.*, 2008; Kinder *et al.*, 2008).

2.3.2(c) Interleukin-1β (IL-1β)

IL-1β is produced largely from activated macrophages and monocytes in inflammatory and immune responses (Ghezzi *et al.*, 1991; Wakabayashi *et al.*, 1991). Previous studies have found the positive relationship between invasiveness of breast cancer and unfavourable prognosis with IL-1β expression levels (Pantschenko *et al.*, 2003; Nicolini *et al.*, 2006). Studies have indicated that 90% of ER-invasive breast carcinomas have increased expression of IL-1β and its expression is related with both breast cancer cells and stromal cells (Barrows and Kreutzer12, 1999; Pantschenko *et al.*, 2003; E Goldberg and L Schwertfeger, 2010).

Furthermore, breast cancer recurrence is found to be associated with the high levels of IL-1β. In addition, study on pre-invasive ductal carcinoma in situ lesions has shown expression of IL-1β which demonstrates the involvement of IL-1β in premalignant breast cancer (Nicolini *et al.,* 2006). Thus, based on these findings, it is suggested that breast cancer development and progression can be determined by IL-1β as a mediator. *In vitro* study of IL-1β in MCF-7 breast cancer cell lines has shown that IL-1β can induce the phosphorylation of focal adhesion kinase which plays an important role for modulating cell adhesion, and expression of matrix metalloproteinase-9. This demonstrates that IL-1β is related with breast cancer cell invasion (Wang *et al.,* 2005). In addition, the migration of other cell also has been linked to IL-1β via up-regulation of intercellular adhesion molecule-1 (Rosette *et al.,* 2005). Studies also have found that, the expression of cyclooxygenase-2 which is known to promote inflammation and has been linked to breast cancer progression is shown to be regulated by IL-1β via activation of the NF-κB signalling pathway (Dinarello, 2002; Howe, 2007).

2.3.2(d) Tumour necrosis factor-alpha (TNF-α)

TNF-α was discovered in the late 1970s as a multi-functional cytokine secreted by macrophages and has the ability to inhibit proliferation of tumour cells and generate tumour regression (Matthews and Watkins, 1978; Green *et al.*, 1982). However, TNF-α also has the capacity to raise the potential of malignancy. The formation of angiogenesis and stimulation of proliferation, survival and migration which promotes formation of the tumour stroma have been shown to be induced by TNF-α in tumour cells that show resistance towards TNF-α induced cytotoxicity. The expression of matrix metalloproteinases is increased by TNF-α which subsequently raises invasive cavity and metastatic potential and eventually the osteoclastic

resorption of bone is stimulated via a prostaglandin-mediated mechanism (Fiers, 1991). Therefore, TNF-α can be identified as either has pro- or anti-tumorigenic property.

TNF-α is useful to be used as cancer indicator, therapy response, and prognosis for cancer patients. Previous studies have indicated that the level of TNF-α concentration is high in cancer patients (Ahmed *et al.*, 2001; Ferrajoli *et al.*, 2002). The expression of TNF-α is increased and accompanied the progression of not only in breast cancer (García-Tuñón *et al.*, 2006) but also in other types of cancer for examples in Barrett's adenocarcinoma (Tselepis *et al.*, 2002), prostate cancer (Michalaki *et al.*, 2004), and cervical carcinoma (Ahmed *et al.*, 2001). Other than that, TNF-α can be a remarkable indicator for chemotherapy response and prognosis as the level of TNF-α is markedly dropped in patients with breast and prostate cancer after undergoing chemotherapy (Ferrajoli *et al.*, 2002; Berberoglu *et al.*, 2003; Michalaki *et al.*, 2004). In addition, it is indicated that the increased tumour grade and node involvement have a correlation with the number of cells expressing TNF-α in inflammatory breast carcinoma, thus concludes that it plays a major role in tumour metastatic behaviour (Miles *et al.*, 1994).

The pathogenesis of breast cancer and inflammation is summarised in Figure 2.1.

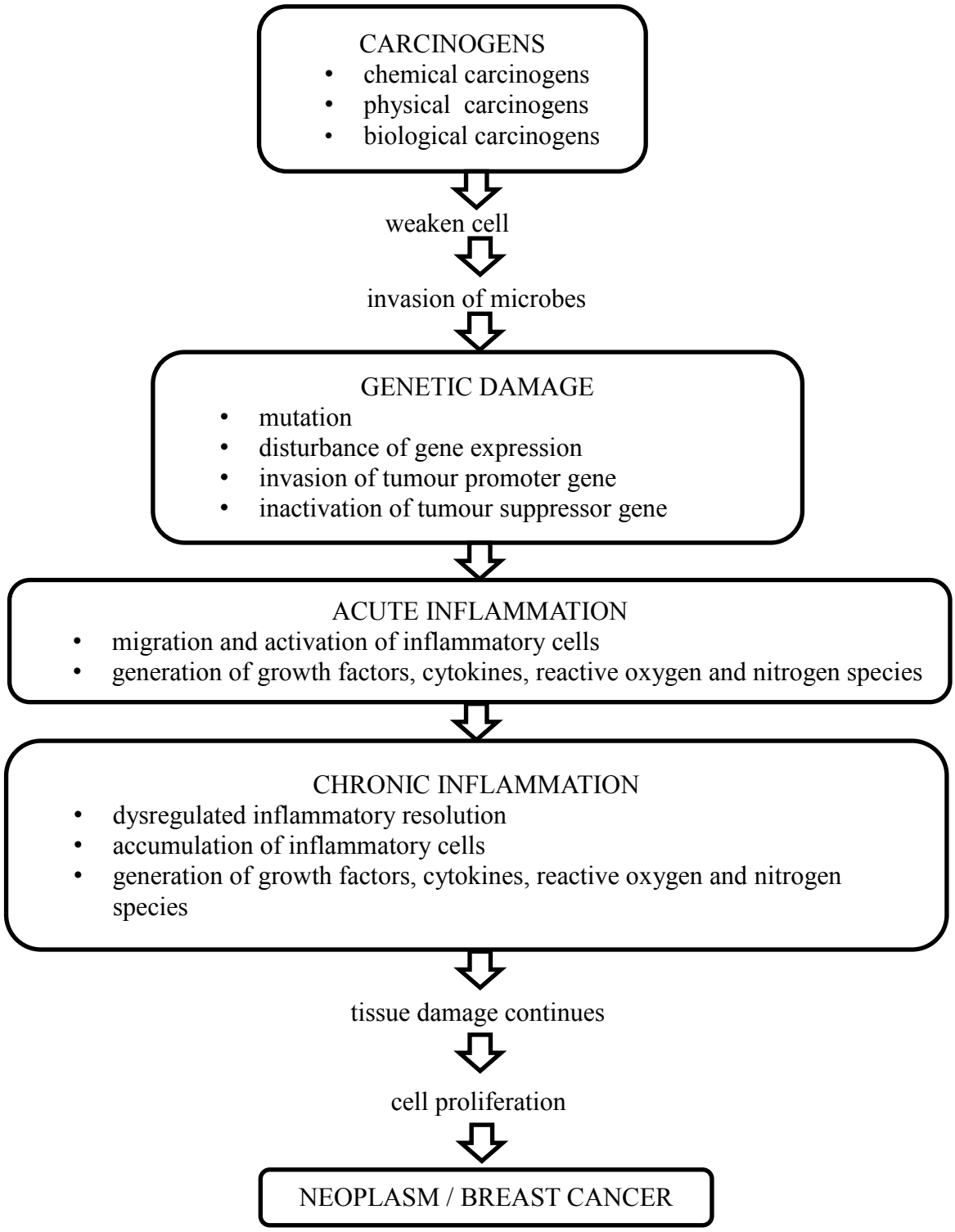

Figure 2.1 Pathogenesis of breast cancer and inflammation

2.4 Breast cancer screening and diagnosis

Breast cancer usually consists of a pre-invasive stage where the carcinoma cells are in the duct system within the breast. Then, they become invasive and invade the surrounding tissue, and thereafter possibly spread to the lymph nodes or other parts of the body. Ideally, screening should detect small tumours and before the metastasis has developed (Tabar *et al.*, 1992), therefore, the prognosis can be improved. There are three basic potential mass screening tools for breast cancer which include mammography, clinical breast examination (CBE) and breast self-examination (BSE).

Mammography has been shown to provide affectivity in reducing the mortality of breast cancer (Gøtzsche and Nielsen, 2009). A mammogram or x-ray of the breast is done with a special type of x-ray machine which produces a picture of the breast. The mammogram is also used in diagnostic mammography when there is a problem, for example, there is the suspicious area in the breast that needs further investigation. During mammography session, the breast is sandwiched between two plastic x-ray plates. The compression is done on the breast in order to spread out the tissue so that the best image can be provided to help detection of any abnormalities in breast tissues (Institute, 2014).

Breast self-examination (BSE) is very difficult to access by the public as the only intervention is education and failure to practice the BSE accordingly seems to be a major disadvantage to the use of BSE as a sole screening method. The frequent BSE practice among women will help them to detect cancer earlier and it can be applied by themselves at their home. The early diagnosis can be gained immediately, free of charge, kept in privacy and a non-invasive process (Uzun *et al.*, 2004). Furthermore, BSE is also the most common screening tool practised by among

women compared with another two methods; mammography and CBE (Dunn and Tan, 2011). A study done on 2093 breast cancer patients shows that 76.7% of women who do self-examination survive for 5 years compared with 60.9% of women who do not do BSE (Huguley *et al.*, 1988).

Clinical breast examination (CBE), on the other hand, is done by trained staff to search for any abnormalities on the breast or assess patient's reports of symptoms to find obvious or noticeable breast cancers at an earlier stage of progression. CBE adopt standards that have a stepwise progression of elements consisting of clinical history, visual inspection and palpation (Saslow *et al.*, 2004). In addition, the CBE's sensitivity and specificity determined by test characteristics (search pattern, palpation, pressures, duration), characteristics of patients (tissue density and nodularity), and carcinoma characteristics (size, depth, mobility) (McDonald *et al.*, 2004). Many previous studies have found that mammography did in routine and frequent clinical breast examinations are effective to lower the mortality in between 50 and 69 years of breast cancer women (Kerlikowske *et al.*, 1995; Barton *et al.*, 1999; Humphrey *et al.*, 2002).

Diagnosis of breast cancer is carried out after there is a problem that needs further investigation which includes mammography, ultrasound, magnetic resonance imaging (MRI), and breast biopsy. In breast cancer diagnosis, the similar mammography method used in screening also will be applied to diagnose the breast cancer patients. MRI, on the other hand, is a breast-imaging technique that captures multiple cross-sectional pictures of the patient's breast. MRI is also used for screening when the mammography is unable to visualise cancer especially in young women, women with dense breast tissue, and suspected breast cancer susceptibility

gene (*BRCA*) either *BRCA1* or *BRCA2* genes carrier women (Lakhani *et al.*, 1998; Brekelmans *et al.*, 2001; Kuhl *et al.*, 2005; Pisano *et al.*, 2005).

A breast biopsy is a small operation done to discover the tissue from the concerned area in the patient's body after the imaging study and will be examined under a microscope by a pathologist to determine the presence of cancer cells. There are three breast biopsy procedures namely fine-needle aspiration, core biopsy, and surgical biopsy which consists of incisional biopsy and excisional biopsy. Fine-needle aspiration is a type of biopsy that uses a very thin needle with a syringe to aspirate (withdraw) suspected tissue. It is a dependable procedure consequent after palpable and/or mammographically detectable breast lesions (Denning *et al.*, 1987; Halevy *et al.*, 1987; Sheikh *et al.*, 1987). The tissue aspirated will be assessed for the morphological pattern and the biomarkers DNA ploidy, ER, epidermal growth factor receptor, p53 protein, and protein of HER2/NEU (Fabian *et al.*, 1993; Zalles *et al.*, 1995). In addition, the procedure has 52% to 93% sensitivity to detect the present of carcinoma (Horgan *et al.*, 1991; Vetrani *et al.*, 1992).

The second type of breast biopsy is a core needle (CN) biopsy which involves removing a core (small cylinders) of tissue from the suspected area. The needle is slightly larger and provides a bigger amount of tissue sample than fine-needle aspiration. The sensitivity of core biopsy, on the other hand, is between 88% and 97% (Baildam *et al.*, 1989; McMahon *et al.*, 1992). A greater diagnostic accuracy of breast cancer can be achieved with the combination of the core biopsy and the needle-aspiration biopsy which is complementary to each other (Dennison *et al.*, 2003).

The surgical biopsy is taken when the suspected area of the breast cannot be figured out by doing a needle biopsy. In this procedure, breast cutting is needed to remove all or part of the lump and will be identified under the microscope. The surgical biopsy may be divided into incisional biopsy which only requires the removal of the suspicious area, however, the excisional biopsy will need the removal of the entire tumour or abnormal area (Rovere *et al.*, 2006).

2.5 Types of breast cancer

There are many different types of breast cancer and mainly they are named based on part of the breast that cancer most resembles, for examples, ductal and lobular tumours. Ductal tumours start in ductal tissue while lobular tumours start in the smaller terminal lobules. Ducts and lobules are kinds of glands and the prefix *adeno* means "related to a gland". This is why breast cancer is often referred as *adenocarcinoma* (Society, 2016b). The most common type of breast cancer is ductal carcinoma in situ, invasive (or infiltrating) ductal carcinoma, and invasive lobular carcinoma. Carcinoma in situ is a precancer and is more contained within the duct or lobules. The cells have cancerous features due to abnormal look, however, have not invaded tissue outside the ducts or lobules. "*In situ*" come from Latin that means "in its original place" (Kosir, 2013). Other than that, there are also types of breast cancer that are sub-types of invasive carcinoma and named after features seen when they are viewed under the microscope. These include adenoid cystic carcinoma, metaplastic carcinoma, medullary carcinoma, mucinous (colloid) carcinoma, papillary carcinoma, tubular carcinoma, micropillary carcinoma, tubulolobular carcinoma, cribriform carcinoma, apocrine carcinoma, inflammatory carcinoma, microinvasive carcinoma, Paget's disease of the nipple and others (Jacobs and Finlayson, 2011).

2.6 Tumour stage

The stage of breast cancer is based on the size of the tumour, invasive or non-invasive, lymph nodes involvement, and whether it has spread beyond the breast and nodes (metastases). According to Sobin *et al.* (2011), in order to classify the cancer progression, Pierre Denoix had devised the TNM staging systems for all solid tumours between the year 1943 and 1952. The tumour size is "T", the lymph node status (the number and location of lymph nodes with cancer) is "N" and the metastases (the degree of cancer has spread to other areas of the body) is "M" (Society, 2016a). Each stage of breast cancer is given in Table 2.1 and details of the TNM staging system are given in Table 2.2.

Table 2.1 Staging system for breast cancer (Hunt *et al.*, 2001)

Stages of Breast Cancer			
Ductal carcinoma in situ			
Stage 0	Tis	N0	M0
Early breast cancer			
Stage IA	T1	N0	M0
Stage IB	T0	N1mi	M0
	T1	N1mi	M0
Stage IIA	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
Locally advanced breast cancer			
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2	M0
	T1	N1	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage IIIC	Any T	N3	M0
Metastatic breast cancer			
Stage IV	Any T, any	Any N	Any M1

Table 2.2 Details of the TNM staging system (Hunt *et al.*, 2001)

Categories	Details
T (Primary tumour)	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ (DCIS, LCIS, or Paget disease of the nipple with no associated tumour mass)
T1	Tumour 2 cm or less in greatest dimension
	Tmic Microinvasion 0.1 cm or less in greatest dimension
	T1a Tumour more than 0.1 but not more than 0.5 cm in greatest dimension
	T1b Tumour more than 0.5 but not more than 1 cm in greatest dimension
	T1c Tumour more than 1 cm but not more than 2 cm in greatest dimension
T2	Tumour more than 2 cm but not more than 5 cm in greatest dimension
T3	Tumour is more than 5 cm across
T4	Tumour of any size growing into the chest wall or skin. This includes inflammatory breast cancer
	T4a Extension to chest wall
	T4b Oedema (Including peau d'orange) or ulceration of skin of the breast or satellite skin nodules confined to the same breast
	T4c Both (T4a and T4b)
	T4d Inflammatory carcinoma
N (Regional lymph node)	
NX	Nearby lymph nodes cannot be assessed (e.g., previously removed)
N0	Cancer has not spread to nearby lymph nodes
N1	Cancer has spread to 1 to 3 axillary (underarm) lymph node(s), and /or tiny amounts of cancer are found in internal mammary lymph nodes (those near the bone) on sentinel lymph node biopsy.

Table 2.2 Continued

Categories	Details
N1mi	Micrometastases (tiny areas of cancer spread) in 1 to 3 lymph nodes under the arm. The areas of cancer spread in the lymph nodes are 2 mm or less across (but at least 200 cancer cells or 0.2 mm across).
N2	Cancer has spread to 4 to 9 lymph nodes under the arm, or cancer has enlarged the internal mammary lymph nodes.
N3	Cancer has spread to 10 or more axillary lymph nodes, with at least one area of cancer spread greater than 2 mm, or Cancer has spread to the lymph nodes under the collar bone (infraclavicular nodes), with at least one area of cancer spread greater than 2 mm
M (metastasis)	
MX	Distant spread (metastasis) cannot be assessed.
MO	No distant spread is found on x-rays (or other imaging tests) or by physical exam.
M1	Cancer has spread to distant organs (most often to the bones, lungs, brain, or liver).

2.6.1 Tumour size

Study has shown that size, nodal status and histological grade are the three prognostic factors of a breast cancer that are most commonly considered (Blamey, 2002). The tumour size can be one of the important factors in choosing the appropriate treatment especially for adjuvant chemotherapy and breast conserving therapy with lumpectomy. However, size does not determine the aggressiveness or progressiveness of the tumour and sometimes small tumour can be aggressive or a large tumour can be mild-mannered. Size prediction is done primarily by mammography, ultrasound, and core biopsy. Most tumours cannot be felt until they are about 10 mm in size and can become visible on an x-ray between 5 mm to 10 mm diameter. The exact size of the tumour can be measured accurately after being removed surgically and examined by the pathologist. However, due to irregular size of the tumour and different options from different pathologists, a lot of errors occur in tumour measurement size (Fisher *et al.*, 1969).

2.6.2 Tumour grade

The grade of the tumour will be determined by the pathologist by looking further into the cells as the grade will indicate the progression or the aggressiveness of the cancer cells (Virnig *et al.*, 2010). The differentiation of normal and cancerous breast tissues will be compared. Normal cells of the mammary system will differentiate and gain specific shapes and forms to carry out the normal function. Cell differentiation depends on the cell division. If cell division is uncontrollable, there will be possibility towards an absent of differentiation. The pathologist usually will grade the tumours from low grade to high grade. Grade 1 tumours have the best prognosis, as they tend to be slow growing (well differentiated), intermediate grade or grade 2 (moderately differentiate), whereas high grade or grade 3 (poorly