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Toremifene in Premenstrual Mastalgia

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CONTENTS

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals. Some unpublished data are also presented.

I   Oksa S, Luukkaala T, Mäenpää J (2006): Toremifene for premenstrual mastalgia: a randomized, placebo-controlled crossover study. BJOG 113:713-718.

II   Oksa S, Luukkaala T, Mäenpää J (2008): The antioestrogen toremifene has luteotropic effects in women suffering from premenstrual mastalgia. Acta Endocrinol 4:425-431.

III   Oksa S, Luukkaala T, Mäenpää J (2010): Toremifene use does not alter serum inhibin A and B levels during mid-luteal phase in women with premenstrual mastalgia. Gynecological Endocrinology 26:114-117.

IV   Oksa S, Parkkola R, Luukkaala T, Mäenpää J (2009): Breast Magnetic Resonance Imaging findings in women treated with toremifene for premenstrual mastalgia. Acta Radiol 50:984-989.

V   Oksa S, Luukkaala T, Mäenpää J: Effect of toremifene on premenopausal breast as evaluated by three dimensional power Doppler ultrasound. Preliminary findings. Submitted.

ABBREVIATIONS

AF	activation function
BMI	body mass index
BI-RADS	American College of Radiology Breast Imaging Reporting and Data System
DHEAS	dehydroepiandrostenedione sulphate
CV	coefficient of variation
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders 4th edition
ELISA	enzyme-linked immunosorbent assay
FI	flow index
FSH	follicle stimulating hormone
GABA	gamma-aminobutyric acid
GnRH	gonadotrophin releasing hormone
GP	general practitioner
HDL-c	high density lipoprotein cholesterol
LH	luteinizing hormone
LNG-IUS	levonorgestrel-releasing intrauterine system
MR	magnetic resonance
MRI	magnetic resonance imaging
OR	oestrogen receptor
PCNA	proliferating cell nuclear antigen
PgR	progesterone receptor
PMS	premenstrual syndrome
PMDD	premenstrual dysphoric disorder
PRL	prolactin
QoL	quality of life
SERM	selective oestrogen receptor modulator
SHBG	sex hormone binding globulin
SSRI	selective serotonin reuptake inhibitor
3D US	three dimensional ultrasound
TRH	thyreotrophin-releasing hormone
VAS	visual analogue scale
VEGF	vascular endothelial growth factor
VFI	vascularisation flow index
VI	vascularisation index

ABSTRACT

Cyclic mastalgia, by definition, occurs in premenopausal women and connotes breast pain that is clearly related to the menstrual cycle. Premenstrual mastalgia may be severe enough to interfere with usual daily activities, and its effect on quality of life (QoL) is often underestimated. Hormonally active treatments are effective for patients with cyclic mastalgia. This book consists of five studies and presents the effects of the anti-oestrogen toremifene on mastalgia in the premenopausal breast.

The aim of the first study was to evaluate the potential role of toremifene in the treatment of mastalgia, and to investigate its mechanism of action in this indication. The next aim was to evaluate if there are any measurable changes in luteal phase sex hormones attributable to toremifene in women with premenstrual mastalgia. The third aim was to find out if the effect of toremifene on the breast would be detectable with dynamic magnetic resonance imaging (MRI). The fourth aim was to investigate the vascular and volume effects of toremifene on healthy premenopausal breasts by three dimensional ultrasound (3D US).

In the first study, a total of 62 premenopausal women suffering from cyclic mastalgia were randomized to receive toremifene 20 mg daily or placebo from day 15 of the menstrual cycle until menstruation for three consecutive cycles. After a wash-out cycle, the women were crossed over to receive placebo or toremifene, respectively, for three additional cycles. In the second and third study, the population was the same as previously described. However, only those participants who had given blood samples at all measuring points were included in the study. Forty-eight patients gave three blood samples during the luteal phase of the menstrual cycle: the first at baseline, the second during the third toremifene/placebo cycle and third during the third placebo/toremifene cycle, respectively. The investigated hormones in the second study were: follicle stimulating hormone (FSH), oestradiol, progesterone, prolactin (PRL), androstenedione, total and free testosterone and in the third study inhibin A and B were investigated.

In the fourth study, ten women with marked premenstrual mastalgia were randomized to receive either toremifene 20 mg or placebo from cycle day 15 until the next menstruation for three menstrual cycles. After a washout period, the treatment was crossed over for three additional cycles. The MRI evaluations were performed prior to menstruation at the end of each treatment phase. Breast pain and QoL scores were collected from one baseline cycle and from all treat-

ment cycles. Nine participants were included in the final analysis. In the fifth study, twenty healthy premenopausal women were recruited. Following a single non-medicated baseline menstrual cycle, the participants received toremifene 20 mg/day from cycle day 15 until the second examination. The breast 3D US evaluations were performed within 5 days prior to menstruation. Both breasts were assessed as four quadrants. The power Doppler setting was used and standardised with twenty degrees volume angle and maximum quality.

In Study I, there was a 64 % reduction in median pain scores in the toremifene-treated cycles compared with a 26 % reduction in placebo-treated cycles. The median pain scores were 1.8 (during toremifene treatment), 3.7 (during placebo), and 5.0 (baseline). Although the placebo effect was also significant, toremifene reduced the pain significantly more than placebo. The adverse reactions turned out to be mild and infrequent during the treatment and were evenly distributed between the arms. The overall QoL scores remained unchanged. In study IV, the median Visual Analogue Scale (VAS) scores were 1.83 during toremifene and 6.33 during placebo. Once again, toremifene alleviated breast pain, but the difference was only of borderline significance (P=0.078). However, the QoL scores appeared to be slightly lower during toremifene when compared with placebo (P=0.047). This is likely to be a coincidental result due to the much smaller sample size in Study IV compared with Study I (9 vs. 56 patients, respectively).

The median serum oestradiol and progesterone levels were significantly higher in the toremifene-treated cycles. The median serum PRL concentration was significantly higher during the toremifene treatment when compared with the baseline. There were no significant changes in other hormone concentrations evaluated. The concentrations of both inhibins were within normal range for the luteal phase and remained unchanged throughout the treatment cycles. The mechanism by which toremifene has a therapeutic effect in cyclic mastalgia does not seem to involve inhibition or stimulation of ovarian inhibin production.

Both the enhancement ratio and the maximum slope of enhancement tended to be lower during the toremifene cycles when compared with placebo. These findings indicate that the therapeutic effect of toremifene in alleviating premenstrual mastalgia may at least partly be mediated though diminished blood flow to the breast. The 3D measured volumes and all vascular indices in healthy breasts remained unchanged during the treatment with toremifene when compared with the baseline.

The finding that toremifene significantly alleviated premenstrual breast pain over placebo with few mild adverse effects encourages its use for this indication. The short-term safety of 20 mg of toremifene administered daily during the luteal phase was addressed. The present results imply that the mechanism of action of toremifene in mastalgia is at least partly based on local effects, although the luteotropic effect was also evident.

TIIVISTELMÄ

Syklistä mastalgiaa esiintyy premenopausaalisilla naisilla, joilla rintojen kipeytyminen on selkeässä yhteydessä kuukautiskierron vaiheeseen. Kuukautisten edellä kipu voi olla niin sietämätöntä, että se haittaa naisen päivittäistä toimintakykyä. Mastalgian elämänlaadullista merkitystä aliarvioidaan usein. Tämä väitöskirja koostuu viidestä eri osatyöstä ja esittelee antiestrogeeni toremifeenin vaikutuksia mastalgiassa ja premenopausaalisessa rinnassa. Tämän tutkimuksen ensimmäinen tavoite oli selvittää toremifeenin teho premenstruaalisen mastalgian hoidossa ja tutkia sen vaikutusmekanismeja rintarauhaseen tässä indikaatiossa. Seuraava tavoite oli selvittää lisääntymishormoneissa tapahtuvia muutoksia toremifeenin käytön aikana syklisestä mastalgiasta kärsivillä potilailla kuukautiskierron luteaalivaiheessa. Kolmas tavoite oli selvittää näkyykö toremifeenin vaikutus rinnan magneettitutkimuksessa. Tutkimuksen neljäs tavoite oli selvittää toremifeenin vaikutuksia terveen premenopausaalisen rinnan verenkiertoon ja tilavuuteen kolmiulotteisen (3D) ultraäänen avulla.

Ensimmäisessä osatyössä kaikkiaan 62 premenopausaalista, syklisestä mastalgiasta kärsivää naista satunnaistettiin saamaan joko toremifeenia 20 mg päivässä tai placeboa kuukautiskierron 15. päivästä kuukautisiin saakka kolmen perättäisen kuukautiskierron ajan. Yhden kuukautiskierron pituisen tauon jälkeen hoito vaihtui toiseksi seuraavien kolmen kuukautiskierron ajaksi. Kolmannessa ja neljännessä osatyössä tutkittavina olivat samat potilaat kuin edellä mainittiin. Kuitenkin vain ne naiset, jotka olivat antaneet kaikki verinäytteet oikeaan aikaan hyväksyttiin mukaan lopulliseen analyysiin. Kaikki kolme näytettä saatiin kerättyä 48 potilaasta. Ensimmäinen verinäyte otettiin hoitoja edeltävässä kuukautiskierrossa, toinen kolmannen toremifeeni/placebo hoitosyklin aikana ja kolmas placebo/toremifeenin aikana. Toisessa osatyössä määritetyt hormonit olivat: follikkelia stimuloiva hormoni (FSH), estradioli, progesteroni, prolaktiini (PRL), androstendioni ja kokonais/vapaa testosteroni sekä inhibiinit A ja B kolmannessa osatyössä.

Neljännessä osatyössä kymmenen merkittävästi rintojen premenopausaalisesta kivusta kärsivää naista satunnaistettiin saamaan joko toremifeenia 20 mg päivässä kuukautiskierron 15. päivästä alkaen kolmen kierron ajan tai placeboa vastaavan ajan. Yhden kuukautiskierron mittaisen tauon jälkeen hoito vaihdettiin toiseksi seuraavien kolmen kierron ajaksi. Rintojen kivun määrää ja potilaan elämänlaatua

mitattiin yhden kuukautiskierron ajalta ennen hoitoja ja jokaisella hoitokierrolla. Rintojen magneettikuvaukset suoritettiin kolmannella hoitokierrolla juuri ennen kuukautisia molemmilla hoitojaksoilla. Yhdeksän naista hyväksyttiin mukaan lopulliseen analyysiin. Viidenteen tutkimukseen osallistui 20 tervettä naista. Yhden lääkkeettömän kuukautiskierron jälkeen tutkittavat aloittivat toremifeenin annoksella 20 mg päivässä kuukautiskierron 15. päivästä alkaen. Rintojen 3D-mittaukset suoritettiin enintään 5 päivää kuukautisia edeltävästi. Molemmat rinnat mitattiin neljänä osana. Ultraäänilaitteen power Doppler toimintoa käytettiin maksimaalisella kuvanlaadulla ja kulmaksi asetettiin 20 astetta.

Ensimmäisessä osatyössä rintojen kipu aleni 64 % toremifeenin käytön aikana ja 26 % placeboa käytettäessä. Mediaani Visual Analogue Scale (VAS) kipuarvot olivat 1.8 (toremifeeni), 3.7 (placebo) ja 5.0 (ennen lääkitystä). Vaikka placebolla saavutettiin tehoa, niin toremifeeni osoittautui silti merkittävästi paremmaksi premenstruaalisen mastalgian hoidossa. Toremifeenin käytön aikana havaittiin vähän sivuvaikutuksia ja ne olivat lieviä. Potilaiden elämänlaadussa ei todettu eroja näiden hoitojen välillä. Neljännessä osatyössä mediaani kipu oli 1.83 toremifeenin aikana ja 6.33 placeboa käytettäessä. Jälleen rintojen kipu väheni toremifeenin aikana mutta tulos oli lähellä merkittävää. Toisaalta elämänlaadussa saatiin merkittävä tulos toremifeenin hyväksi. Tämä tulos voi kuitenkin olla sattumaa huomioiden neljännen osatyön pieni potilasmäärä verrattuna ensimmäiseen työhön (9 vs. 56 potilasta).

Seerumin mediaani estrogeeni ja progesteronitasot olivat merkittävästi korkeammat toremifeenihoidon aikana. Myös seerumin mediaani PRL arvot olivat korkeammat toremifeenia käytettäessä. Muissa hormonitasoissa ei havaittu muutoksia. Seerumin inhibiini A ja B pitoisuudet luteaalivaiheessa olivat lähtötilanteessa normaalit ja ne pysyivät muuttumattomina toremifeeni ja placebolääkityksien aikana. Toremifeenin terapeuttinen vaikutus ei siis näyttäisi olevan sidoksissa munasarjan inhibiinituotannon stimulaatioon tai inhibitioon.

Sekä enhancement että enhancement ratio olivat alhaisempia toremifeenihoidon aikana. Nämä löydökset osoittavat, että toremifeenin teho syklisessä mastalgiassa perustuu ainakin osittain rinnan vähentyneeseen verenkiertoon. 3D-ultraäänellä tutkitut terveen rinnan verenkierrolliset indeksit ja tilavuus pysyivät ennallaan toremifeenihoidon aikana.

Havaitsemamme toremifeenin hyvä teho rintojen syklisen kivun hoidossa rohkaisee sen käyttöön tässä indikaatiossa. Lyhytkestoisen toremifeenihoidon turvallisuus 20 mg päivittäisellä annoksella osoitettiin tässä tutkimuksessa. Esitetyt tulokset osoittavat, että toremifeenin teho selittyy ainakin osittain paikallisilla vaikutuksilla rintarauhaseen, vaikka lääkityksen luteotrooppinen vaikutus tuli myös selvästi esiin.

Sinikka Oksa

1   INTRODUCTION

Breast pain is the most common breast symptom causing women to consult physicians and surgeons. It is rarely a sign of cancer. However, concern about cancer is the primary reason for most women to seek medical evaluation and treatment for this syndrome (Eberl et al. 2008). Breast pain is classified as cyclic mastalgia, noncyclical mastalgia, and chest wall pain. Cyclic mastalgia is a breast pain occurring in a predictable pattern with the menstrual cycle, often a few days before menstruation. Noncyclical mastalgia is described as a constant or intermittent pain with irregular exacerbations and no relationship to menstruation. Although women with chest wall pain may complain of breast pain, the actual source of the pain is not the breast tissue but rather the costochondral joint underlying it. Mastalgia is cyclic in two-thirds of the patients (Mansel 1994). Cyclic pain is often bilateral, usually most severe in the upper outer quadrants of the breast, and may be referred to the upper arm and axilla. According to severity breast pain can be classified as mild, moderate or severe. Mild premenstrual discomfort lasting for one to four days can be considered normal. Research criteria for the diagnosis of severe cyclic mastalgia include pain severity greater than 4 cm measured with a 10 cm VAS and pain duration of at least 7 days per month (Ader and Browne 1997a). Approximately 8-10 % of premenopausal women experience moderate to severe breast pain monthly during the premenstrual period (Ader et al. 1999). Premenstrual mastalgia has been reported to interfere remarkably with women's lives, relationships, work and sleep (Ader and Shriver 1997b).

The evaluation of mastalgia begins with a thorough history and a careful physical examination. Special attention should be given to the type of pain, its location and its relation to the menstrual cycle. Women with noncyclical mastalgia will benefit from a physical examination to determine whether the pain comes from the breast or chest wall. Although cyclic mastalgia is a well-known somatic component of the premenstrual syndrome (PMS), more than 80 % of women with premenstrual mastalgia do not meet the criteria for PMS (Ader et al. 1999). A higher use of mammography among young women with mastalgia compared with age-matched women without mastalgia has been observed (Ader and Browne 1997a). The particular value of breast imaging in breast pain is reassurance for both the patient and physician.

Before beginning any therapeutic interventions for breast pain, patients should be asked to document the frequency and severity of their pain for at least two menstrual cycles (BeLieu 1994). Accurate assessment of mastalgia requires a prospective diary evaluation of pain with the VAS scale. Approximately 15 % of women with mastalgia require further pain-relieving therapy (Gateley et al. 1992b). Mild symptoms can be managed with mechanical breast support (Hadi 2000) and topical nonsteroidal anti-inflammatory agents (Colak et al. 2003). Because of the variability in mastalgia and a remarkable placebo effect, only treatments that have been tested in randomized, controlled trials can confidently be considered as beneficial. Several hormonal imbalances as potential causative roles in cyclic mastalgia have been investigated. Cyclic breast pain is worsened by exogenous oestrogens and improved with the use of the anti-oestrogenic tamoxifen (Shrivastava et al. 2007). Toremifene and tamoxifen are chemically related compounds with comparable activity in breast cancer (Hirsimäki et al. 2002, Holli et al. 2000). Consequently, when planning the present study, it was reasonable to assume that toremifene would have a similar kind of antimastalgic effect as tamoxifen.

2 REVIEW OF THE LITERATURE

2.1 Development and anatomy of the breast

The mammary gland, unlike other branched organs, undergoes most of its branching during adolescent rather than foetal development (Sternlicht 2006). It has long been known that ovarian and pituitary hormones are essential for post-pubertal mammary morphogenesis and that oestrogens can rescue the mammary development in ovariectomised (Daniel et al. 1987), but not hypophysectomised, animals (Kleinberg et al. 2000). The major role of progesterone is in enhancing ductal length and branching, while oestradiol partly stimulates the ductal morphogenesis by enhancing insulin-like growth factor-I action, but having a greater effect on the terminal end bud formation and side branching (Ruan et al. 2005). Progesterone has been shown to act by a paracrine mechanism on a subset of mammary epithelial cells to allow for alveolar growth. The absence of progesterone from all cells of the mammary epithelium results in a failure of side-branching and lobuloalveolar growth (Brisken et al 1998).

Locally produced inhibin B may modulate mammary development and functional differentiation through an autocrine, paracrine or intracrine mechanism. Alternatively, mammogenesis may be dependent on the systemic effects of inhibin B. Inactivation of the activin/inhibin B gene results in incomplete mammary development and failure of lactation. The ductal elongation is incomplete, end buds persist and the morphogenesis of secretory alveoli is reduced (Robinson and Hennighausen 1997).

The main ducts arise from the lactiferous sinuses in the nipple. Subsidiary ducts arise from the sides of the main stems and quickly break up into short branches. Lobules resembling clusters of grapes are found at the end of these branches. Each lobule, usually 1 to 2 mm in diameter, contains a complex duct system composed of ductules which branch several times before terminating blindly in slightly dilated, club-shaped endings. The main stroma of the breast, called the peri-lobular connective tissue, is situated around the ducts and lobules. It is collagenous and contains a varying amount of fat. The intra-lobular connective tissue found within the lobules surrounding the ductules contains no fat (Parks 1959). The glandular tissue of the breast is suspended within the superficial

fascia of the anterior chest wall, extending roughly from the second to the sixth or seventh anterior intercostal space and from the edge of the sternum to the midaxillary line. About two-thirds of it rests upon the fascia covering the pectoralis muscle and the rest upon the fascia of the serratus anterior muscle. Toward the axilla, the axillary tail of Spence passes through an opening in the pectoral fascia, the foramen of Langer, into the axilla. The breast is fixed to the overlying skin and the underlying pectoral fascia by fibrous bands known as Cooper's ligaments (Gray's Anatomy 1980).

The female breast is composed of 15 to 20 sections called lobes, with each lobe ending in many smaller lobules. Connected together by a loose connective tissue, blood vessels and ducts, lobules are also known as terminal ductal lobular units. The lobules terminate into numerous tiny bulbs that produce milk during lactation. The lobes, lobules and bulbs are all linked together by thin tubes called ducts. Because breasts vary greatly in the amount of adipose tissue they contain, they vary considerably in size. The nipple, where the various ducts eventually terminate is composed of a dense connective tissue. Pigmentation varies greatly in both the nipple and the areola (the pigmented area around the nipple). Both the nipple and the areola contain numerous sebaceous glands and milk ducts (Gray's Anatomy 1980, Powell 1990). The anatomy of the breast is presented in Figure 1.
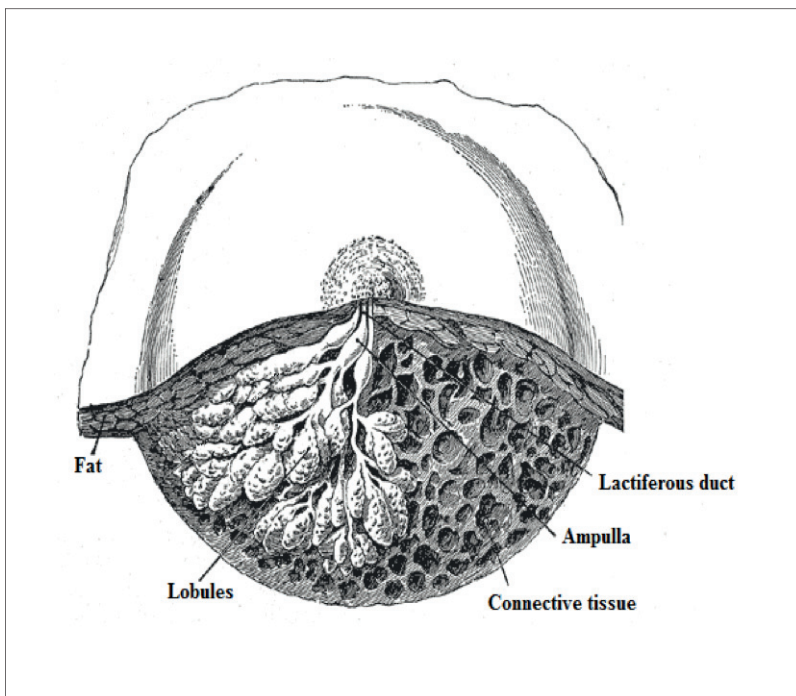
Figure 1.   Anatomy of the breast

2.1.1 Arterial supply and venous drainage

The blood supply to the breast is provided mainly by the anterior and posterior medial branches of the internal mammary artery and the lateral mammary branch of the lateral thoracic artery (Cunningham 1977, Grays´s Anatomy 1980, Sobotta 1982). The blood supply varies markedly. According to Doughty et al. (1996), the proportion of the breast being supplied by the internal mammary artery and lateral thoracic artery varies from 20 to 95 % and from 0 to 35 %, respectively. In 33 % of the subjects, the lateral thoracic artery did not contribute to the breast perfusion at all, but a large area of the lateral aspect of the breast was perfused from a further branch of the subclavian or axillary artery. The venous drainage of the breast is divided into a deep and a superficial system which are joined by short connecting veins. Both systems drain into the internal, thoracic, axillary and cephalic veins (Cunningham 1977).

2.1.2 Lymphatic drainage

The lymphatic vessels of the mammary gland originate from a plexus in the interlobular connective tissue and from the walls of the lactiferous ducts. There are two main pathways via which lymph is drained from the breast. One is to the axillary nodes and the other is to the parasternal nodes. The internal mammary nodes receive lymph from the deep portion of the breast. Specifically, the majority or 75 % of the lymphatic drainage from both the medial and lateral portions of the breast is directed to the axillary nodes (Gray´s Anatomy 1980, Sobotta 1982).

2.1.3 Innervation

An intercostal nerve runs along the inferior edge of each of the first 11 ribs. A subcostal nerve is located beneath each of the first 12 ribs. These nerves innervate the muscles that join the ribs and provide sensory input from the overlying skin of the chest. The first three intercostal nerves also mediate sensation from the upper extremities and axilla. The costal nerves further mediate sensation from the adjacent lower parietal pericardium, the parietal pleura, and the peripheral segment of the intrathoracic diaphragm. The breast receives its innervation from the lateral and anterior cutaneous branches of the second to the sixth intercostal nerves and from the supraclavicular nerves. The extent of the contribution of each nerve is variable, differing even between the left and right breast of an individual (Gray´s Anatomy 1980, Sobotta 1982, Sarhadi et al. 1996).

2.2   Mastalgia

2.2.1 Classification

2.2.1.1 Cyclic pain

This most common type of breast pain is associated with menstruation, and is also called a premenstrual or cyclic mastalgia. Mastalgia is cyclic in two-thirds of patients. Cyclic pain is often bilateral, usually most severe in the upper outer quadrants of the breast, and may be referred to the upper arm and axilla (Gateley and Mansel 1991, Ader and Shriver 1997b). The cyclic pain may, however, be more severe in one breast (Rea et al. 1997, Smith et al. 2004). The pain is associated with a diffuse nodularity. There is no measurable relationship, however, between pain severity and the extent of nodularity (Fentiman and Hamed 1989). In order of decreasing frequency, premenstrual breast symptoms reported by women are tenderness, swelling, pain, and lumpiness. As mentioned earlier, the diagnostic criteria for severe cyclic mastalgia include a pain severity greater than 4 cm measured with a 10 cm VAS, and a pain duration of at least seven days per cycle (Ader and Browne 1997a).

2.2.1.2 Noncyclical pain

A noncyclical mastalgia is described as a constant or intermittent breast pain with irregular exacerbations and no relationship to menstruation. True noncyclical mastalgia commonly tends to be bilateral and located within one quadrant of the breast (Maddox et al. 1989b). Some women have a localised tender area in the breast, known as the trigger spot (Mansel 1994).

2.2.1.3 Extramammary pain

A musculoskeletal pain tends to be almost always located along the lateral chest wall or on costochondral joints, and moreover to be unilateral in 92 % of cases. In a study of Maddox et al. (1989a), patients responded very well to steroid and local anaesthetic injection (97 %) when the noncyclical breast pain was musculoskeletal in origin. The pain is typically exacerbated by movements, coughing and deep breathing. Costochondritis is a self-limited condition defined as an inflammation of the costochondral joints of ribs or chondrosternal joints, usually at multiple levels and lacking swelling or induration (Fam 1988). Although the second to fifth costochondral joints are the ones most often affected, especially ribs three and four, any of the seven costochondral joints can be involved. It is often confused with the Tietze's syndrome, a similar but rarer disorder (Proulx

and Zryd 2009). Tietze's syndrome is an inflammatory process causing an even visible enlargement of the costochondral joint. It usually occurs in a single rib (70 % of the time), typically within the costal cartilages of the upper ribs two through three, predominantly in rib two (Gregory et al. 2002). Other extramammary causes of mastalgia include, e.g. cervical and thoracic spondylosis, thoracic outlet syndrome, lung disease, and gall stones (Mansel 1994).

2.3 Prevalence and clinical importance

According to severity, breast pain can be classified as mild, moderate or severe. The reported prevalence among premenopausal women ranges from 41 % to 79 %. Ader and South-Paul (2001) reported that 70 % of premenopausal women experience some degree of a premenstrual discomfort. In a large clinic-based trial of 1,171 women attending a gynaecologic clinic for any reason, 69 % suffered from regular discomfort, which was judged as severe by 11 % of women, and 36 % had consulted a doctor for the breast pain (Ader and Browne 1997a). Moderate-to-severe breast pain during the premenstrual period is experienced monthly by approximately 8–10 % of premenopausal women (Ader et al. 1999). In another clinic-based trial of 231 women, cyclic mastalgia was reported to interfere with sexual activity by 48 %, with physical activity by 36 %, with work, school or social activities by 6 % to 13 %, and with sleep by 10 % of the women, respectively. Moreover, 79 % of the participants had regularly experienced cyclic breast pain and nearly half of the study population had asked some healthcare provider about their symptoms (Ader and Shriver 1997b).

The average age of the patients with the cyclic type of breast pain is 34 years. Noncyclical mastalgia presents at a later age, with most women being at the fourth or fifth decade of life at presentation (BeLieu 1994, Mansel 1994). An Australian study of 170 mastalgia patients reported that the average age of the women was 42 years and that the breast pain was cyclic in 59 % of the cases. The patients of this trial attended a specific mastalgia clinic over a three-year period (Wetzig 1994). Current moderate to severe premenstrual mastalgia lasting for five days or more monthly was reported by one third of the study population. An onset of cyclic pain before the age of 20 years was followed by a prolonged course. An improvement in the pain was spontaneous in 22 % of the cases and resulted from a hormonally related event like menopause, pregnancy, or the use of oral contraceptives (Wisbey et al. 1983). In a long-term follow up study of 212 women, Davies et al. (1998) found that the patients with cyclic mastalgia tended to develop their pain earlier than noncyclical mastalgia patients, in accordance with BeLieu (1994) and Mansel (1994). Although breast pain was long-lasting in both groups (median 12 years), it was longer in patients with cyclic mastalgia,

who also developed breast pain earlier. In the noncyclical mastalgia group, the rate of spontaneous resolution (40 %) was higher than in the cyclic group (14 %). The resolution of cyclic mastalgia was associated with the menopause in most patients. In a prospective study of 72 patients, 35 patients were suffering from the noncyclical mastalgia and 37 from the musculoskeletal pain, the latter including Tietze's syndrome and other causes of chest wall pain. The mean age of the participants at presentation was greater in the musculoskeletal group (39.3 vs. 33.9 years) with a shorter mean duration of pain (14.7 vs. 35.4 months) as compared with the noncyclical mastalgia group (Maddox et al. 1989a).

Breast pain is the most common breast symptom causing women to consult primary care physicians and surgeons. A retrospective cohort study with 2,400 pre- and postmenopausal women found that women younger than 50 years of age presented with breast symptoms to their primary care physician twice as often as older women did. Additionally, subjects with a family history of breast cancer (22 %) were more likely to present with breast complaints compared with those without a family history (14 %) (Barton et al. 1999a). On the other hand, Nichols et al. (1980) reported that approximately one third of the breast symptom visits to general practices were made by women younger than 35 years of age. In the primary care setting, breast symptoms were reported in about 3 % of all visits by female patients. The breast pain (48 %) and a breast mass (29 %) were the most common breast-related complaints. Complaints of breast symptoms were most common among women aged 25 to 44 years (48 of 1,000) and among women aged 65 years and older (33 per 1,000), respectively. This 17-year data on the prevalence of breast symptoms is based on a population of 84,285 female patients (Eberl et al. 2008). In a prospective study with 30 women, it was found that most women with mastalgia had often also other menstrual complaints. However, 12 % of the patients with a severe cyclic mastalgia had relatively minimal associated symptoms (Tavaf-Motamen et al. 1998).

2.4   Aetiology

2.4.1 Health, lifestyle, behavioural, and dietary factors

Perceived stress was associated with cyclic mastalgia in a population-based study (Ader et al. 2001). The extent to which psychological distress has a causal or consequential relationship to mastalgia is unclear. However, substantial improvements in social impairment and depression were found in women whose breast pain was treated successfully (Ramirez et al. 1995). Studies have revealed increased depression and anxiety among women with mastalgia (Ramirez et al. 1995, John-

son 2006). Comparable clinical levels of emotional distress have been reported in women with severe mastalgia and women with breast cancer undergoing surgical treatment (Ramirez et al. 1995). Colegrave et al. (2001) found that women with breast pain had increased anxiety, depression, somatisation, and history of emotional abuse compared with women with lumps alone. In a study of 20 women with cyclic mastalgia, levels of depression and anxiety scores were found to be higher and changes in anxiety and depression scores were found to be greater between the luteal and follicular phases when compared with asymptomatic women (Downey et al. 1993). The association between the psychological factors and mastalgia will be discussed further in the chapters on PMS and PMDD (premenstrual dysphoric disorder).

Active breast movements on its weak suspensory ligament may contribute considerably to mastalgia. Physical activity may increase cyclic breast pain experienced especially by women whose occupations include lifting and prolonged use of the arms (Mansel 1994). On the other hand, increasing exercise levels may improve chronic pain via the release of endorphins (Sullivan et al. 1991). In a cross-sectional study of 502 women, it was found that more educated women from urban areas reported breast tenderness more often as a premenstrual symptom. Aerobic exercise increased breast tenderness in this trial (Huerta-Franco and Malacara 1993). The absence of previous breast feeding and low levels of regular physical exercise were identified as significant factors in the history of the women suffering from mastalgia (Wetzig 1994).

Some authors have found an association between the consumption of caffeine and breast pain (Bullough et al. 1990), but opposite reports have also been published (Schairer et al. 1986, Allen and Froberg 1987). Ader et al. (2001) found a small but significant association between some lifestyle factors and mastalgia: smoking (RR 1.52), a high intake of caffeine (RR 1.53), and perceived stress (1.88). Frequent mastalgia complaints were reported to be associated with alcohol misuse (Johnson et al 2006). Patients with cyclical mastalgia have shown to have reduced plasma proportions of the esters of the unsaturated fatty acids when compared with controls (Gateley et al. 1992a). Reductions in dietary fat intake have been associated with improvements in cyclic breast pain (Sharma et al. 1994). However, Mansel (1989) did not find any correlation between weight and cyclic breast pain. Soya protein and isoflavones have been reported to reduce breast tenderness when compared with placebo. The traditional diets of Asian populations contain moderate to high levels of isoflavones derived mainly from soy. This could be one of the reasons why cyclic mastalgia is more common in Western populations (Ingram 2002, Bryant et al. 2005). A high intake of calcium and vitamin D probably reduce the risk of PMS (Bertone-Johnson et al. 2005).

2.4.2 Hormonal and histological findings

2.4.2.1 Normal menstrual cycle

Oestrogen, progesterone and testosterone are the main steroid hormones that play an essential role during the follicular and luteal phases of the menstrual cycle. The granulosa cells are the main source of ovarian oestradiol production, which results from conversion of theca cell-derived androgens. Although ovarian theca cells also contribute circulating androgens, the adrenal cortex is the major source (Kushnir et al. 2009). Oestradiol and progesterone are the principal mediators of the suppressing effect on gonadotrophin secretion during the normal menstrual cycle. Oestradiol is the main component of the positive feedback mechanism that induces the midcycle luteinizing hormone (LH) surge (Messinis 2006). The main function of the corpus luteum is to synthesize and secrete progesterone under the control of LH (Chin and Abayasekara 2004). PRL is mainly secreted by the anterior pituitary cells. PRL influences the gonads either directly or indirectly. Direct action results in a decreased sensitivity of the LH and follicle stimulating hormone (FSH) receptors in the ovaries. The indirect effect is exerted by a reduction of gonadotrophin releasing hormone (GnRH) secretion (Ignacak et al. 2012). Oestrogen is a positive regulator of PRL synthesis (Faupel-Badger et al. 2006).

Inhibins are principally produced in the ovary by granulosa cells of developing follicles. Both inhibin forms have the capacity to suppress FSH secretion by pituitary cells in culture without affecting LH secretion. Inhibin B rises from the late luteal phase to the mid-follicular phase in concert with FSH, whereas inhibin A increases in the late follicular phase along with LH, and peaks in the mid-luteal phase (Groome et al. 1996). Declining inhibin A levels during the late luteal phase seem to be the predominant regulator of rising FSH levels. In contrast, high inhibin B concentrations during the early follicular phase are responsible for the decline in FSH serum levels (Burger et al. 1998). The hormonal profiles of the normal menstrual cycle are shown in Figure 2.

2.4.2.2 Hormones in mastalgia

A hormonal aetiology of cyclic mastalgia is suggested by its onset at menarche, its relation to the menstrual cycle and its resolution with the menopause. However, any consistent hormonal abnormalities have not been identified. Several hormonal imbalances with potential causative roles in premenstrual mastalgia have been investigated, and each of them has yielded supporting and opposing evidence (Smith et al. 2004). A case-control study compared personal and hormonal variables among 30 women experiencing cyclic mastalgia with those of 77 asymptomatic women. The urine levels of LH and FSH were found to be significantly
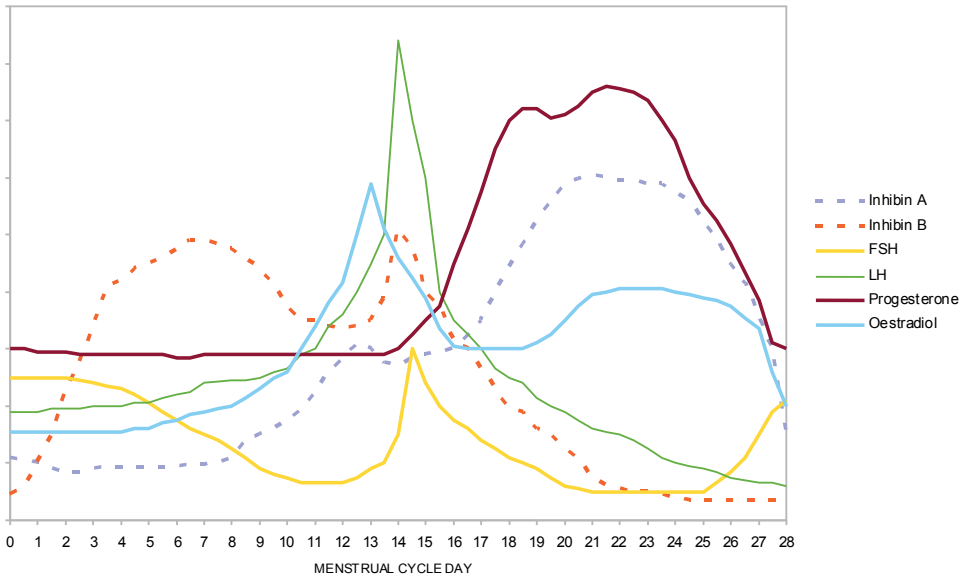
Figure 2.   Hormone fluctuations throughout the menstrual cycle.

higher in cyclic mastalgia cases throughout the cycle (Ecochard et al. 2001). Enhanced PRL responsiveness to thyreotrophin-releasing hormone (TRH) has been found in about 50 % of cyclic mastalgia patients. The serum baseline PRL levels can be normal, but dynamic testing of the pituitary using TRH has demonstrated an increase in the dynamic release of PRL (Kumar et al. 1984). A PRL response to TRH is considered to be increased when the serum levels exceed the upper normal limit of PRL with a factor of three after a TRH injection (Dogliotti et al. 1985). The progesterone metabolite allopregnanolone fluctuates very similarly to progesterone, with higher levels in the luteal phase than in the follicular phase of the menstrual cycle (Genazzani et al. 1998). In women with premenstrual symptoms, however, serum levels of allopregnanolone and progesterone during the luteal phase have been found to be lower than in symptom-free women (Monteleone et al. 2000).

Roszkowski et al. (1997) evaluated the cytotoxic activity of peripheral blood natural killer cells in relation to serum levels of sex hormones in patients with mastopathy. The study included 37 patients classified into mastalgia, fibrosis, fibrocystic disease, and fibroadenoma groups, and 19 healthy age-matched volunteers. Serum levels of oestradiol, progesterone, LH, FSH, and PRL were measured with specific radioimmunoassays. The natural killer cell activity was evaluated by means of a 51Cr-release assay. In all patient groups, progesterone levels were sig-

nificantly decreased. There were also more patients than controls with low levels of oestradiol (< 50 pg/mL). On the other hand, LH levels were significantly increased only in patients with fibrocystic disease and fibroadenomas. Natural killer cell activity was within normal range in all patient groups. In individual women, natural killer cell cytotoxicity did not correlate with the levels of the studied hormones. However, in patients with low (< 50 pg/mL) and high (> 200 pg/mL) oestradiol levels, an increase and a decrease of the natural killer cell activity was observed, respectively. This suggests that in patients with mastopathy, oestradiol may directly or indirectly affect the natural killer cell cytotoxicity.

2.4.2.3 Neurosteroids

Many researchers have suggested that premenstrual complaints are elicited by the drop in progesterone concentrations in the late luteal phase. Circulating steroid hormones have been shown to serve as precursors for the synthesis of neurosteroids, which can be produced locally in the hippocampus and other brain structures (Baulieu and Robel 1990). The steroid precursors are mainly synthesized in the gonads and the adrenal glands (Do Rego et al. 2009). Neurosteroids can also be produced de novo in the brain (Paul and Purdy 1992). They modulate brain excitability primarily by an interaction with neuronal membrane receptors and ion channels, principally gamma-aminobutyric acid (GABA) receptors. Neurosteroids do not activate intracellular steroid receptors (Akk et al. 2009). Women with premenstrual symptoms have been reported to have decreased serum GABA levels in the late luteal phase (Halbreich et al. 1996). On the other hand, Epperson et al. (2002) found, using the magnetic resonance (MR) spectroscopy, abnormally low GABA levels in the occipital cortex during the follicular phase of the menstrual cycle and abnormally increased levels during the luteal phase in women with severe premenstrual complaints.

2.4.2.4 Local breast hormones

The mammary gland has a capability to produce a significant proportion of tissue oestrogens locally. Hence, the plasma oestrogen levels do not correlate with simultaneous tissue oestrogen concentrations. Aromatase is an enzyme that catalyses the conversion of other steroids into bioactive oestrogens and aromatase activity has been found in the breast (Geisler 2003). Within the breast adipose tissue, stromal cells are the primary aromatase expressing cells. Local oestrogen biosynthesis can be highly variable. The activity of aromatase has been reported to increase with age. The enzyme activity has been shown to be higher in fat from breast cancer patients when compared with women with a benign breast disease. However, some level of oestrogen biosynthesis was detected in all specimens of

breast adipose tissue examined (O'Neill and Miller 1987, Miller 1991, Kulendran et al. 2009).

The levels of oestradiol in the nipple aspirate of premenopausal women have been shown to be significantly associated with oestrogen precursor steroids within the same breast. However, the associations between these hormones in the nipple aspirate or oestradiol in the serum (measured on the same midluteal day) were negligible. By contrast, progesterone levels in the nipple aspirate were fairly strongly correlated with progesterone levels in the serum. In addition, the nipple aspirate fluid concentrations of epidermal growth factor and cathepsin D were positively associated with oestradiol and its precursors in the aspirate, but not with steroid levels in the serum or saliva (Gann et al. 2006). Potential oestradiol precursors or oestrone sulphate, dehydroepiandrostenedione sulphate (DHEAS), and androstenedione, were present in the breast in concentrations that were many times greater than those of oestradiol (Chatterton et al. 2004).

In a report of sex steroid variations in the nipple aspirate from 40 healthy premenopausal women, it was found that progesterone reached the mean peak concentration in the luteal phase, which was significantly higher than at midcycle. There was a delay in the peak oestradiol concentration, as opposed to the serum, where the peak occurred at midcycle. Oestradiol concentrations in the nipple aspirate fluid were significantly lower at midcycle than in the luteal phase of the menstrual cycle. The serum oestradiol concentration was highest at midcycle, with a serum: nipple aspirate fluid ratio of 1: 6.7, whereas in the midluteal phase this ratio was 1: 31.4. In contrast, androstenedione, DHEAS, and oestrone sulphate in the nipple aspirate fluid tended to decline from the midcycle to the luteal phase. The concentration of the vascular endothelial growth factor (VEGF) has been shown to increase in the extracellular fluid of the normal breast to two-fold from the follicular to the luteal phase of the menstrual cycle. The resulting enhanced rate of diffusion from the blood offers an alternative explanation for the concentration gradient for oestradiol between the circulation and breast (Chatterton et al. 2010).

An oestradiol exposure has been shown to increase extracellular angiogenin levels and the consequent angiogenesis in the normal breast tissue of healthy volunteers (Nilsson et al. 2010). The anti-oestrogenic tamoxifen has been found to decrease the concentration of pro-angiogenic factors angiogenin and VEGF, and to increase the levels of the anti-angiogenic endostatin in the normal breast tissue (Åberg et al. 2011). Rose et al. (1987) reported that serum PRL and growth hormone concentrations were similar in 46 healthy women and in 36 patients with cyclic mastalgia or cystic breast disease. However, the mean PRL levels in the breast aspirate tended to be higher in patients than in controls. Summarized concentrations of PRL and growth hormone from the breast fluid were significantly elevated in patients.

Di Loreto et al. (1999) found activin A, inhibin A and inhibin B subunits from the cyst fluid of women with the fibrocystic disease. The concentrations of the local activin A and inhibins showed no variation during the menstrual cycle. This lack of variation is in contrast to the cyclic changes found in serum inhibin levels. The authors concluded that this may reflect a local production of these peptides.

2.4.2.5 Histological findings

The proliferative activity of breast tissue has been reported to be highest between days three and seven of the menstrual cycle, while the active secretory phase with a stromal oedema and venous congestion peaks between days 21 and 27, respectively (Vogel et al. 1981). Ferguson and Anderson (1981), on the other hand, found that mitotic and apoptotic indices were highest around cycle days 25 and 28, respectively. They suggested that the increase in cell size or number may cause an increase in parenchymal breast volume. Most patients with mastalgia also have breast nodularity and tenderness. There is, however, no consistent relationship between the histology and breast pain symptoms. Previously, this clinical spectrum used to be called fibroadenosis and was considered to be synonymous with the fibrocystic disease of the breast. Fibrocystic changes have later been associated rather with a normal breast involution than to any disease (Love et al. 1982, Hughes 1989). In a study of 39 women with cyclic breast pain, all subjects had fibrocystic changes in breast biopsies. However, similar histological changes were found in 61 of 68 women without breast pain (Jørgensen and Watt-Boolsen 1985).

A patient with a persistent burning pain situated behind the nipple or nipples associated with palpable ectatic ducts and with a green- or yellow-stained nipple discharge can be classified as having ductectasia. Peters and Diemer (2003) demonstrated with 335 premenopausal women (136 with cyclic pain) that the degree of the ductal dilatation of the mammary gland correlates positively with the intensity of the breast pain.

Khanna et al. (2002) evaluated the oestrogen receptor (OR) status from breast specimens in 50 women with benign breast lesions. They found that 32 % of the lesions were OR positive and that the incidence was highest in fibroadenomas. Patients with the cyclic pattern of pain were more likely to have OR positive lesions when compared with those with noncyclical pain, but the difference between these groups was not significant. Mastalgia was reported by 26 patients in this study, and treatment with danazol 100–200 mg daily was initiated. All patients with an OR positive breast disease responded to danazol treatment irrespective whether the mastalgia was cyclic or noncyclical. In a cross-sectional study with 56

premenopausal women, it was demonstrated that tamoxifen significantly reduced the ORα and progesterone receptor (PgR) expressions by a normal breast tissue. An important finding was also that low doses of tamoxifen (5 and 10 mg) decreased the receptor expression to the same levels as a standard dose of tamoxifen (20 mg). The percentage of the cells expressing ORα was approximately 43 % and PgR 27 %, respectively, in this study. Tamoxifen was administered for 50 days before assessment, and mammary tissue specimens were collected during the luteal phase of the menstrual cycle (deLima et al. 2003).

The potential role of inflammation and inflammatory cytokines in mastalgia was evaluated in 29 premenopausal women with mastalgia and in 29 asymptomatic controls. No significant differences were found between the groups regarding the degree of inflammatory cell infiltration or cytokine expression in breast tissue specimens (Ramakrishnan et al. 2003). Gopinath et al. (2005) recently reported that the number of vanilloid thermoreceptor 1 positive intra-epidermal fibres was significantly increased in breast skin of patients with breast pain and tenderness. The vanilloid thermoreceptor 2 and 3 fibres were also significantly elevated in breast skin keratinocytes in patients with breast pain when compared with healthy women. Nerve growth factors and the expression of the vanilloid receptor in nociceptor fibres are considered key molecules in mediating pain and hypersensitivity.

2.4.3 Associated diseases

Bhargav et al. (2009) found a high prevalence of hypothyroidism (23.2 %) in 210 patients with benign breast disorders. Cyclic mastalgia was the most common predominant complaint (70 %), followed by noncyclical mastalgia (30 %), nipple discharge (29.4 %) and lumps (17 %). A correction of hypothyroidism with thyroxin replacement resulted in a significant improvement in mastalgia patients. Adamopoulos et al. (1986) also reported an increased (13 %) rate of thyroid dysfunction among patients with benign breast complaints. However, Giustarini et al. more recently (2006) found that only 4 % of 25 benign breast disease patients had hypothyroidism.

Genc et al. (2011) have studied the association between mastalgia and fibromyalgia. They found that 36 % of patients with mastalgia fulfilled the criteria of fibromyalgia, while 42 % of women had mastalgia in the fibromyalgia group. Johnson et al. (2006) noticed in a cross-sectional trial with 1,219 women that mastalgia was strongly associated with fibromyalgia, chronic pelvic pain and irritable bowel syndrome.

2.4.3.1 Breast cancer

The adjusted relative risk of breast cancer has been found to increase significantly with a longer duration of cyclic mastalgia (Goodwin et al. 1995, Plu-Bureau et al. 2006). Milanese et al. (2006) reported in a cohort study of women with a previously diagnosed benign breast disease that the relative risk of breast cancer was 1.4 when compared with age-matched women in the general population. Deschamps et al. (1996) suggested that cyclic mastalgia and breast swelling may carry an increased breast cancer risk. Opposing results have been also published. Khan and Apkarian (2002) reported that women who complained of breast pain were less likely to be diagnosed with breast cancer than women without mastalgia, regardless of age or other risk factors. However, the classification or minimum duration of breast pain were not defined in this study.

Aiello and Buist (2004) have shown that the presence of a lump is associated with a two- to three-fold greater risk of breast cancer irrespective of the presence of any other symptoms. According to several studies, a lump accompanying any other breast complaint increases the risk of cancer (Barton et al. 1999a, Seltzer 2004, Eberl 2008). However, if breast pain is the primary complaint, the probability of a malignancy seems to be low (Lumachi et al. 2002a, Lumachi et al. 2003, Howard et al. 2012). Lumachi et al. (2002b) showed that the relative risk of breast carcinoma ranged between 0.3 and 0.7 in patients with breast pain. Studies focusing on the connection between breast pain and cancer are summarized in Table 1.

2.4.3.2 PMS and PMDD

Premenstrual disorders are likely to start in adolescence. The reported prevalences based on the diagnostic criteria for PMS and PMDD are summarized in Table 2. The lower prevalence rates of PMS are found in women suffering from moderate to severe symptoms.

Table 1.   Frequency of cancer in patients presenting with breast pain

Study	No.of patients with breast pain	No. (%) of patients with breast cancer	Comments
Preece et al. (1982)	536	36 (6.7 %)	Review of patients focal pain as primary symptom
Smallwood et al. (1986a)	209	8 (3.8 %)	Review of 460 patients focal pain as primary symptom
Duijm et al. (1998)	987	4 (0.4 %)	Observational follow up study of 987 women referred because of pain, and 987 controls
Barton et al. (1999a)	169	2 (1.2 %)	Retrospective cohort study of 2,400 women
Lumachi et al. (2002b)	1141	36 (3.2 %)	Review of 2,879 patients with breast symptoms
Seltzer (2004)	501	8 (1.6 %)	10,000 women referred for consultation regarding a breast complaint
Howard et al. (2012)	916	6 (0.6 %)	Retrospective cohort study of breast pain as primary complaint

Table 2.   Prevalences of PMS and PMDD.

Study	Prevalence		n	Comments
	PMS	PMDD		
Sveindóttir and Bäckström (2000)		2–6 %	83	Women, Iceland
Perkonigg et al. (2004)		3 %	1488	German,14 –24 years
Rizk (2006)	16.4 %		115	Girls, 12–18 years United Arab Emirates
Takeda et al.(2006)	5.3 %	1.2 %	1187	Japanese students
Fujiwara and Nakata (2007)	48.3 %		522	Japanese students at least one symptom
Potter et al. (2009)	12.2 %		2863	French women
Joshi et al. (2010)	52.3 %	4.7 %	197	Indian women three symptoms or more
Lete et al. (2011)	8.9 %	1.1 %	2018	Spanish, 15–49 years
Pinar et al. (2011)		72.1 %	316	Turkish college students

2.4.3.2.1 Genetic background

The results of twin and family studies suggest that PMS is a heritable disorder (Kendler et al. 1992, Condon 1993, Treloar et al. 2002, Jahanfar et al. 2011). Huo et al. (2007) performed a haplotype analysis of ORα and ORβ in 91 women with severe premenstrual symptoms and in 56 controls. Significantly different genotype and allele distributions were found for ORα but not for ORβ between the cases and controls.

The association between breast pain and PMS is controversial. Although cyclic mastalgia is a well-known component of PMS, more than 80 % of women with premenstrual mastalgia do not meet the criteria for PMS (Ader et al. 1999). Takeda et al. (2006) reported that the physical symptoms were most common and severe among patients with PMDD. Kiesner and Pastore (2010) demonstrated with 92 healthy women that day-to-day variations in physical symptoms were significantly associated with day-to-day variations in psychological symptoms of the menstrual cycle. Joshi et al. (2010) investigated the prevalence of premenstrual symptoms in perimenopausal women. Mastalgia was the most common symptom (50.5 %), followed by mood changes (46.7 %), depression (7.5 %) and attacks of anger (6.5 %). Warner and Bancroft (1990) found, based on self-reporting by women that mood symptoms and irritability (65 %), anger for no apparent reason (64 %), and getting easily upset (60 %) were the most common symptoms. The most prevalent physical symptoms were abdominal bloating (45 %) and breast tenderness (40 %). There was a remission of mood and physical disturbances postmenstrually in 79 % and 94 % of sufferers, respectively. Ader et al. (2001) found no association between the premenstrual breast pain and PMS. Eighty-two percent of women with clinical cyclic mastalgia did not have PMS. If both mastalgia and PMS are present simultaneously, they may not be of equal severity, and breast symptoms and other components of PMS may respond differently to treatment.

The typical symptoms of PMS and the clinical criteria of PMDD are given in Tables 3 and 4, respectively.

Table 3.   Common symptoms of PMS

Physical	Behavioral	Mood
Swelling	Sleep disturbances	Irritability
Breast tenderness	Appetite changes	Mood swings
Aches	Poor concentration	Anxiety/tension
Headache	Decreased interest	Depression
Bloating/weight gain	Social withdrawal	Feeling out of control

Adapted from American College of Obstetricians and Gynecologists 2000

Table 4.   Clinical criteria of PMDD

Depressed mood	Fatigue
Anxiety/tension	Appetite changes/food cravings
Mood swings	Insomnia/hypersomnia
Irritability	Feeling out of control
Decreased interest	Physical symptoms (e.g., breast tenderness, bloating)
Concentration difficulties	

Adapted from the American Psychiatric Association 2000

2.4.3.2.2 History

The potential psychological origin of breast pain has been explored throughout the history of medical literature. In 1829, Sir Astley Cooper wrote that women seeking advice for breast pain usually had a nervous and irritable temperament. Although endocrine and neuralgic aspects of the breast pain were also considered, similar opinions predominated for many years (Atkins 1938). Preece et al. (1978) finally challenged the previous opinion that breast pain was an expression of psychoneurosis. They found that women with mastalgia and a control group of women with varicose veins had very similar psychological complaints.

2.5   Evaluation of a patient with mastalgia

2.5.1 Anamnesis

The evaluation of mastalgia begins with a thorough history followed by a careful physical examination. Special attention should be given to the type of pain, its location and its relation to the menstrual cycle. Menstrual irregularity, emotional stress and changes in medication have been shown to exacerbate mastalgia (Be-Lieu 1994). In obtaining the history, questions should be directed at identifying problems in these areas. In addition, a full breast history should be taken, including any family history of breast cancer. Taking a history of personal risk factors acknowledges the concerns of the patient and prepares the physician to counsel her concerning future expectations. The majority of mastalgia patients can be managed by exclusion of cancer and reassurance (Hamed and Fentiman 2001).

2.5.2 Clinical examination

A justification for performing clinical examination of the breasts, although high-quality mammography is widely available, is that this practice can detect lesions missed by mammography (Barton and Elmore 2009). The accuracy of the clinical examination may be affected by the examiner's experience, duration of the examination and technique used (Barton et al. 1999b). Women with noncyclical mastalgia will benefit from a careful physical examination to determine whether the pain comes from the breast itself or from the chest wall (Hamed and Fentiman 2001).

An adequate breast examination is performed by careful inspection and palpation of each breast, the nipple-areolar complex, and regional lymph nodes. This requires a routine planned procedure with several changes in the patient's position and a meticulous palpation of the entire extent of the breasts. An adequate breast examination also includes a careful palpation of the local and regional lymph nodes (Powell 1990). Typically the upper outer quadrant is denser than the upper inner or the lower outer quadrants and is much denser than the lower inner quadrant (Goodson 1996). A comparison of the physical findings of one segment of the breast with the adjacent breast tissue and the corresponding segment of the opposite breast is often helpful (Powell 1990).

2.5.3 Pain

Retrospective reporting of breast pain characteristics by the patient has been found to be of low to moderate validity (Tavaf-Motamen et al. 1998). A diagnosis of cyclic mastalgia is best achieved through a daily rating of symptoms over at least two menstrual cycles (Ader et al. 1999, Tavaf-Motamen et al. 1998). With two months of records, the clinical impression of ovulatory cycles can usually be confirmed and the timing of pain relative to the menses assessed. Typically, women have the same kind of breast symptoms from one cycle to the next. There must be a symptom-free interval just after menstruation. Instruments that can help the diagnostic process are frequently used in clinical trials. These include the use of VAS, a daily record of the severity of problems, a record of the impact and severity of menstruation, a calendar of premenstrual experiences, and a daily symptom report (Futterman and Rapkin 2006). An accurate assessment of mastalgia requires a prospective diary evaluation with a daily VAS. This equipment provides reproducible results and is easy for patients to use (Tavaf-Motamen et al. 1998, Mansel et al. 2007, Aydin 2010). Patients may also keep an informal diary of symptoms throughout the month as an alternative to formal rating scales (Steiner et al. 1999). All types of prospective scales allow for patients to accurately review their symptoms. After several months of personal records, up to 20 % of patients will experience spontaneous symptom relief (Gateley et al. 1992).

2.5.3.1 Pain threshold

Sex steroids influence the central nervous system and their receptors are indeed expressed in many areas of the brain, including those involved in pain transmission and modulation (De Tommaso 2011). Both oestrogen (Craft 2007) and progesterone (Coronel 2011) have been shown to have both pro-nociceptive and anti-nociceptive effects. Recent studies performed on pain perception during the menstrual cycle have, however, found only a modest or no effect of gonadal hormones (Kowaltczyk et al. 2006, Stening et al. 2007, Klatzkin et al. 2010). Veith et al. (1984) found that beta-endorphin levels were relatively stable across the menstrual cycle, pain thresholds did not fluctuate in any consistent pattern. Bartley and Rhudy (2012) compared pain thresholds between mid-follicular and late-luteal phases in healthy women. They found no menstrual phase effects on any pain threshold outcomes.

2.6   Imaging

The most common breast problems for which women consult a physician are breast pain, nipple discharge and a palpable mass (Barton et al. 1999, Lumachi et al. 2002b, Lumachi et al. 2003). A large number of women with breast symptoms are referred for mammography or sonography. The utility of mammography and sonography in the evaluation of a palpable mass is well established, with negative predictive values ranging from 99.8 % to 100 % (Soo et al. 2001, Dennis et al. 2001). Most women with these complaints have a benign breast disease. In a retrospective analysis with 698 women who spontaneously underwent breast examination it was found that breast pain was significantly the most common complaint in younger patients (under 50 years of age) and a breast lump in patients over 60 years of age (Lumachi et al. 2003).

Regardless of the type of breast problem, the goal of the evaluation is to rule out cancer and address the patient's symptoms. Women younger than 35-year-old with mastalgia were more likely to have had a mammogram than those without mastalgia (Ader and South Paul 2001). In an observational follow up study of Duijm et al. (1998), the reason for mammography was pain alone in 15 % of the patients. They compared a group of 987 women with breast pain and another group of 987 asymptomatic controls. Half of their subjects were under 50 years of age.

Mammography is the gold standard to detect early stage breast cancer before the lesions become clinically palpable. Two-dimensional (2D) ultrasound has been used as an adjunct to mammography for clinical examination in the assessment of both palpable and impalpable breast lesions. Tumyan et al. (2005) reported that the negative predictive value of mammography and sonography combined in patients with focal breast pain was 100 %. These imaging modalities were found to be reassuring to the physician in the absence of a breast mass in women with painful breasts. Howard et al. (2012) showed that initial imaging in women with breast pain actually led to additional evaluation, e.g. additional imaging, biopsies, and follow-up visits without an increased breast cancer detection.

2.6.1 Mammography

A higher use of mammography among young women (under 35 years) with mastalgia compared with age-matched women without mastalgia has been confirmed (Ader and Browne 1997a, Ader and Schiver 1997b). This rate is greater in clinic-based studies (4.5 times higher) than in population-based studies (two times higher) (Ader et al. 2001). If the clinical breast examination is normal, most patients younger than 35 years do not need any further diagnostic evaluations

(Duijm et al. 1998, Morrow 2000, Lumachi et al. 2003). On the other hand, women older than 35 are recommended to have a mammogram even if the physical examination seems normal (Faiz and Fentiman 2000, Morrow 2000).

Leinster et al. (1987) reported that the incidence of cyclic mastalgia in healthy women presenting for breast screening was 69 %. There was a higher incidence of 'high risk' mammographic patterns and a lower incidence of 'low risk' patterns in women with cyclic mastalgia compared with the rest of the screened population. This finding correlated with breast pain severity, duration and need for treatment. Deschamps et al. (1996) investigated the association between the mammographic patterns and clinical manifestations of benign breast disease among 1,394 women. The relative risk for mammary dysplasia involving half or more of the breast parenchyma was highest in women with cyclic mastalgia.

2.6.2 Ultrasound

Ultrasound is the method of choice for differentiating solid from cystic lesions, for further characterizing mammographic findings, and better appreciating palpable breast lesions (Watermann et al. 2005). B-mode ultrasonography is used in every day practice. Colour Doppler is used for studying lesion vascularisation (Athanasiou et al. 2009). Ultrasonography is a very useful modality for breast imaging in young and pregnant women. Breast cancer is a disease that can elude detection on a mammogram, especially in women with dense breast tissue. Kaplan (2001) reported that the bilateral whole-breast ultrasound can be an effective adjunct imaging examination in women with dense breast tissue in mammography.

It has been shown that the basal breast blood circulation flow values were much higher in women suffering from severe breast pain than in asymptomatic women. A significant dose-dependent decrease in breast vascularity was detected as a response to a luteal phase gestagen in women with cyclic mastalgia. The highest circulatory rates were found in the most severe mastalgia patients (Madjar et al. 1993).

2.6.3 3D US

3D US is one of the most recent developments in the breast imaging, providing additional aspects to conventional 2D sonography, such as the ability to study a breast mass and the surrounding tissues in three orthogonal planes (Weismann et al. 2011). The combined volume and vascular data that the 3D US can offer has made it popular in the clinical setting. 3D power Doppler imaging makes it possible to detect even minimal blood flow in the breast, and to assess changes in

flow and vascularity that occur in response to therapeutic efforts. Conventional 2D ultrasound is very useful for distinguishing breast cysts (Bilali et al. 2009), but 3D US has the capacity to demonstrate lesion margins and topography, thereby helping to differentiate benign from malignant breast masses, especially in a dense mammary tissue (LeCarpentier et al. 2008, Huang et al. 2009, Kalmantis et al. 2009). In addition 3D US can help in facilitating the needle localisation and guidance during the breast biopsy, thereby reducing the number of core samples that are needed to achieve a reliable histological diagnosis (Weismann et al. 2000).

It is possible to examine the vascularisation of a breast lesion using the 3D power Doppler technique. Neovascularisation, irregular vascular pattern, arterio-venous shunts and missing vessel autoregulation converge to a carcinoma diagnosis. Further advantages of the 3D power Doppler technique include the detection of even a minimal blood flow, the reliable analysis of vessel architecture, the extent of the vascularisation pattern and the definition of the number of vascular poles (Kalmantis et al. 2009). With 3D power Doppler it is possible to demonstrate flow in breast arteries with a diameter less than 1 mm. 3D US is also used to quantify the volume of organs and pathology (Fenster et al. 2001). 3D US and volume data can be more complete than 2D ultrasound image data because the entire surface of the mass can be evaluated with dynamic and multisectional capabilities (Cho et al. 2006).

2.6.4 MRI

MRI is an adjunct tool for evaluating breast tissue. MRI has been used as a screening tool for patients determined to be at a high risk of breast cancer. MRI has been shown to be more accurate than mammography in the annual breast cancer surveillance of women with a hereditary risk of breast cancer (Stoutjesdijk et al. 2001). Other indications for MR mammography are the preoperative estimation of the extent of the disease in a newly diagnosed cancer, patients with breast implants, cancer of unknown primary and the differentiation between a tumour recurrence and scarring (Sohns et al. 2011).

There are also drawbacks associated with breast MRI. MRI uses an intravenous contrast agent, is more expensive and time-consuming than mammography, is affected considerably by the patient's menstrual cycle, and is problematic for claustrophobic patients (Stoutjesdijk et al. 2001). However, the gadolinium-based contrast agent is not iodinated, and adverse events are very rare (Shellock and Kanal 1999). Hussain et al. (1999) measured variations in breast volume during the menstrual cycle using MRI. The total breast volume was reported to be at the maximum in the week before menstruation and at the minimum during the mid-cycle. The mean volume at ovulation is 5.5 % less than the mean volume

at menses. The overall variation in the average breast volume was found to be 76 ml (Hussain 1999). Fowler et al. (1990) estimated breast volume on MR images. The total volume was again reported to be at the maximum in the week before menstruation and at the minimum during the mid-cycle. Müller-Schimpfle et al. (1997) reported that breast parenchymal enhancement in MRI was significantly higher during cycle days 21-6 than during cycle days 7-20. Patients aged 35-50 yielded a greater parencymal enhancement than younger or older women. During the latter half of the menstrual cycle there is a significant increase in enhancement and in the extraction flow product (Kuhl et al. 1997, Delille et al. 2005). Delille et al. (2005) concluded that a dynamic breast MRI should be performed during the first half of the menstrual cycle in order to minimize interpretative difficulties related to the uptake of gadolinium in normal breast tissue due to cycle-related hormonal fluctuations. The breast MRI volume decreased while mammographic density remained unchanged after treatment with selective oestrogen receptor modulator (SERM) raloxifene at a dose of 60 mg in premenopausal women at an increased risk for breast cancer (Eng-Wong et al. 2008). The clinical assessment of the MRI images and dynamic scans, and enhancement curves are performed according to the American College of Radiology Breast Imaging Reporting and Data System (BI-RADS) lexicon for MR mammography. The standardised terminology of the BI-RADS lexicon enables a quantification of the likelihood of malignancy for detected lesions (Mahoney et al. 2012).

2.7 Treatment

The treatment of the cyclic mastalgia and PMS/PMDD will be discussed simultaneously, focusing on the breast-related symptoms in the case of PMS/PMDD. Mastalgic symptoms should be treated when they are severe enough to interfere with a woman's lifestyle and occur for several days each month. Several treatment options are available (BeLieu 1994). After a thorough history, examination and imaging, reassurance has been reported to have a high success rate in mastalgia. Barros et al. (1999) observed in a trial with 121 women that reassurance was effective in 85.7 %, 70.8 %, and 52.3 % of the patients with mild, moderate or severe mastalgia, respectively. McFayden et al. (1992) concluded that reassurance is a fundamental form of treatment and that the use of medical therapy would be necessary only in a small proportion of mastalgia patients and would result in a considerable incidence of side-effects.

Approximately 15 % of women with mastalgia have been reported to require further pain-relieving therapy (Gateley et al. 1990, Gateley et al. 1992b). Treatment should be considered when there is a history of mastalgia for at least six months and pain charts show more than seven painful days per cycle (Mansel

1994). If breast pain, especially the cyclic mastalgia, does not develop until near the menopause, drug treatment may be delayed (Davies et al. 1998). A well-fitting support bra can provide substantial pain relief for women who exercise vigorously (McFayden et al. 1992, Hadi 2000) and topical or systemic nonsteroidal anti-inflammatory agents are reasonable first-line treatments for mild breast pain (Rosolowich et al. 2006).

A clinically useful response to therapy can be reached in 92 % of patients with cyclic and in 64 % of those with noncyclical mastalgia, respectively (Gateley et al. 1992b). When mastalgia is severe and begins at an early age, the patient may need multiple courses of drug treatment or a continuous low-dose regimen (Davies et al. 1998). Wetzig (1994) observed that the overall response rate to all mastalgia treatments was 65 % with a mean follow up of 15.5 months. Using bromocriptine, danazol and evening primrose oil, 77 % of patients treated can obtain useful relief of their breast pain symptoms (Gateley and Mansel 1990).

2.7.1 Placebo effect

Because of the marked variability of mastalgia and a remarkable placebo effect, only treatments that have been tested in randomized, controlled trials can be confidently considered as beneficial. There is a placebo effect of at least 20 % (range 10 % - 40 %) in mastalgia treatment (Mansel et al. 2004, Goyal and Mansel 2005, Kaviani et al. 2008). Placebo has also been shown to be effective as a therapeutic tool in PMS (Sampson et al. 1988, Freeman and Rickels 1999a). In a longitudinal study, Magos et al. (1986) found an initially strong placebo response rate that gradually waned.

2.7.2 Lifestyle and dietary

Based on epidemiological studies, regular, moderate physical exercise has a positive effect on menstrual cycle-related symptoms and mood states (Aganoff and Boyle 1994, Johnson 1998). Dietary changes often recommended as a treatment of premenstrual symptoms include reduction of salt, sugar, alcohol, and caffeine intake. Consumption of an evening meal rich in carbohydrate and poor in protein during the late luteal phase seems to be effective in PMS-related mood disorders (Wurtman et al. 1989). Avoidance of caffeine has been shown to have no benefit (Allen and Froberg 1987).

A lack of essential fatty acids, or linoleic and gamma linoleic acid, has been implicated in the aetiology of perimenstrual symptoms. Linoleic acids, found in fish oil, inhibit arachidonic acid formation at the beginning of the prostaglandin

cascade, and have been found to relieve pain symptoms. Although gamma linoleic acid is a precursor for prostaglandins, some studies have shown that increasing gamma linoleic acid intake reduces premenstrual breast tenderness and fluid retention. Treatment with primrose oil has found to produce a significant increase in the proportion of plasma essential fatty acid esters. Clinical response to primrose oil in mastalgia was better in patients with lower pretreatment levels of the esters of the unsaturated fatty acids (Gateley et al. 1992a).

Evening primrose oil is a rich dietary source of essential fatty acids (Bayles and Usatine 2009). It is a perennial herb that is named based on its unusual habit of opening its flowers in the evening. The plant is rich in oil containing gamma-linoleic acid, an essential fatty acid. Mansel (1994) noticed that an abnormal profile of certain essential fatty acids might explain the response of breast pain to evening primrose oil.

Supplements have been found to have a positive clinical effect on fluid retention and mood changes (Facchinetti et al. 1991, Walker et al. 1998). Thys-Jacobs et al. (1995) have reported that women with PMS are vitamin D deficient with midcycle elevations of parathyroid hormone. Studies which have investigated vitamin supplements, primrose oil, as well as caffeine avoidance in the mastalgia treatment are summarized in Table 5.

Table 5.   Treatment of mastalgia with dietary changes and supplements

Study	Outcome for mastalgia	
London et al. 1983	Vitamin E/placebo	Significant benefit
Ernster et al. 1985	Vitamin E/placebo	No benefit
London et al. 1987	Vitamin E/placebo	Slight benefit
Meyer et al. 1990	Vitamin E/placebo	No benefit
Parsay et al. 2009	Vitamin E/placebo	Significant pain reduction
Renwick 1979	B1/placebo	No benefit
Smallwood 1986b	B6/placebo	No benefit
Blommers et al. 2002	Primrose oil/fish oil/placebo oil	No benefit
Pruthi et al. 2010	Vitamin E/primrose oil/placebo	No benefit
Allen and Froberg 1987	Avoidance of caffeine/placebo diet	No benefit
Thys-Jakobs et al. 1989	Calcium 1000 mg/placebo	Benefit
Thys-Jakobs et al. 1998	Calcium 1200 mg/placebo	Benefit

2.7.3 Anti-inflammatory drugs

Topical application of diclofenac and piroxicam gave relief to 21 of 26 women with severe cyclic, noncyclical and scar-related breast pain (Ahmadinejad et al. 2010). Colak et al. (2003) evaluated diclofenac diethyl-ammonium gel and placebo gel in mastalgia patients and reported significantly reduced breast pain when compared to placebo at six months. Although a large placebo effect was demonstrated in both cyclic and noncyclical mastalgia groups, the change in pain scores was significantly smaller than in the diclofenac group. In an open, non-randomized study, piroxicam gel was found to be more effective than evening primrose oil in 50 women with moderate or severe mastalgia, with no adverse reactions (Qureshi and Sultan 2005). In a randomized, placebo-controlled trial, oral naproxen 250 mg was compared with placebo in 81 women suffering from noncyclical mastalgia for three months. Pain scores decreased significantly in both groups already after one month. Significant differences in pain or QoL between the two treatment groups were not found. The placebo response was notable (Kaviani et al. 2008).

2.7.4 Diuretics

Women with cyclic mastalgia often report breast swelling and abdominal bloating in the luteal phase of the menstrual cycle (Mansel 1994). In a normal breast, the mean total change in volume has been shown to be 100 ml. During normal ovulatory cycles, the smallest breast volumes were found on cycle days 9-17, with a steep rise up to day 25, followed by a subsequent gradual decrease up to and during the menstruation (Milligan et al. 1975). However, studies measuring total body water have shown no difference between mastalgia patients and controls (Preece et al. 1975). This is consistent with the observation that diuretics are of no value in the treatment of mastalgia in general (Bundred 2007).

There is conflicting evidence as to the efficacy of the aldosterone antagonist spironolactone administered during the luteal phase. Burnet et al. (1991) reported in a trial of 69 women that spironolactone 100 mg daily from day 5 to day 25 of the menstrual cycle did not improve any of the premenstrual symptoms when compared with placebo. However, in another double-blind, placebo-controlled crossover study of 35 women, treatment with luteal phase spironolactone 100 mg a day was associated with an improvement in PMS symptoms as judged by a significant decrease in negative mood symptom scores and somatic symptom scores. Spironolactone was found to improve irritability, depression, feeling of swelling, breast tenderness and food craving significantly when compared with placebo.

The authors concluded that the therapeutic effect of spironolactone is not related to its diuretic action, but rather to its direct interaction with the progesterone metabolites on the central GABAa receptor (Wang et al. 1995).

For patients with severe PMS symptoms, a pharmacological intervention is indicated. There are two general pharmacological strategies for the treatment of PMS/PMDD. One involves the elimination of hormonal cyclicity by a suppression of ovulation, and the other targets central nervous system processes (Halbreich et al. 2006). In a survey by Lete et al. (2011), about 60 % of women who had sought help for PMS had received pharmacologic treatment, usually hormonal contraceptives, followed by analgesics and anti-inflammatory agents. One fifth of women were not treated because their physicians considered that the symptoms were not important and would disappear spontaneously. The specific treatment should be targeted according to the symptom profile of the patient (Halbreich et al. 2006).

2.7.5 Endocrine treatment

2.7.5.1 Selective serotonin reuptake inhibitors and other serotonergic agonists

Selective serotonin reuptake inhibitors (SSRI)s have been shown to be effective and potentially acceptable in the treatment of severe PMS. Their long term safety has been demonstrated in studies on affective disorders. The clinical efficacy of SSRIs is reached not until after 4-8 weeks in psychiatric disorders (Wikander et al. 1998). In PMS, however, they may become effective within a few days, and in most cases within one menstrual cycle after starting the treatment (Steiner et al. 1995). During luteal phase intermittent treatment with paroxetine, the participants experienced a sustained reduction in irritability as early as 14 hours after drug intake. When the different cycles were compared, the difference was significant at the third treatment day. The rapid onset of the efficacy of SSRI treatment allows for the possibility of intermittent or semi-intermittent dosing regimens to induce a temporary luteal-phase increase in serotonin concentrations (Landén et al. 2009).

SSRIs such as fluoxetine (Su et al. 1997), sertraline (Halbreich et al. 2002), paroxetine (Yonkers et al. 1997), and citalopram (Wikander et al. 1998) have been shown to be effective in the treatment of PMS/PMDD. Mental symptoms usually respond markedly. However, the effects on somatic symptoms are more variable. The degree of improvement with respect to somatic symptoms may depend on the compound and regimen (Table 6). Steiner et al. (2001) have proposed that as a result of mood symptom improvements, patients may also perceive somatic

symptoms as less problematic. Reduced libido, insomnia, nausea, gastrointestinal disturbances, and fatigue were the most commonly reported side-effects of the SSRIs (Wikander et al. 1998, Pearlstein et al. 2005b).

Table 6.   Response of the breast and other somatic complaints to different serotonergic agents. Only placebo-controlled trials are shown.

Study	Agent		Breast pain / other somatic complaint
Landén et al. (2007)	paroxetine 10–20 mg	continuous	improved
Yonkers et al. (1997)	sertraline 50–150 mg	continuous	not effective
Freeman et al. (1999)	sertraline 50–150 mg	continuous	not effective
Halbreich et al. (2002)	sertraline 50–100 mg	continuous	not effective
Freeman et al. (2004)	sertraline 50–100 mg	luteal phase continuous	improved not effective
Su et al. (1997)	fluoxetine 20–60 mg	continuous	improved
Steiner et al. (2001)	fluoxetine 20 mg or 60 mg	continuous	improved
Miner et al. (2002)	fluoxetine 90 mg	single dose/twice luteal	not effective
Cohen et al. (2002)	fluoxetine 10 mg fluoxetine 20 mg	continuous continuous	not effective improved
Wikander et al. (1998)	citalopram 10 mg–30 mg citalopram 10 mg–30 mg citalopram 5 mg–30 mg	continuous luteal phase semi-intermittent	not effective not effective not effective
Freeman et al. (2001)	venflaxine 50–200 mg	continuous	improved
Sunblad et al. (1992)	clomipramine 25–75 mg	continuous	improved
Sunblad et al. (1993)	clomipramine 25-75 mg	luteal phase	not effective

Sinikka Oksa

2.7.5.2 Progesterone

Although Euhus et al. (1997) found that medroxyprogesterone acetate effectively suppressed cyclic mastalgia in a survey among 671 case subjects and 1,433 controls; most of the studies on progesterone and mastalgia have been negative. Maddox et al. (1990) randomly allocated women with the cyclic breast pain to oral medroxyprogesterone acetate 20 mg a day or placebo, given during cycle days 10 to 26 for three months, and then crossed over for the remaining three months, but they found no significant pain relief. Pye et al. (1985) also found progestagens ineffective in women with either cyclic or noncyclical mastalgia. In another randomized, controlled trial, women were randomized to daily applications of 1 % progesterone or placebo cream from cycle day 10 to the beginning of the next cycle, for three months. The locally applied progesterone cream was not found to be better than placebo in relieving breast pain (McFadyen et al. 1989). Oral progesterone has not been found to have any clear beneficial therapeutic effect either (Freeman et al. 1995).

2.7.5.3 Oral contraceptives

Studies prospectively comparing combined oral contraceptives with placebo in the treatment of premenstrual symptoms have shown some benefit. Although combined oral contraceptives have been reported to reduce physical symptoms, they do not always reliably improve mood symptoms. Negative mood symptoms, similar to those occurring in women with PMS, are well known adverse effects of some combined oral contraceptives (Walker and Bancroft 1990). The use of oestrogens may be associated with breast tenderness, bloating and nausea, which often cause women to discontinue oral contraceptives. Studies investigating premenstrual breast complaints and different oral contraceptives are summarized in Table 7. The use of a combination of drospirerone and ethinyloestradiol combination or desogestrel as a single agent has, respectively, been shown to improve premenstrual symptoms and breast pain.

Table 7.   Studies with oral contraception and premenstrual symptoms.
                (EO=ethinyloestradiol, DRSP=drospirenone)

Study	Treatment groups	Outcome
Graham and Sherwin 1992	EO 35 µg and triphasic norethisterone vs. placebo	No differences between groups
Foidart et al. 2000	EO 30 µg + DRSP 3 mg vs. EO 30 µg + desogestrel 150 µg	EO+DRSP better
Abraham et al. 2003	Triphasic vs. monophasic pill vs. no hormonal contraception	No differences in mastalgia
Apter et al. 2003	EO 30 µg + DRSP 3 mg	Breast tenderness improved
Sangthawan and Taneepanichskul 2005	EO 30 µg + DRSP 3mg vs. EO 30 µg + levonogestrel 150 µg	Fewer premenstrual symptoms
Pearlstein et al. 2005a	EO 20µg +DRSP 3 mg vs. placebo	Improved physical symptoms
Yonkers et al. 2005	EO 20µg + DRSP 3 mg vs. placebo	Improved physical symptoms
Schultz-Zenden and Boschitsch 2006	EO 30 µg + DRSP 3 mg	Mastalgia improved
Ahrendt et al. 2007	Desogestrel 75 µg	Most breast complaints dissappeared or improved
Machado et al. 2010	EO 30 µg + DRSP 3 mg continuous EO 30 µg + DRSP 3 mg cyclic	No change Mastalgia improved

2.7.5.4 The levonorgestrel-releasing intrauterine system

The levonorgestrel-releasing intrauterine system (LNG-IUS) is a well tolerated and cost-effective treatment for menorrhagia (Hurskainen et al. 2004). Two trials have reported the effects of LNG-IUS use on premenstrual symptoms. One of them was a randomized, controlled trial in 236 women suffering from menorrhagia. The participants were randomized to be treated either by hysterectomy (n= 117) or LNG-IUS (n=119). In the LNG-IUS group, the levels of most premenstrual symptoms decreased significantly from baseline to six months. Breast tenderness, however, did not decrease significantly either from baseline to 6 months or from 6 to 12 months. When the data were analyzed from baseline to 12 months the decrease in mastalgia also became significant. Breast tenderness was reported as a new symptom by 15 women in the LNG-IUS group and by six

of the hysterectomized women (Leminen et al. 2012). Another trial (Barrington and Bowen-Simpkins 1997) also reported a positive effect of LNG-IUS use on PMS symptoms.

2.7.5.5 Dopamine agonists

Dopamine agonists that reduce levels of PRL such as bromocriptine, lisuride and cabergoline have been shown to be effective in the treatment of cyclic mastalgia. Mansel and Dogliotti (1990) reported that bromocriptine 2.5 mg twice daily over six months significantly reduced breast pain, tenderness and heaviness when compared with placebo. During the bromocriptine treatment, serum levels of PRL decreased. The benefits of bromocriptine over placebo persisted for up to six months after the cessation of treatment despite serum PRL levels returning to previous baseline values. In some patients, breast pain did not return during the follow-up period, and these patients were considered to be cured. However, side effects like headache and dizziness resulted in a high drop-out rate (29 %) in this study. Bromocriptine has been reported to prevent exaggerated PRL secretion by the pituitary as a response to TRH in women with breast pain, when compared with no effects in healthy controls (Peters et al. 1985, Rea et al. 1997). The status of a patient's dynamic PRL release has been shown to predict her response to endocrine therapy (Kumar et al. 1985, Rea et al. 1997).

Pye et al. (1985) reported the positive results of a treatment with bromocriptine 2.5 mg in 47 % of cyclic mastalgia patients and in 20 % of patients with noncyclical mastalgia, respectively. A response to bromocriptine is usually seen within three months. The dose should be increased gradually to minimize side-effects. In placebo-controlled trials, a luteal phase treatment with bromocriptine has also been shown to improve non-breast related PMS-type symptoms such as abdominal tension, oedema, weight gain and irritability (Benedek-Jaszmann and Hearn-Sturtevant 1976, Meden-Vrtovec and Vujic 1992). Rea et al. (1997) reported that for patients with an abnormal PRL secretion after TRH stimulation, bromocriptine was more effective in cyclic mastalgia than in patients with a negative TRH test, but the mechanism of action in this setting is unclear. Mansel (1994) has recommended that women treated with bromocriptine should use an adequate mechanical rather than oral contraception. The side-effects of bromocriptine are common and dose-related, and they include nausea, vomiting, headache, dizziness, postural hypotension, and constipation (BeLieu 1994, Mansel 1994, Bundred 2007).

Lisuride 0.2 mg daily throughout the menstrual cycle significantly reduced mastalgia when compared with placebo over two months of treatment, and the resolution of pain also correlated with decreased PRL levels (Kaleli et al. 2001). Subsequently, cabergoline and bromocriptine were compared by Aydin et al.

(2010) in a randomized, open-label trial for the symptomatic treatment of cyclic mastalgia in 140 women. Bromocriptine was administered 5 mg daily to 61 patients and cabergoline 0.5 mg weekly to 67 patients during the luteal phase of the menstrual cycle. Cabergoline was found to be as effective as bromocriptine but with fewer side-effects. The maximum reduction in pain was achieved during the second month of treatment in both groups.

2.7.5.6 Danazol

Danazol is a synthetic androgen that binds to progesterone and androgen receptors. Response rates have been reported to be 70 % in cyclic mastalgia and 31 % in noncyclical mastalgia (Pye et al. 1985). Maddox et al. (1989b) have also shown a good clinical response to danazol therapy in patients with noncyclical mastalgia. The main factor limiting the use of danazol is the spectrum of side effects. Danazol is associated with weight gain, deepening of the voice, menorrhagia, and muscle cramps (Bundred 2007). Adverse effects can be avoided by reducing the dose of danazol to 100 mg daily or confining treatment to the luteal phase (Maddox et al. 1989b, O´Brien and Abdukhalil 1999). Wetzig (1994) reported a complete response rate of 67 % to a low dose of danazol daily with minimal side effects. O´Brien and Abdukhalil (1999) evaluated the efficacy and side-effects of danazol used only during the luteal phase for three menstrual cycles. They demonstrated effective relief of cyclic mastalgia with side-effects comparable to placebo. Konstostolis et al. (1997) compared three treatments in women with severe cyclic mastalgia over six menstrual cycles: danazol 200 mg daily, tamoxifen 10 mg daily, and placebo. Pain relief was achieved in 65 % of those receiving danazol, 72 % of those taking tamoxifen, and 38 % of those taking placebo, respectively, with an equal amount of side-effects in the danazol and tamoxifen groups.

2.7.5.7 GnRH analogues

"A reversible medical oophorectomy" or "a chemical menopause" can be accomplished using GnRH agonists. The agonist analogues of GnRH have been shown to be an effective treatment for PMS and PMDD (Freeman et al. 1997). Available agents include leuprolide, histrelin, goserelin and buserelin. The long-term use of GnRH agonists is hampered by the high cost and the treatment-associated hypo-oestrogenism, bone demineralization and increased cardiovascular risk (Halbreich et al. 2006). There is a widespread consensus that GnRH agonists should be reserved only for severe refractory mastalgia (Bundred 2007). In a randomized, controlled trial with 147 premenopausal women suffering from breast pain, it was found that goserelin injections significantly reduced breast pain when compared with placebo at six months. The women who received goserelin injections had

more side-effects (vaginal dryness, hot flushes, oily hair or skin, breast size reduction, and irritability) when compared with placebo (Mansel et al. 2004).

The risks can be reduced by a concomitant add-back therapy using oestrogen/progestin replacement therapy or tibolone (Bundred 2007). However, the add-back therapy may result in a recurrence of premenstrual symptoms (Leather et al. 1999). The use of tibolone is associated with a smaller risk of recurring symptoms, because it has no progesterone-like effects. The effectiveness of the combination of tibolone and leuprolide was evaluated in patients affected by a severe PMS. During two menstrual cycles, the patients received depot leuprolide acetate at a dose of 3.75 mg intramuscularly. Tibolone at a daily dose of 2.5 mg or placebo tablets were administered at the onset of vasomotor symptoms. All patients showed an improvement in their premenstrual symptoms. A significantly lower number of hot flushes was observed in the tibolone group (Di Carlo et al. 2001).

2.8   Selective oestrogen receptor modulators

There are two receptors for oestrogens, ORα and ORβ. The two receptors are coded by different genes and their tissue expression varies across organs. ORα has been found mostly in the epithelial cells of the acini and ducts of human breast. The expression of ORα is low (6 %–8 %) in premenopausal women. The expression of ORβ is more widespread (70 %–85 %), being detected in most of the epithelial cells throughout the breast but also in stromal cells (Li et al. 2010). Shaw et al. (2002) noticed that ORα was expressed at the lowest level during the luteal phase with no variations in the ORβ expression. On the contrary, ORα is the dominating subtype universally in the uterus, with only a weak immunostaining for ORβ (Pelletier and El-Alfy 2000). In the ovary ORβ is apparently localized to the granulosa cells whereas ORα is detectable in the surrounding thecal cells (Couse and Korach 1999, Pelletier and El-Alfy 2000). In cortical bone ORα is predominantly expressed, whereas in the cancellous bone ORβ shows higher levels of expression (Bord et al. 2001).

Drugs that exert their effect via an interaction with the oestrogen receptors are known collectively as selective oestrogen receptor modulators or SERMs. More than 70 SERMs belonging to five chemical groups have been described: triphenylethylenes, benzotiophenes, tetrahydronaphtylenes, indoles and benzopyrans. The SERMs are structurally diverse non-steroidal compounds that bind to oestrogen receptors and produce oestrogen agonist effects in some tissues and oestrogen antagonist effects in others. They are being evaluated for a number of oestrogen-related diseases, including post-menopausal osteoporosis, hormone-dependent cancers, and cardiovascular disease. Several compounds that exhibit a SERM profile are currently available for clinical use, including clomiphene, tamoxifen,

toremifene (which are triphenylethylenes), and raloxifene (a benzothiophene). Each compound has a specific profile in its target tissue effects, and this may differ between premenopausal and postmenopausal women. Clomiphene is the most widely used agent for the induction of ovulation (Goldstein et al. 2000). Tamoxifen is indicated both for the treatment and prevention of breast cancer. It may have additional effects on the cardiovascular and skeletal systems (Fisher et al. 2005). Toremifene is also used for breast cancer while potentially having even more favourable cardiovascular effects (Harvey et al. 2006). Raloxifene, on the other hand, is approved for the management of osteoporosis with data supporting positive effects on the cardiovascular system and breast tissue (Goldstein et al. 2000). Tamoxifen appears to have stimulatory effects on the uterus, especially the endometrium (Shibutani et al. 2000). A few subjective adverse events have been attributed to these agents, with hot flushes being the most common one. Similar to oestrogens, SERMs increase the incidence of venous thromboembolism (Harvey et al. 2006). The use of tamoxifen in large numbers of premenopausal women in the breast cancer prevention trials has increased familiarity with this medication outside the breast cancer indication (Tan-Chiu et al. 2003, Fisher et al. 2005, Cuzick et al. 2007).

2.8.1 Tamoxifen

Tamoxifen is a triphenylethylene compound (Figure 3) that has been widely used for breast cancer, both in the therapeutic setting and in the chemopreventive setting, for healthy women at high risk for this disease (Fisher et al. 1998). The transcriptional activity of ORα,β is mediated by two activation functions (AFs). AF-1 is located in the amino-terminal and AF-2 in the carboxyl-terminal portion of the molecule (Smith 1998). Oestradiol-17β can function as an agonist in all environments regardless of whether AF-1 or AF-2 is the dominant activator. In contrast, compounds such as tamoxifen inhibit AF-2 activity, and consequently function as antagonists in all environments where AF-2 is required. In contexts where AF-1 is the dominant activator, on the other hand, tamoxifen manifests partial agonist activity. These observations provide at least a partial explanation for the tissue-selective biological activity exhibited by tamoxifen. Moreover, tamoxifen appears to be more anti-oestrogenic when complexed with ORβ than with ORα (Hall and McDonnell 1999).
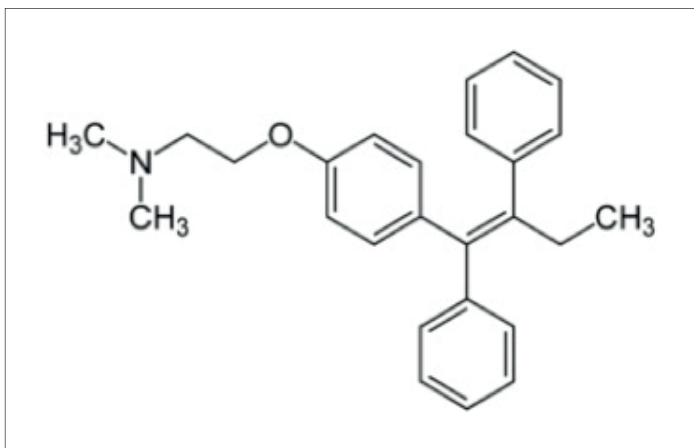
Figure 3.   Structural formula of tamoxifen

2.8.2 Treatment of mastalgia with tamoxifen

Fentiman et al. (1988) randomly allocated 60 women with mastalgia to receive tamoxifen at a dose of either 10 mg or 20 mg daily for either three or six months. The patients had self-rated the presence of a moderate or severe mastalgia over at least six months, with a therapy-free interval of at least three months prior to the study. A response was achieved in 90 % of those receiving 10 mg and in 86 % of those given 20 mg, respectively. Almost half of the patients had, however, a relapse usually within 2-3 months of discontinuing the treatment. The relapse rate was similar for both dosages and durations of the treatment. Side-effects were reported less frequently by patients receiving 10 mg than by those receiving 20 mg (21 % vs 64 %; P<0.001). A prolongation of the treatment from three months to six months did not materially improve the response rate. Tamoxifen was more effective in the relief of cyclic than noncyclical pain, a response in 94 % and 56 %, respectively. Due to the high relapse rate, longer courses of therapy should be considered, although the safety of a more protracted treatment has yet to be determined.

Messinis and Lolis (1988) conducted a randomized, placebo-controlled, trial on 34 women suffering from severe premenstrual mastalgia. The patients were randomized to either placebo or tamoxifen at a dose of 10 mg from cycle day 5 to 24 for six consecutive cycles. In the tamoxifen group, 89 % of the women reported relief of the symptoms, while in the placebo group, 38 % reported a partial improvement (P<0.001), respectively. In the placebo group, severe mastalgia

reappeared in all participants when evaluated at six months after the completion of the treatment. On the other hand, over half of the women in the tamoxifen group were still symptom-free at that time. No significant side effects of tamoxifen were reported. Faiz and Fentiman (2000) have proposed a treatment regimen starting with a daily dose of 10 mg for three months, which should be reviewed and titrated, with the option of either reducing to alternate days or increasing the dose to 20 mg before a further review.

The safety and efficacy of two doses of a topical gel containing 4-hydroxy-tamoxifen was compared with a placebo gel for the treatment of moderate to severe cyclic mastalgia. A total of 130 women were randomized to receive either placebo, or 2 mg or 4 mg of 4-hydroxytamoxifen daily for four menstrual cycles. The higher dose resulted in statistically significant improvements in the signs and symptoms of cyclic mastalgia across the patient- and physician-rated scales. The gel was well tolerated and there were no changes in the menstrual pattern (Mansel et al. 2007).

As mentioned above, Kontostolis et al. (1997) evaluated the benefits and side-effects of tamoxifen 10 mg during cycle days 5 to 24 or danazol 200 mg daily compared with placebo. All patients had severe mastalgia. The success rates were 72 % in tamoxifen group, 65 % in danazol group and 38 % in placebo group, respectively. Both tamofixen and danazol were significantly more effective than placebo, while tamoxifen was even more effective than danazol (P≤0.001). Hot flushes were the most common complaint during tamoxifen treatment (25 %) and weight gain during danazol treatment (31 %), respectively. One year after the end of the trial, 53 % of the women in the tamoxifen group, 37 % in the danazol group (P<0.001vs. tamoxifen), and none in the placebo group were symptom-free.

A randomized, double-blind study was performed on 43 premenopausal women with fibroadenoma of the breast. Treatment with tamoxifen (10 mg or 20 mg a day) or placebo was started on cycle day 1 and a lumpectomy was performed on day 22. The breast tissue samples were processed for routine histology and an immunohistochemistry for the detection of proliferating cell nuclear antigen (PCNA). The percentage of cells expressing PCNA was significantly higher in the group receiving placebo (50.3 %) than in the groups receiving tamoxifen 10 mg (24.1 %) or 20 mg/day (23.2 %), respectively (P<0.001). The serum levels of progesterone, oestradiol and sex hormone binding globulin (SHBG) were significantly higher in both tamoxifen-treated groups when compared with the placebo group. Increasing concentrations of FSH and lower levels of PRL were only found in the group receiving 20 mg/day of tamoxifen (Bernardes et al. 1999). Tamoxifen reduced the expression of PCNA both in the epithelium and in the stroma of the fibroadenoma in the higher dose group (Bernardes et al. 2003).

In another fibroadenoma study, tamoxifen 10 or 20 mg/day significantly reduced the expression of the monoclonal antibody MIB-1 compared with placebo, with no significant differences between the doses in terms of proliferative activity. A significant increase in progesterone (P=0.038), oestradiol (P<0.001), and SHBG (P=0.001) levels was found. An elevation of serum FSH concentration (P=0.0045) and a fall in PRL levels (P=0.0055) were also observed (de Sousa et al. 2001). Tamoxifen at a dose of 10 mg/day during the luteal phase had also previously been shown to increase serum progesterone and oestradiol concentrations (Tajima 1984). Viviani et al. (2002) on the other hand concluded that tamoxifen at a daily dose of 20 mg for 50 days can significantly reduce the volume of fibroadenomas. Tamoxifen treatment has also been demonstrated to reduce the incidence of clinically detected benign breast neoplasms (Tan-Chiu et al. 2003).

In a placebo-controlled trial with 7,152 women at an increased risk of breast cancer, the participants were randomly assigned to take either tamoxifen 20 mg/day or placebo for five years, respectively. The reduction of breast density was greater in younger premenopausal women and in women with a low body mass index (BMI) among whom the baseline breast density values were typically higher (Cuzick et al. 2004). Tamoxifen, when administered only during the luteal phase, significantly reduces the nuclear volume and mitotic activity of the breast epithelium. This data demonstrates an oestrogen-antagonistic action of tamoxifen even when administered for short periods of time (Uehara et al. 1998). De Lima et al. (2003) studied the effects of low doses of tamoxifen (5 and 10 mg/day) for 50 days compared with the standard dose (20 mg/day) on breast biomarkers measured the normal breast tissue from premenopausal women. Differences in the expression of ORα, PgR, Ki-67, apoptotic bodies and mitotic indices between the different groups after treatment could be seen. The authors commented that a lower dose of tamoxifen could minimize the side-effects associated with treatment without affecting its chemopreventive activity in the breast.

Srivastava et al. (2007) conducted a meta-analysis on trials on mastalgia. Their study was restricted to randomized, controlled trials comparing bromocriptine, danazol, evening primrose oil and tamoxifen with placebo. Tamoxifen was associated with fewest side effects and the authors recommended it as a drug of choice in cyclic mastalgia.

In conclusion, tamoxifen seems to be an effective and reasonably well-tolerated treatment option for mastalgia. However, there is a consensus to limit the use of tamoxifen for benign indications, even under expert supervision with an appropriate non-hormonal contraception, to no more than six months at a time. This is because no long term data exists on a chronic use of luteal phase tamoxifen (Bundred 2007).

2.8.3 Toremifene

Toremifene, like tamoxifen, is a nonsteroidal triphenylethylene SERM that binds to the oestrogen receptors. It exerts either oestrogenic or anti-oestrogenic effects, depending on the end organ, dose, and duration of the therapy (Kallio et al. 1986). Toremifene is closely related to tamoxifen but differs from it in the substitution of a chlorine atom for a hydrogen atom in the ethyl group (Figure 4), resulting in a differing metabolism and a potentially more favourable toxicity profile (Hirsimäki et al. 2002, Gennari et al. 2012). The chemical formula of toremifene is C26H28ClNO and the molecular weight is 405.96 g/mol.
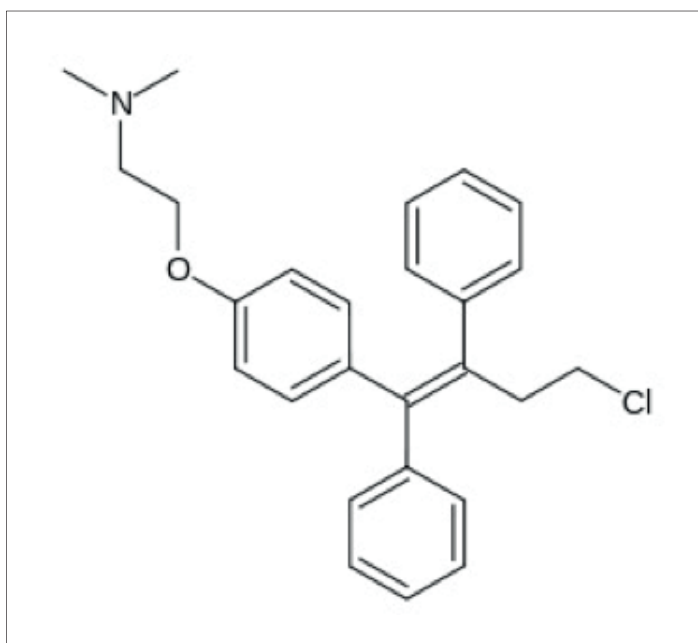

Figure 4.   Structural formula of toremifene

The half-life of toremifene in plasma is five days, and the drug is > 99% bound to plasma proteins. The main metabolites of toremifene are N-demethyl-toremifene and deaminohydroxy-toremifene (Mäenpää and Ala-Fossi 1997). Toremifene is highly metabolized in the liver and is eliminated primarily in the faeces following enterohepatic circulation (Gennari et al. 2012). Consequently, altered liver- but not kidney-function affects the pharmacokinetics of toremifene (Mäenpää and

Sinikka Oksa

Ala-Fossi 1997). Previous studies indicate that toremifene inhibits cell proliferation in both breast and endometrial tissues (Gershanovich et al. 1997) and acts as an anti-oestrogen in breast tissue, inducing apoptosis and inhibiting cells from entering into mitosis in human breast cancer cells, like tamoxifen (Wärri et al. 1993). Tamoxifen has been shown to be genotoxic and carcinogenic in experimental animals, and carcinogenic in humans (Williams et al. 1998). Toremifene has also been shown to be genotoxic, but to a far lesser extent (Hirsimäki et al. 2002). Many trials have confirmed that the efficacy of toremifene and tamoxifen in breast cancer is equivalent (Buzdar 1998, Pagani 2004). In a recent retrospective trial of 452 premenopausal women with breast cancer, the survival outcomes and side-effects of tamoxifen 20 mg and toremifene 60 mg were compared. The clinical efficacy and side effects of tamoxifen and toremifene were reported to be similar (Holli et al. 2000, Su et al. 2012). In a large, prospective, randomized trial of 1,813 women, Lewis et al. (2010) confirmed the similar efficacy and side-effect profile between toremifene 60 mg and tamoxifen 20 mg in early stage breast cancer.

The study of Gianni et al. (2009) confirmed previous findings of a lack of significant difference in endometrial cancer incidence, with 1.1 % and 1.0 % in toremifene and tamoxifen treated patients, respectively. Toremifene at a dose 40 mg/day has been reported to have a bone-preserving effect, but this is weaker than that of tamoxifen (20 mg/day) (Marttunen et al. 1998, Tiitinen et al. 2004). Toremifene seems to have a more favourable effect on the lipid profile than tamoxifen. In a study of Saarto et al. (1996), toremifene 60 mg/day increased the concentration of high density lipoprotein cholesterol (HDL-c) significantly more than tamoxifen 20 mg/d in postmenopausal patients with breast cancer. Toremifene also increased the apolipoprotein (apo) A-I level and apo A-I-to-A-II ratio. In another study, HDL-c levels in the toremifene group began to increase in the third month of use and continued to increase for two years, at which point the increase was 18.0 % (Tominaga et al. 2010). Toremifene improves overall lipid profiles, particularly as a potent HDL-C enhancer (Christodoulakos et al. 2006). Sawaki et al. (2011) reported that during toremifene treatment the intracellular concentrations of triglyceride remain unchanged, while conversely, during tamoxifen treatment the triglyceride levels increase. Tominaga et al. (2010) also reported that toremifene showed better effects on overall lipid profiles when compared with tamoxifen.

Most of the adverse effects of toremifene are related to its activity at oestrogen receptors and include hot flushes, vaginal discharge and nausea. Although toremifene decreases antithrombin III levels slightly, the incidence of thromboembolic complications is low (Mäenpää and Ala-Fossi 1997).

Literature on the use of toremifene in the treatment of mastalgia is discussed later in comparison to the findings of the present study.

2.8.4 Side effects of SERMs

Holli et al. (2000) reported no major differences between tamoxifen and toremifene in the frequency or severity of subjective side effects, and both were generally well tolerated. Tamoxifen was given at a dose of 20 mg daily and toremifene at 40 mg daily. Sweating (in 53.8 % of the toremifene-treated and in 51.1 % of the tamoxifen-treated patients) and hot flushes (51.6 % versus 47.5 %, respectively), were the most common side effects, but they frequency diminished with the continuation of therapy, and approximately only half of the symptom occurrences may be estimated to have been caused by the therapy. Vaginal dryness was found in 26.1 % of patients in the toremifene group and in 26.6 % of those in the tamoxifen group. The only significant difference between the drugs was a higher incidence of leucorrhoea in the patients treated with toremifene (42.0 % versus 35.5 %, P=0.05). The incidence of thromboembolic events was slightly more common in the tamoxifen group than in the toremifene group, but the difference was not significant (5.9 % versus 3.5 %, respectively). Tamoxifen and toremifene have been reported to cause similar vasomotor and vaginal symptoms (Marttunen et al. 2001, Pagani et al. 2004, Lewis et al. 2010). Ocular toxicity due to either toremifene or tamoxifen seems rather rare (Gianni et al. 2006, Parkkari et al. 2003).

Besides the significant benefits, long-term tamoxifen treatment increases the risk of developing endometrial cancer (Van Leeuwen et al. 1994). Endometrial thickness significantly increases during the course of treatment with either drug, with no differences between tamoxifen and toremifene (Tomás et al. 1995, Hachisuga et al. 2005). However, toremifene has been reported by Shibutani et al. (2001) to be less genotoxic than tamoxifen. The level of tamoxifen adducts found, coupled with the previous demonstration of their mutagenicity, suggests that a genotoxic mechanism may be at least partly responsible for tamoxifen-induced endometrial cancer. According to Hachisuga et al. (2005), K-ras mutations correlate with the phenotypic progression from atypical hyperplasia to endometrial cancer. K-ras mutations were found more often in tamoxifen-treated endometrium samples, than in toremifene-treated samples. A high incidence of mutations in codon 12 of the K-ras gene was also found in endometrial polyps during the tamoxifen treatment (Hachisuga et al. 2003). However, Wallén et al. (2005) concluded that the significance of the K-ras changes during SERM remains to be elucidated.

2.9 Non-responders to mastalgia treatment

Drug treatment is more beneficial in cyclic mastalgia than in noncyclical mastalgia (Tavaf-Motamen 1998). Although noncyclical pain responds poorly to treatment, it tends to resolve spontaneously in half of women (Gateley and Mansel 1991, Bundred 2007). A group of mastalgia patients exist which do not respond to any therapy. The management of a patient with mastalgia who fails to respond to first line therapy is problematic. Gateley et al. (1990a) studied, in an uncontrolled trial, mastalgia patients (n=126) who failed to respond to first line therapy. With cyclic mastalgia, the response rate to second line therapy was 57 %, and to third line therapy 25 %, and the equivalent figures for noncyclical mastalgia were 24 % and 21 %, respectively. They failed to identify which patients would respond to therapy. Danazol may be used as a salvage treatment after the failure of other drugs, whereas the second line response to bromocriptine and evening primrose oil is poor (Gateley et al. 1992b). However, no randomized, controlled trials of second and third line therapies have been found.

2.10 Surgery and acupuncture

2.10.1 Breast surgery

Breast surgery has no role in the management of breast pain in the absence of a dominant mass. Even when the pain appears to be localized, an excision is practically never therapeutic (Morrow 2000). Some women may even develop de novo breast pain at the site of previous breast operations (Mansel 1994). In a retrospective study of 282 women, it was found that the use of breast implants for augmentation, and in particular the submuscular placement of implants, was associated with increased pain (Wallace et al. 1996).

2.10.2 Hysterectomy

In a study by Davies et al. (1998), hysterectomy improved symptoms in 33 % of patients with cyclic mastalgia. Surprisingly, whether they also had a bilateral salpingo-oophorectomy did not seem to make a difference. Very little data is available on the effect of hysterectomy combined with the bilateral oophorectomy on severe PMS. Casson et al. (1990) purported that the operation has a favourable effect based on a small study of 14 patients with physical and psychological PMS symptoms. In another small study of 14 women, Casper and Hearn (1990) also reported positive postoperative effects. When medical treatments have failed

and the patient has completed her family size, the operation has been suggested as a suitable course of action. Cronje et al. (2004) evaluated the effectiveness and patient satisfaction with hysterectomy and bilateral oophorectomy with a subsequent oestrogen replacement therapy in 47 women suffering from severe PMS. The median age of the patients was 42 years. Seven women were younger than 40 years and two women were under 30 years of age. The patients had suffered from symptoms for nearly ten years and had received different treatments for approximately six years before the operation. Only two women expressed any dissatisfaction postoperatively. A total relief of symptoms was reported by 93.6 % of these women.

As mentioned above, even hysterectomy alone without salpingo-oophorectomy has been found to decrease PMS symptoms in women treated for menorrhagia. In a randomized, controlled trial of 236 women, all investigated symptoms of headache, breast tenderness, irritability, fatigue and swelling were alleviated significantly from baseline to six months in the hysterectomy group (Leminen et al. 2012). Other trials have also reported reductions in premenstrual symptoms after hysterectomy (Braiden and Metcalf 1995, Osborn and Gath 1990).

2.10.3 Acupuncture

Acupuncture can be considered as an effective treatment modality for PMS (Habek et al. 2002). The most obvious changes were observed in myalgia, mastalgia and dysmenorrhoeal complaints among PMS patients (Anil 2012). Acupuncture has also been found to be an effective treatment option in noncyclical breast pain. Pain scores decreased significantly in a study with 37 patients with noncyclical pain (Thicke et al. 2011).

3 AIMS OF THE STUDY

The present study was undertaken to evaluate the efficacy of toremifene in premenstrual mastalgia, and the factors underlying the mechanism of its action in this indication. The specific aims were:

1. To investigate the efficacy and tolerability of toremifene when compared with placebo, administered during the luteal phase for premenstrual mastalgia (I).

2. To detect any measurable changes in hormonal profile during toremifene or placebo medication (II, III).

3. To investigate the mammary effects of cyclically administrated toremifene in cyclic mastalgia by dynamic contrast-enhanced MRI (IV).

4. To detect breast vascular and volume changes attributable to toremifene treatment as compared with an untreated cycle by 3D power Doppler ultrasound (V).

4   PATIENTS AND METHODS

4.1   Patients

Women with marked premenstrual mastalgia were recruited to the Study I by means of either newspaper advertisements or referral from their general practitioner (GP) or hospital clinician, followed by a brief telephone interview. The participants were recruited between April 2000 and December 2002 from Pori, Tampere and their neighbouring areas in Finland. After a telephone pre-screening, 62 women met the inclusion criteria and were followed up for one baseline cycle. The telephone pre-screening eliminated women using hormonal contraception or treatment; those reporting irregular menstrual cycles, a history of breast or endometrial cancer, radiation, hysterectomy and/or bilateral oophorectomy, pregnancy, lactation, or a desire for pregnancy; and those with serious health problems. Women with a history of thromboembolic disease were also excluded (I–V); and were those with an artificial cardiac pacemaker or metallic prostheses (Study III).

Thirty-two participants were randomized to receive toremifene and 30 to receive placebo for the first three subsequent menstrual cycles (Study I). Six women (three from each group) were excluded from the analysis: three discontinued the trial for personal reasons; one had no symptoms anymore; for one participant the study instructions were too difficult to follow; and one had menstrual disturbances before starting the medication (Figure 5). The remaining 56 participants provided complete data for the final evaluation.
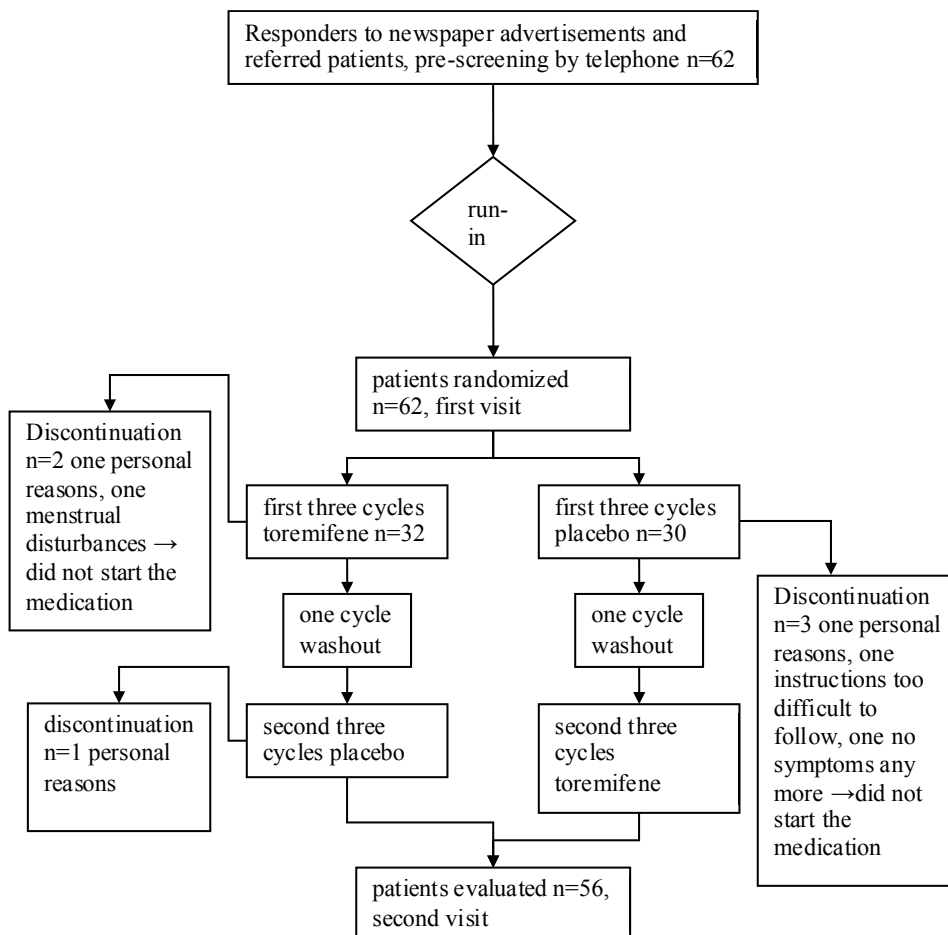
Figure 5.   Flow of the participants through the study I.

The venous blood samples for Studies II and III were obtained from the same study population as in study I. Only those participants, (48 women), who gave a blood sample at all measuring points were included to Studies II and III.

Ten women with marked cyclic mastalgia were recruited to Study IV between March and May 2007 by means of newspaper advertisements or referral from their gynaecologist or GP. Study V was performed on twenty healthy premeno-pausal women from Pori and neighbouring areas from August to December 2010.

The inclusion criteria for Studies I–IV were: 1) mastalgia lasting for more than 5 days during the luteal phase of the menstrual cycle and severe enough to interfere with social life and to cause a desire to use medication for it; 2) at least a

three-month history of symptoms; 3) an age between 25–45 years (Studies I–III), or 20–45 (Study IV); and 4) regular menstrual cycles. The participants in Study V were healthy volunteers, with the inclusion criteria of an age of 20–45 years and use of reliable non-hormonal contraception. In all studies, the women, except those with a history of surgical sterilization, were advised to use non-hormonal contraception until the first post-treatment menstrual period. The main characteristics of the study population and design of the studies are summarized in Table 8.

Table 8.   The main characteristics of the study population and study design.

Study		Number of patients	Mean [median] age/years	Mean weight kg,[BMI]	Number of parous patients	Design
I	Placebo first	30	40.4	68.3	27	Randomized, controlled crossover
	Toremifene first	32	40.8	69.6	28	Randomized, controlled crossover
II		48	[42]	67.8	37	Randomized, controlled crossover
III		48	[42]	67.8	37	Randomized, controlled crossover
IV		10	42	70.0	6	Randomized, controlled crossover
V		20	[39.5]	[23.9]	16	Prospective

4.2 Study medication

After a single non-medicated baseline menstrual cycle, the women were randomly allocated to receive either toremifene 20 mg daily or placebo during the luteal phase of the cycle for three consecutive cycles. After an untreated washout period lasting the length of one menstrual cycle, the participants were crossed over to placebo or toremifene, respectively, for three additional cycles. The study medication was given in a tablet form, and the participants were instructed to take one tablet daily from cycle day 15 until the next menstruation (Studies I–IV).

The active (toremifene 20 mg) and control (placebo) tablets were identical, white and round in appearance, and supplied in identical bottles that were labelled with the patient number and the appropriate period of the study. A computerized block-randomization system was allocated to ensure an even distribu-

tion of toremifene and placebo. The system was independent of the investigators. The codes were kept confidential until the end of the study. All participants and study investigators were blinded as to the randomization codes, and the screening and the follow-up study assessments were completed by study personnel blinded to the randomization code. Orion Pharma (Espoo and Turku, Finland) provided the double blinding and the dispensing of medication (Studies I–IV).

In study V, following a single non-medicated baseline menstrual cycle, the participants received during the next cycle 20 mg toremifene in tablet form from cycle day 15 until the second breast examination. The first breast examination had taken place during the non-medicated cycle. The compliance of the participants was assessed by counting the returned tablets at the end of each study.

4.3 Assessments

4.3.1 Pain and QoL (I, IV)

In Studies I and IV, the intensity of mastalgia was self-assessed with a numeric rating scale (0–10) VAS by the participants. They completed a breast pain VAS at the late luteal phase of the baseline cycle and at the luteal phase of each subsequent menstrual cycle until the end of the trial. The days of the menstruation were recorded during the study by the participants. The QoL scores were recorded from the late luteal phase of the baseline cycle and also from the third and sixth treatment cycles. A modified 36-item Finnish Depression Scale (Salokangas et al. 1995), with a score ranging from 0 to 108, was used to estimate the QoL. In the original study of Salokangas et al. (1995) the incidence of depressive symptoms rose sharply with rising scores.

The first visit included a detailed history, and the inclusion and exclusion criteria were also checked. The participants underwent a careful clinical examination at baseline and at the end of the study, including a vaginal ultrasound examination.

4.3.2 Laboratory assays (II–III)

In Studies II and III, venous blood samples for the analysis of serum hormone levels were obtained on three occasions during the luteal phase (cycle days 20 - 22) of the menstrual cycle, at baseline, and during the third cycle of toremifene and placebo treatment, respectively. Samples were collected from each subject while fasting, and the serum was separated and frozen at -20 °C until assayed.

In Study II, the assays were performed at Medix Laboratories Ltd, Espoo, Finland. In Study III the assays for serum inhibin B were performed at the laboratory of the University Hospital of Helsinki, Helsinki, Finland and the assays for inhibin A at Limbach laboratories, Heidelberg, Germany. The reference ranges of the hormones assayed during the luteal phase, detection limits, laboratory methods, interassay coefficients of variation, and the test manufacturers are shown in Table 9.

Table 9.   Laboratory assays. *Enzyme linked immunosorbent assay.

Hormone	Reference range for luteal phase	Detection limit	Method	Interassay coefficients of variation	Manufacturer
Oestradiol nmol/L	0.25–0.90	0.020	competitive radioimmunological	10 % low/ 9 % medium 8 % high	DiaSorin S.p.A Saluggia, Italy
FSH IU/L	2.0–8.0	0.1	automated time-resolved immunofluorometric assay	3 % low and medium 2 % high	AutoDELFIA, PerkinElmer Turku, Finland
PRL mU/L	70–500	9	automated time-resolved immunofluorometric assay	4 % low, medium, and high	AutoDELFIA, PerkinElmer Turku, Finland
Progesterone nmol/L	12–69	1	automated time-resolved immunofluorometric assay	6 % low/ 2 % high	AutoDELFIA, PerkinElmer Turku, Finland
Androstenedione nmol/L	3.0–11.0	0.8	in-house competitive 3H-radioimmunological	15 % low/ 13 % medium 10 % high	Antiserum by INC Biomedical Inc. CA,USA
Total testosterone nmol/L	<2.8		automated competitive luminoimmunological		ADVIACentaur, Bayer Cooperation NY, USA
Free testosterone pmol/L	2–20		radioactive (5 α-dihydroxyl[1,2,4,5,6,7-3H] testosterone, directly as %		Pharmacia Biothec England
Inhibin A pg/mL	13–160	10	two-site ELISA*	3.3 % low/ 4.6 % high	Diagnostic System Laboratories Texas, USA
Inhibin B ng/L	5–200	16	two-site ELISA*	<12 %	Oxford Bio-Innovation Oxford, UK

4.3.4 Imaging (IV, V)

4.3.4.1 MRI

The MRI investigations were performed on both breasts during the premenstrual period, within 5 days prior to menstruation, on two occasions, on the third toremifene or placebo cycle, respectively.

The imaging was performed using Philips Gyroscan Intera 1.5 T equipment (Philips Medical Imaging, Best, the Netherlands). All sequences covered the whole of the breasts, except the T2 calculation single slice. After imaging, subtraction of the dynamic images was performed, and dynamic enhancement curves of the retromamillar glandular tissue were determined with a GE workstation (GE AWS 4.3; GE Medical Imaging, Milwaukee, Wisk., USA) using a circular region-of-interest (ROI) tool. The clinical assessment of the images and dynamic scans, and of the enhancement curves was performed according to BI-RADS lexicon for magnetic resonance (MR) mammography, released in November 2003 (American College of Radiology 2003). One experienced radiologist assessed the MR images and made the post processing analysis blindly, without any information regarding the symptoms or medication of the patient. The imaging was performed at Turun Tesla Vagus Oy, Turku, Finland.

4.3.4.2 3D

At baseline, the participants gave a detailed medical history, and underwent a careful physical examination, including a breast palpation and pelvic examination. Moreover, a vaginal 2D scan was taken using a Voluson 730 PRO device equipped with a transvaginal transducer 3.3–10 MHz (GE Medical Systems Kretztechnik GmbH & co, Zipf, Austria).

The breast 3D US examinations were performed within 5 days prior to menstruation, on two occasions, at the baseline and during the treatment phase using the Voluson i device with a relative stopping power index (RSP) 3D transducer; RSP 6 - 16 frequency of 5.6 - 18.4 MHz and rotational virtual organ computer aided analysis (GE Medical Systems Kretztechnik GmbH & co, Zipf, Austria). The probe was held still and the patients were asked to stop breathing for a few seconds to allow the ultrasound unit to generate the 3D data. The imaging was performed with the patient in the supine position with the ipsilateral arm extended overhead. The power Doppler setting was standardized with a twenty-degree volume angle and the maximum quality. The measurements were made using a customized breast programme preset. For both breasts, four quadrants were measured as shown in Figure 6. One side of the probe was placed on the edge of the areola and the other side was pointed towards eleven, two, five and seven o'clock.

The frame for the region of interest was adjusted by manual tracing to include skin at the top and fascia of the pectoral muscle at the bottom. The 3D volume mode was switched on. Manual drawing of the contour of each quadrant was performed in plane A. At the end of the 180° rotation, the built-in software calculated the volume, and the three vascularity indices, namely the vascularisation index (VI), the flow index (FI) and the vascularisation flow index (VFI) automatically. The imaging was performed by one investigator at Porin Lääkäritalo, Pori, Finland. All collected data were saved on the hard disc of the equipment for further analysis.

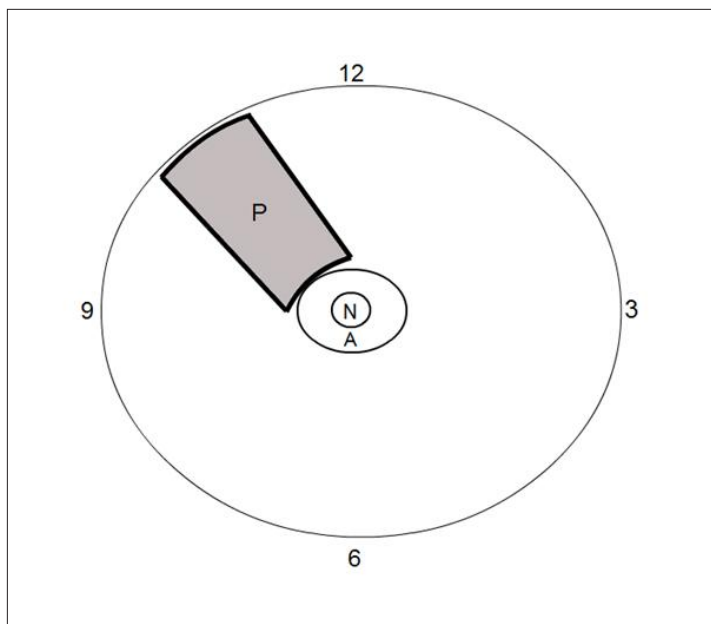## 4.4 Statistical analysis

Figure 6.   The position of the ultrasound probe (P) measurement on the upper lateral quadrant of the right breast at the 11 o'clock position (A, breast areola, N, nipple).

In Study I, it was estimated that the efficacy of placebo would be approximately 25 % in relieving premenstrual mastalgia. To be able to show a more than 33 % improvement in the efficacy of toremifene over placebo, 62 patients were needed (α = 0.05, power 80 %). The normality of the distribution of the continuous variables studied was tested by the Kolmogorov-Smirnov test. Treatment, carryover, and period effects of the VAS scores were calculated between the first and second treatment cycles in view of the crossover design. Differences between the baseline period and the placebo and treatment groups were tested by the Sign test due to the different shape of the distributions. Differences in quality-of-life scores in cycles between the placebo and toremifene groups, treatment, carry-over, and period effects were tested by the Mann-Whitney U test.

In Studies II and III, normality of the distribution of the continuous variables studied was tested by the Shapiro-Wilk's test. The distributions of all variables were skewed. Differences between baseline and toremifene and between placebo and toremifene, respectively, were tested by the Wilcoxon Signed Ranks test. The statistical analyses were performed by SPSS version 14.0.2, for Windows (SPSS Inc., Chicago, IL, USA).

Due to the small sample size, non-parametric exact tests were also used in Study IV. Differences between toremifene and placebo cycles were analyzed by the Wilcoxon signed-ranks test. The statistical analysis was performed using SPSS for Windows, version 16.0.2. (SPSS Inc., Chicago, IL, USA).

Due to the small sample size, nonparametric exact tests were also used in Study V. Differences between the toremifene and baseline cycles, and between the right and left sides, respectively, were analyzed by the related samples Wilcoxon signed-ranks test. Statistical analyses were performed by SPSS for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA).

In the statistical evaluations, p values less than 0.05 were considered statistically significant.

4.5   Ethical considerations and trial registration

The Ethics Committees of Tampere University Hospital and Satakunta Central Hospital approved the study protocols. Written informed consent was obtained from each patient before entering the study. Study IV was registered at Clinicaltrials.gov (identifier NCT00534846) as was Study V (identifier NCT01417754).

5 RESULTS

5.1 Pain (Study I, IV)

There were significant reductions in VAS scores in the toremifene and placebo groups after three treatment cycles. In the group where toremifene was given first (n=29), there was a significant reduction in mastalgia between the baseline cycle and the toremifene cycles (P=0.014). During the subsequent placebo cycles, breast pain reappeared (toremifene versus placebo, P=0.038; baseline versus placebo, P=0.441). Conversely, in the placebo first group (n=27), the pain scores continued to decline throughout the study (baseline versus placebo, P=0.035; toremifene versus placebo, P= 0.078; and baseline versus toremifene, P< 0.001) (Figure 7).
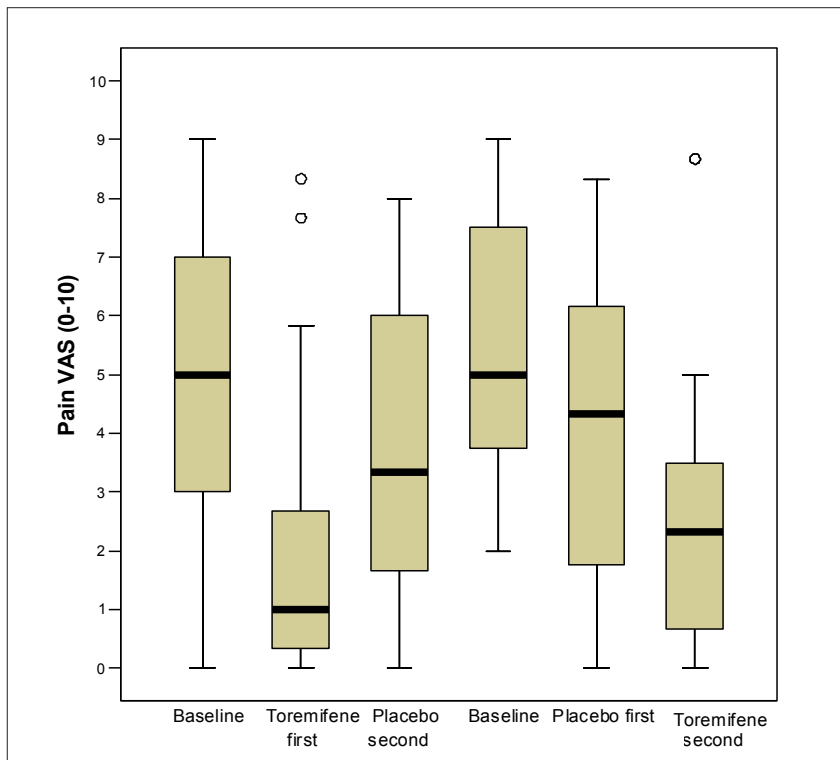

Figure 7.   The medians, quartile ranges and ranges of the pain VAS scores from the baseline cycles and the treatment cycles.

When all toremifene cycles were compared with all placebo cycles and with the baseline cycle, the median VAS scores were 1.8, 3.7, and 5.0, respectively (baseline versus toremifene, P<0.001; baseline versus placebo, P=0.034; and toremifene versus placebo, P=0.004). The reduction in median pain scores was 64 % in the toremifene-treated cycles compared with a 26 % reduction during placebo cycles. The treatment effect between the treatment cycles was statistically significant (P=0.001), with no significant carry-over effect (P=0.706) or period effect (P=0.876).

The medians, quartile ranges, minimum and maximum of pain VAS scores in the baseline cycles and the both treatment cycles are presented in Table 2 of original Study I.

In study IV, the median VAS scores during the placebo cycles were 6.33 and only 1.83 during the toremifene cycles (Table 10) but the difference was only of borderline significance (P=0.078).

Table 10.   Median, minimum and maximum pain VAS scores (IV).

	Baseline	Placebo	Toremifene
Median	4	6.33	1.83
Minimum	1	3	0
Maximum	9	9	8

5.2 Adverse events (Studies I, IV, V)

The days of menstruation and adverse events were recorded during the Studies by the participants. At the end of each Study, the participants were interviewed individually and study-related material was collected.

In Study I, adverse reactions were mild and evenly distributed between the groups. There were neither menstrual disturbances nor hot flushes during the treatment periods. Mild headache was reported by three women during the toremifene cycles and by two women during the placebo cycles. The corresponding figures for nausea were two and one, respectively.

There were not any reported adverse events during the treatment periods in Study IV. Two participants had mild nausea during the toremifene treatment cycle in Study V.

5.3   QoL (Studies I, IV)

The participants completed three QoL questionnaires, at the late luteal phase of the baseline cycle, and from the both treatment cycles. These questionnaires were collected at the end of the Studies.

In Study I, the QoL scores remained unchanged, and there were no significant differences between the scores of the run-in, placebo and toremifene cycles. The medians, quartile ranges, minimum and maximum of QoL scores in the baseline cycles and the both treatment cycles are seen in Table 11. The median QoL score appeared to be slightly lower during toremifene cycles when compared with the placebo cycles (Study IV). The difference was statistically significant (P=0.047).

Table 11.   The medians, quartile ranges, minimum and maximum of QoL scores during baseline cycles and two treatment cycles (Study I).

	QoL score	P value
Toremifene first		
Baseline	46 (14–83)	0.7*
Toremifene	38 (13–80)	0.327**
Placebo	42 (12–75)	0.7***
Placebo first		
Baseline	42 (20–64)	0.424**
Placebo	39 (8–62)	1*
Toremifene	40 (7–62)	0.69***

*Baseline compared with toremifene, **Baseline compared with placebo, ***Toremifene compared with placebo

In Study IV, the median QoL scores were 39.5 (range 18–66) during the toremifene cycles and 41 (range 31–68) during the placebo cycles. The difference was statistically significant (P=0.047).

5.4   Laboratory evaluations (Studies II, III)

Twenty-four women randomized to toremifene first and 24 women randomized to placebo first gave blood samples for analysis. Three patients had serum PRL values above normal at baseline and during the both treatments. One patient had an androstenedione level above normal at all measuring points. Three patients had both total and free testosterone values higher than normal. That all women were premenopausal and ovulatory was confirmed according to the baseline FSH and progesterone levels.

The median serum oestradiol and progesterone concentrations were significantly higher in the toremifene treatment cycles. The median PRL level 268 mU/L was significantly higher during toremifene treatment when compared with the baseline 222 mU/L. However, there was no significant difference between the toremifene and placebo treatment cycles, or between the baseline and placebo. The median concentrations of FSH, androstenedione, and total and free testosterone were similar at baseline and during the treatment cycles. All analyzed inhibin A and B levels were within normal range for premenopausal women. The median inhibin A concentration was 40 pg/mL during the toremifene cycles, while it was 38 pg/mL during the placebo cycles and 42 pg/mL at baseline, respectively. The median inhibin B level was during toremifene 17 ng/L, during placebo 20 ng/L and at baseline 19 ng/L, respectively. The medians of the serum hormone levels obtained on three occasions during the luteal phase (cycle days 20–22) of the cycle are presented in Table 12.

Table 12.   Serum luteal hormone levels (medians [Md] with interquartile ranges [Iq]). Differences between baseline and placebo (p1), between baseline and toremifene (p2) and between placebo and toremifene (p3).

Hormone	Baseline Md (Iq)	Placebo Md (Iq)	Toremifene Md (Iq)	p1	p2	p3
FSH IU/L	3.35 (2.20–4.30)	3.00 (2.15–4.25)	3.45 (2.75–4.20)	0.349	0.305	0.430
Oestradiol nmol/L	0.31 (0.24–0.41)	0.27 (0.21–0.34)	0.36 (0.29–0.43)	0.095	0.005	<0.001
Androstenedione nmol/L	5.50 (4.05–7.53)	5.95 (4.23–7.35)	5.95 (4.15–7.58)	0.996	0.121	0.479
PRL mU/L	222 (167–333)	242 (170–355)	268 (198–397)	0.157	0.046	0.583
Progesterone nmol/L	32.5 (21.3–45.0)	34.5 (24.8–46.5)	42.5 (29.5–65.0)	0.802	0.002	0.002
Total testosterone nmol/L	1.88 (1.59–2.26)	1.93 (1.50–2.31)	1.94 (1.62–2.39)	0.960	0.395	0.135
Free testosterone pmol/L	16.0 (13.0–22.8)	15.9 (13.0–19.0)	15.5 (12.0–21.2)	0.100	0.078	0.852
Inhibin A pg/mL	42 (27–53)	38 (15–67)	40 (19–62)	0.468	0.638	0.365
Inhibin B ng/L	19 (15–30)	20 (15–29)	17 (15–27)	0.519	0.983	0.880

5.5   Imaging (Studies IV, V)

Six participants had normal MRI findings, while two were found to have small benign fibroadenomas 7 and 6 mm in diameter, respectively. One patient discontinued her participation after the first appointment. The MRI data on two patients during the placebo cycles are incomplete. Patient no. 6 withdrew her consent after the first MRI because she felt claustrophobic during the imaging. The MRI data on patient no. 4 are missing due to technical problems with the MRI equipment during her appointment. Nine patients were assessable for the final evaluation.

The median enhancement ratio of the right breast during the toremifene versus placebo cycles was 8.50 % and 12.50 %, and for the left breast 7.50 % and 17 %, respectively. The median maximum slope of enhancement in the right breast during toremifene treatment versus placebo was 5.9 versus 6.7, and the corresponding figures for the left breast were 5.2 and 6.5, respectively. The difference for the left breast was statistically significant (P=0.047). The median T2 relaxation times varied between 55 and 79 ms with no differences between the treatment cycles. The dynamic MRI results are summarized in Table 13.

Table 13.   Results of the dynamic MRI

Patient number	Cycle	Enhancement ratio, right	Enhancement ratio, left	Max. slope of enhancement, right	Max. slope of enhancement, left	T2 relaxation time, right	T2 relaxation time, left
1	TOR	7 %	9 %	7.5 (3.8)	6.7 (3.9)	64 ms	63 ms
1	Placebo	10 %	19 %	6.6 (4.5)	6.6 (3.1)	69 ms	72 ms
3	TOR	12 %	10 %	5.9 (2.7)	4.7 (2.3)	68 ms	80 ms
3	Placebo	6 %	5 %	6.4 (2.6)	5.4 (2.8)	72 ms	79 ms
4	TOR	14 %	22 %	7.2 (3.9)	7.9 (4.7)	58 ms	58 ms
4	Placebo	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5	TOR	22 %	20 %	5.7 (2.0)	5.5 (1.9)	55 ms	65 ms
5	Placebo	20 %	22 %	7.6 (4.5)	9.5 (6.3)	66 ms	59 ms
6	TOR	6 %	6 %	9.9 (5.6)	6.2 (2.2)	71 ms	72 ms
6	Placebo	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	TOR	14 %	22 %	5.8 (2.4)	5.8 (3.1)	60 ms	64 ms
7	Placebo	26 %	32 %	7.5 (2.9)	6.4 (2.0)	63 ms	70 ms
8	TOR	3 %	3 %	4.3 (1.5)	3.7 (1.2)	72 ms	72 ms
8	Placebo	6 %	10 %	6.6 (3.8)	7.0 (2.7)	72 ms	71 ms
9	TOR	6 %	6 %	4.6 (2.2)	4.6 (1.9)	62 ms	69 ms
9	Placebo	18 %	15 %	6.8 (3.9)	5.6 (2.5)	67 ms	73 ms
10	TOR	10 %	6 %	6.2 (3.3)	4.8 (2.2)	72 ms	73 ms
10	Placebo	11 %	6 %	6.1 (2.3)	4.9 (2.1)	70 ms	71ms

All 20 women recruited were included in the final analysis in Study V. Thirteen women were using a barrier method for birth control, while 7 had been surgically sterilized. Nineteen women had normal vaginal ultrasound findings, while one patient was found to have a small benign submucosal myoma, 25 mm in diameter. All participants had normal breasts on palpation, and there were neither abnormal 2D nor 3D breast findings. The volume results from the four quadrants of both breasts were summarized. The combined median volumes of the both breasts tended to be slightly higher during the toremifene treatment compared with baseline (Figure 8), but the differences were not significant. The only significant difference was found between the combined median volumes between the right and left breast during the treatment cycle (P=0.009). The differences of the vascularisation indices VI, VFI, and FI from each of the four quadrants of both breasts were not significant between the baseline cycle and toremifene treatment cycle. The distributions, medians and ranges of the vascularisation indices with p-values are summarized in the Table 1 of the original publication (V).
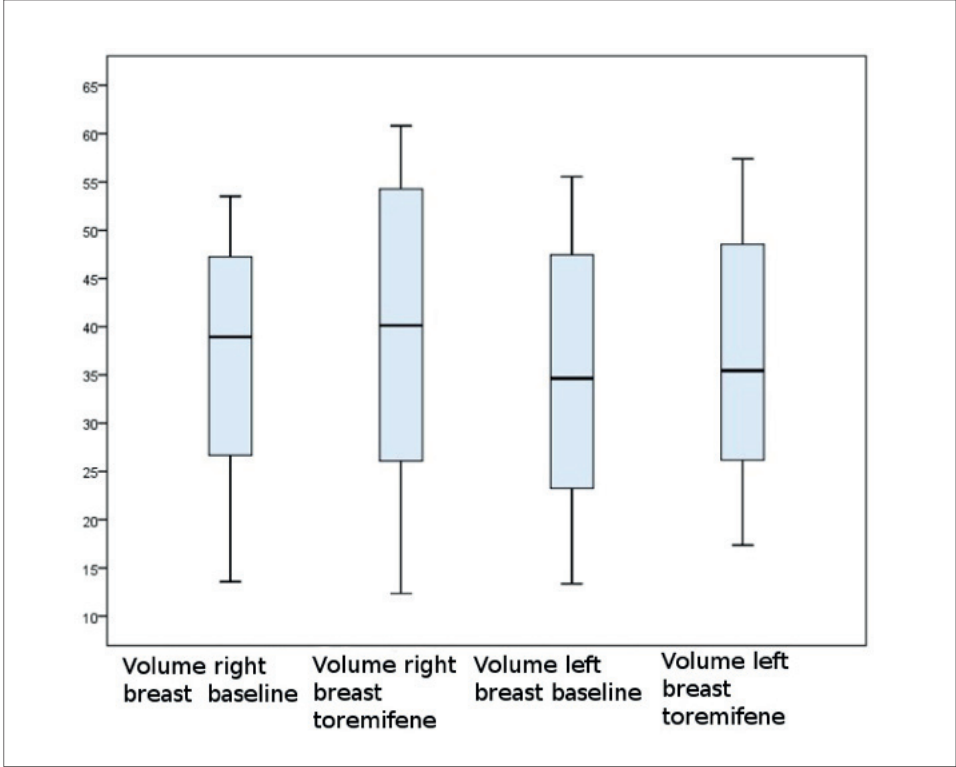

Figure 8.   The combined volumes (cm^3) of the four breast quadrants from both sides are shown by median (black line), interquartile range (box), and range (line bar) at the baseline cycle and during the toremifene cycle. The differences were tested by the Wilcoxon signed rank test.

Sinikka Oksa

6 DISCUSSION

6.1 The efficacy of toremifene in the treatment of premenstrual mastalgia

The randomized, crossover design was selected because this arrangement allowed an excellent differentiation of the effect of toremifene from that of placebo. In view of the crossover design, the treatment, carry-over and period effects of the VAS scores were calculated between the placebo and toremifene treatment periods. While the treatment effect between the treatment cycles was significant, there was neither a significant carry-over effect nor period effect. The response rate to placebo proved to be of the magnitude anticipated, or 26 %. The clinical benefit of toremifene was even better than expected. The prestudy assumption was that toremifene would be 30 % better than placebo. Actually, the final difference was greater, or 38 %.

The VAS scale is an established method for evaluation of mastalgia (Tavaf-Motamen et al. 1998, Futterman and Rapkin 2006). The simple self-assessed numeric VAS scale for the pain responses to medication was quite easy for the patients to assess and record. The assessment of breast pain by VAS scale is also recommended by other authors. The luteal phase administration of toremifene and the selected dose 20 mg were successful. The rationale for giving the treatment during the luteal phase only came from former studies on the pharmacological treatment of PMS and mastalgia (Landén et al. 2009). The efficacy of luteal phase toremifene (64 %) on cyclic mastalgia based on this study seems to be of a similar magnitude to that of tamoxifen 10 mg during cycle days 5 to 24 (72 %) (Konstostolis et al. 1997).

No earlier studies exist on the administration of toremifene for premenstrual mastalgia during the luteal phase only. However, a continuous administration of toremifene at a higher dose of 30 mg and 60 mg has been reported to be very effective for breast pain, with apparently more adverse events (Hamed et al. 2004, Gong et al. 2006). In a randomized, placebo-controlled trial, Gong et al. (2006) gave toremifene 30 mg or placebo daily continuously for three menstrual cycles to 143 women for moderate to severe premenstrual mastalgia. The response rate to toremifene was 76.7 %, while it was 34.8 % to placebo. Both response rates are comparable to the response rates seen in the present study. Gong et al., on the

other hand, treated 52 patients with noncyclical mastalgia with either toremifene or placebo. Among them, the response rates were smaller, or 48.1 % and 24.0 %, respectively. Treatment was switched to danazol 200 mg/day for non-responders for two menstrual cycles in both groups. 75 % of the patients from the placebo group and 40 % from the toremifene group responded to danazol.

Toremifene has also been used at a clearly higher dose level, or 60 mg daily. In a small open study, Hamed et al. (2004) studied 17 women complaining of moderate to severe mastalgia. 70 % of the participants had cyclic mastalgia and 30 % noncyclical mastalgia. The treatment period was 12 weeks. All the women with cyclic mastalgia responded to toremifene 60 mg, and also 75 % of those with noncyclical mastalgia.

6.2   Side effects

In Study I, the adverse reactions turned out to be mild and infrequent during the toremifene treatment and they were evenly distributed between the treatment arms. The drop-out rate of 9.7 % can be considered to be quite low in this setting. No participant discontinued her treatment because of adverse reactions. The side-effects reported with tamoxifen have been more frequent than the side-effects in the present study (Fentiman et al. 1988, Konstostolis et al. 1997).

Gong et al. (2006) reported that adverse events with the continuous toremifene treatment were equal to placebo. However, side effects were observed quite often in women receiving placebo (42.9 %) and in women receiving 30 mg toremifene daily (50.5 %), respectively. The main adverse reactions in both groups were menstrual disturbances, dizziness, vaginal discharge, and nausea. The incidence of vaginal discharge was noticed to be slightly higher in the toremifene group.

Hamed et al. (2004) found that all women encountered side effects during treatment with 60 mg of toremifene, with hot flushes, sweating, fatigue, and nausea being those most frequently reported. Four, or 23.5 %, of the participants withdrew their consent because of troublesome adverse events. Thus, a continuous administration of toremifene seems to cause more side effects than a cyclic administration.

6.3   QoL

The QoL scores remain unchanged in Study I, and there were no significant differences between the scores of the run-in, placebo, and toremifene cycles. In Study I, our instrument for measuring QoL, the modified Finnish Depression Scale, seemed to be less than ideal because it failed to detect any differences in

well-being between the untreated, placebo, and toremifene cycles, in spite of the marked differences in the pain scores. An alternative explanation, although less plausible, is that the reduction in breast pain was not marked enough to be reflected in the QoL in general. However, in Study IV with the same QoL instrument, the median QoL score appeared to be slightly lower during toremifene treatment cycles when compared with placebo. This is likely to be a coincidental result due to the much smaller sample size in Study IV than in Study I (nine vs. 56 patients, respectively).

6.4 Mechanism of action

6.4.1 Endocrine effects

Interestingly, both toremifene in the present study and tamoxifen (Tajima 1984) seem to have a luteotropic effect when given during the luteal phase only, as reflected in increasing serum oestradiol and progesterone concentrations. SERMs are known to have an anti-oestrogenic effect on the hypothalamus or hypophysis, resulting in, for example, hot flushes associated with their use, and in the induction of ovulation by clomiphene given in the early follicular phase of the cycle. Giving toremifene or tamoxifen during the luteal phase could antagonize the negative feedback by oestradiol and progesterone on the hypothalamic-hypophyseal axis. One possible mechanism is mediation by the increase in LH secretion. Indeed, in the study by Tajima (1984), the mean serum LH and FSH concentrations were slightly higher in the tamoxifen treatment cycles than in the control cycles. This may indicate that tamoxifen has anti-oestrogenic actions at the level of the hypothalamus or pituitary gland. However, even when the LH increase was not seen in the study by Tajima, the serum progesterone and oestradiol concentrations rose nonetheless. Similarly in our study, there were not any significant changes in median FSH levels during the toremifene cycles compared with the placebo cycles. These findings suggest that the site of the luteotropic action of anti-oestrogens may also be at the ovarian level. Tamoxifen and toremifene may compete with oestradiol for binding to the ORs of corpus luteum and thus inhibit the luteolytic action. This speculation is supported by a study by Fromson and Sharp (1974), demonstrating a selective uptake of tamoxifen by the corpus luteum.

Another somewhat surprising phenomenon found in Study II was the significant, albeit quite slight, increase in PRL level during toremifene treatment cycles, when the opposite would have been expected to happen with diminishing mastalgia. That finding may, however, be biased due to the fact that 6 % of the Study II participants were actually hyperprolactinemic throughout the study period. Tajima (1984) did not find any significant changes in luteal phase PRL

concentrations during a luteal phase administration of 10 mg of tamoxifen. The findings in Study II are in line with the connection between luteal insufficiency and mastalgia (Ayers and Gidwani 1983). This mechanism could compensate for a progesterone deficiency and be contributory to the anti-oestrogenic effect of toremifene and tamoxifen in the elimination of cyclic mastalgia.

Gong et al. (2006) found a positive correlation between the baseline breast pain score and serum oestradiol level in patients with cyclic mastalgia. Conversely, the serum baseline levels of progesterone and PRL and patient age have not been found to have any correlation to basal breast pain. There were not any significant differences in the mean serum levels of oestradiol, progesterone, and PRL between the toremifene-treated and placebo groups. Gong et al. administered toremifene throughout the menstrual cycle to women with mastalgia at a dose of 30 mg. In their study, the clinical response to toremifene treatment was not related to serum oestradiol, progesterone or PRL, or the age of the women with either cyclic or noncyclical mastalgia.

The findings of Study III imply that the therapeutic effect of toremifene in premenstrual mastalgia is not linked to any alterations in the inhibin production by the ovary. All women had both inhibin A and B concentrations within the normal premenstrual reference range at all measuring points. Di Loreto et al. (1999) have found activin A, inhibin A, and inhibin B subunits in the cyst fluid of women with fibrocystic disease of the breast. The concentrations of these breast inhibins showed no cyclic variation during the menstrual cycle in contrast to the cyclic changes found in serum inhibin levels, implying that there may be local production of these peptides in the breast.

Because of the endocrine activity of the breast itself, it is perhaps not reasonable to measure plasma concentrations of sex hormones at all while evaluating the aetiology of cyclic breast pain and treatment effects. The puzzling and negative findings of Studies II and III rather highlight the importance of focusing on local factors of the breast gland when trying to understand the mechanism of action of toremifene and other SERMS on the premenopausal breast. 4-hydroxytamoxifen gel has been reported to be effective in severe cyclic mastalgia (Mansel et al. 2007). The efficiency of this local administration of the anti-oestrogenic medication would probably be explained by the direct effects on the breast.

6.4.2 Local breast effects of toremifene

6.4.2.1 MRI

In Study IV, toremifene seemed to reduce both the enhancement ratio and maximum slope of enhancement, when compared with placebo, while diminishing premenstrual pain scores. These findings indicate that the therapeutic effect of toremifene may at least partly be mediated by diminished blood flow to the breast. On the other hand, tamoxifen 20 mg/day has been reported to decrease the concentration of pro-angiogenic factors angiogenin and VEGF, and to increase the levels of the anti-angiogenic endostatin in the normal breast. Åberg et al. (2011) found a significant correlation between extracellular VEGF and oestradiol levels in breast tissue but not in the plasma. Tamoxifen used to prevent premenopausal breast cancer decreases mammographic breast density, especially in premenopausal women (Cuzick et al. 2004, Cuzick et al. 2007). Chen et al. (2011), using 3D MRI have found a reduction in both the volume and relative density of breast fibroglandular tissue in women receiving continuous tamoxifen 20 mg /day. On the other hand, Eng-Wong et al. (2008) reported that even though the mammographic density did not increase, the breast MRI decreased as a response to raloxifene 60 mg a day in premenopausal women. These reports reinforce the interpretation that, at least in the premenopausal setting, the effects of SERMs on the breast are mediated though a local rather than systemic effect. The diminished proliferative and secretory activity could translate into reduced blood flow and, hence, to a diminished congestion, resulting in the alleviation of premenstrual mastalgia.

6.4.2.2 3D US

MRI has been found to be problematic for e.g. claustrophobic patients (Stoutjesdijk et al. 2001). Unfortunately, one of our patients also withdrew her consent after the first MRI because she felt claustrophobic during the imaging. Because MRI is also quite expensive and because 3D power Doppler US has recently been found to be a useful method in assessing flow patterns in organs like the uterus (Saarelainen et al. 2012), we wanted to explore the applicability of 3D US in evaluating the effect of toremifene on the breast. Unfortunately, 3D US failed to demonstrate any significant effects of toremifene on mammary volume or blood flow. The volume of the breast evaluated with 3D US prior to menstruation remained unchanged between the toremifene-treated cycle and baseline cycle. Neither did the vascular indices assessed alter when compared between the untreated and toremifene cycles.

It is necessary to perform measurements with the 3D probe as four divided segments in order to overcome the problem that the entire mammary gland cannot be scanned and measured simultaneously. The volumes of the four breast quadrants were easy to summarize, but the results concerning the vascular indices had to be reported from four separate areas rather than extrapolated for the whole breast because the indices are presented as averages. Moreover, the 3D technique presented may not cover the entire breast gland, and it was quite challenging to get optimal and similar adjustments in separate measurements.

The basal circulation of the normal breast has been reported to be low. However, it is the most severe mastalgia patients who have the highest breast basal circulatory rates (Madjar et al. 1993). Yakut et al. (2009) found significantly higher venous impedance indices during the premenstrual period in patients with cyclic mastalgia. Consequently, an explanation for the negative findings in Study V could be that the participants were healthy volunteers rather than mastalgia patients. Had the participants been women suffering from mastalgia as were the participants in Study IV, the findings would probably been different. On the other hand, the small sample size and short follow-up time may also have had influence on the results of Study V.

6.5 Strengths and limitations of the study

A placebo-controlled, prospective, randomized design is considered to be the most reliable method to study the efficacy of drugs in general. A placebo-controlled setting is particularly important in the study of drugs for mastalgia because many studies have confirmed the important role of the placebo effect in the relief of breast pain (Goyal and Mansel 2005, Gong et al. 2006, Kaviani et al. 2008). The crossover design we used not only allowed for limiting the number of patients needed without compromising the power of the study, but also demonstrated the temporary nature of the placebo effect as contrasted with the more sustained effect of the active drug. There was only one clinical investigator who coordinated the contacts in all sub-studies and performed the assessments in Studies I and V. The drop-out rate in general can be considered low, taking into account that the study design demanded the intensive and accurate cooperation of patients.

This study also had some limitations. The results of this study are based on three-month long treatment periods and only a one-month long baseline period and, thus, they may not be extrapolatable for longer treatment durations. The rationale for only one baseline cycle was to keep the untreated period short, thus decreasing the number of potential withdrawals. On the other hand, because no studies on the use of toremifene existed at the time when Study I was designed, and no long-term data on the chronic luteal phase use of any SERM was available,

a short- rather than long-term use of toremifene was considered to be safer in this setting. The study populations in Studies IV and V were small and the obtained results can be considered only as a guide to future evaluations. However, our goal here was to preliminarily test new methods for breast examination during treatment with an anti-oestrogen. The instrument we used for evaluating QoL was obviously less than ideal, but we were not able to find more suitable instruments in the previous literature on the use of SERMs for cyclic mastalgia.

6.6   Future Prospects

The administration of medication for cyclic mastalgia only during the luteal phase seems rational in the future, taking into account the maximum appearance of breast symptoms premenstrually. The long-term efficacy and safety of toremifene in premenopausal women was not addressed on the grounds of this study. Toremifene was, however, very effective in premenstrual mastalgia, with only few mild adverse effects. This encourages future research into its long-term use for this indication. A randomized comparison of toremifene with other drugs such as tamoxifen and dopamine agonists is also warranted. Raloxifene, which seems to lack adverse effects on the endometrium, could also be a good candidate for treating cyclic mastalgia. The breast 3D US assessments should be repeated with mastalgia patients during treatment with placebo and toremifene, preferably using MRI as a control. The role of cytokines in the pathology of breast pain warrants further research. The investigation of local breast hormone levels in relation to these pain modulators or tumour necrosis factor-alpha, interleukin-1 beta, and interleukin-6 with mastalgia patients could lead to better understanding of the mechanisms of mastalgia induction and improved and specific therapy options.

7   SUMMARY AND CONCLUSIONS

The present study was designed to investigate the effect of toremifene on premenstrual mastalgia. To accomplish this, 62 women suffering from marked premenstrual mastalgia were prospectively randomized to receive toremifene 20 mg/day or matching placebo during the luteal phase of the menstrual cycle for three consecutive cycles, using the double-blind, crossover design. The endocrine response during the mid-luteal phase attributable to toremifene was also evaluated with mastalgia patients. The mechanism of the effects of toremifene on breast tissue was further evaluated by means of contrast enhanced MRI on another smaller (n=10) group of mastalgia patients, also using a double-blind, randomized design. Finally, vascular and volume changes in the breast attributed to toremifene were assessed by 3D US. The 3D evaluations were performed in a prospective trial design with 20 healthy volunteers.

Treatment with an anti-oestrogen (toremifene or tamoxifen) should be targeted at the luteal phase only in patients with cyclic mastalgia in order to minimize adverse reactions. Additionally, a lower dose of each drug has been shown to be better tolerated. An ideal starting dose of luteal phase toremifene is probably 20 mg. For non-responders, toremifene with higher dose of 40 mg could be studied. However, the risk of adverse effects seems to increase with higher dose of 60 mg, based on the study of Hamed et al. (2004).

The main findings and conclusion of the study are:

1. Toremifene 20 mg/day administered during the luteal phase significantly alleviates premenstrual breast pain. Adverse reactions reported by the participants were mild and evenly distributed between the toremifene and placebo groups. The overall QoL scores remained unchanged. Toremifene may be considered as a valid and safe treatment option for premenstrual mastalgia. However, this trial did not address the long-term safety of toremifene in premenopausal women.

2. Toremifene, given during the luteal phase only, exerts a luteotropic effect by increasing serum progesterone and oestradiol levels. The mid-luteal concentrations of androstenedione, total and free testosterone remained unchanged. The PRL levels increased significantly.

Sinikka Oksa

3.   A luteal administration of toremifene does not seem to result in any changes in mid-luteal concentrations of inhibin A and B in the serum. All measured inhibin A and B levels were between normal values. The mechanism by which toremifene has a therapeutic effect in cyclic mastalgia does not seem to involve inhibition or stimulation of ovarian inhibin production.

4.   Toremifene reduced both the MRI enhancement ratio and the maximum slope of enhancement, when compared with placebo, in the breasts of patients with remarkable premenstrual mastalgia. The therapeutic effect of toremifene may at least partly be associated with diminished blood flow to the breast.

5.   The study introduced a novel method for assessing breast changes as a response to toremifene in healthy premenopausal women by using 3D US. However, no significant changes in vascularity indices or total volume of the breast were detected in premenstrual 3D evaluations between the baseline and toremifene cycles.

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Sinikka Oksa

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ORIGINAL COMMUNICATIONS

Toremifene for premenstrual mastalgia: a randomised, placebo-controlled crossover study

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Objective To investigate the efficacy of toremifene in the treatment of premenstrual mastalgia.

Design Double-blind, placebo-controlled crossover study.

Setting Three Finnish general practices from the districts of Satakunta Central Hospital and Tampere University Hospital.

Population A total of 62 women aged 25–45 years with premenstrual mastalgia during at least three previous menstrual cycles.

Methods Women were randomised to receive toremifene 20 mg daily or placebo from day 15 of the menstrual cycle until menstruation for three consecutive cycles. After a wash-out cycle, the women were crossed over to receive placebo or toremifene for three additional cycles.

Main outcome measures Cyclic breast pain relief assessed by visual analogue scale (VAS) score. Quality-of-life scores assessed by a modified 36-item Finnish Depression Scale, with a score ranging from 0 to 108. Acceptability of treatment.

Results About 32 women were randomised to receive toremifene first and 30 to receive placebo first. Twenty-nine and 27 participants in the groups treated with toremifene first or placebo first completed the treatment, respectively. There were significant reductions in VAS scores in both groups after three treatment cycles. This was significantly greater in the toremifene-treated group (VAS: 1.8 in the toremifene group and 3.7 in the placebo group, $P = 0.004$). Treatment effect between treatment cycles was significant ($P = 0.001$). Quality of life was similar during the toremifene and placebo cycles.

Conclusion This study demonstrates that the antiestrogenic compound, toremifene, is able to relieve premenstrual breast pain without major adverse effects. There was a 64% reduction in median pain scores in the toremifene-treated cycles compared with a 26% reduction in placebo-treated cycles.

Keywords Mastalgia, premenstrual syndrome, selective oestrogen receptor modulators, toremifene.

Introduction

Benign breast pain is a common complaint among women in western countries. Between 41 and 69% of women reported having mastalgia sufficient to interfere with their daily routines.[1] Two-thirds of breast pain is cyclic. Discomfort lasting for 1–4 days can be considered 'normal'.[2] However, approximately 8–10% of premenopausal women suffer monthly from moderate to severe breast pain that lasts for more than 5 days. Cyclic mastalgia frequently presents as part of the premenstrual syndrome (PMS).[3,4] Pain usually occurs during the luteal phase of the menstrual cycle and disappears with the onset of menstruation.[3,5] It classically affects the outer half of the breast and is associated with a diffuse nodularity.[6,7] Hormonal studies have failed to reveal any specific endocrine cause of cyclic mastalgia, despite the close relation to the menstrual cycle.[2,7,8] However, premenstrual mastalgia is worsened by exogenous estrogen and improved by suppression or elimination of the ovarian cycle.[7]

The treatment options for premenstrual mastalgia include bromocriptine,[8] danazol,[9] gonadotrophin-releasing hormone (GnRH) analogues,[10] and the antiestrogen tamoxifen.[11,12] Tamoxifen reduces pain in 71–96% of symptomatic women,[5] danazol in 47–88%,[13] and bromocriptine in 47–88%.[2,5,8] All these drugs are associated with troublesome adverse effects: danazol causes androgenic adverse effects, menstrual disturbances,

headache, and nausea.[5,9,13] Adverse effects of bromocriptine[2] and GnRH analogues[5] are frequent. Tamoxifen causes hot flushes in 36% of women and menstrual disturbances in 22%. Also, ovarian cyst formation and growth of uterine fibroids have been reported.[14] In postmenopausal women, long-term use of tamoxifen is associated with a two- to three-fold increased risk of endometrial cancer.[15]

Toremifene is another triphenylethylene derivative, similar to tamoxifen, which is as effective as tamoxifen in the treatment of postmenopausal breast cancer at a dose of 60 mg daily.[16,17] Although toremifene has tamoxifen-like effects in postmenopausal endometrium,[18,19] its genotoxic and mutagenic potential seems to be far less than that of tamoxifen.[20] So far, no excess of endometrial cancers has emerged among women treated with toremifene: the cumulative incidence of endometrial cancer in women with breast cancer receiving toremifene by the year 2002 with more than 300 000 patient years was 20 cases or 0.07/1000 patient years.[21–23]

Consequently, toremifene is potentially an attractive treatment option for premenstrual mastalgia. The aim of this study was to evaluate the efficacy and tolerability of toremifene for this purpose.

Methods

Women with marked premenstrual mastalgia were recruited to the study by means of either newspaper advertisements or referral from their GP or hospital clinician, followed by a brief telephone interview. Telephone prescreening eliminated women using hormonal contraception or treatment; those reporting irregular menstrual cycles, history of breast or endometrial cancer, hysterectomy and/or bilateral oophorectomy, pregnancy, lactation, or a desire for pregnancy; and those with serious health problems. Women with a history of thromboembolic disease were also excluded. The inclusion criteria included: 1) a mastalgia severe enough to interfere with social life and to cause a desire to use medication against it, lasting for more than 5 days during the luteal phase of the menstrual cycle; 2) at least a 3-month history of symptoms; 3) age between 25 and 45 years; and 4) regular menstrual cycles. The women, except for those with a history of surgical sterilisation, were advised to use nonhormonal contraception until the first post-treatment menstrual period. The study protocol was approved by the Regional Ethics Committees of Pori Central Hospital and Tampere University Hospital, and all the women gave a written informed consent before enrolment.

After a single nonmedicated baseline menstrual cycle, the participants were randomly allocated to receive either toremifene or placebo for three cycles. After an untreated wash-out cycle, the women were crossed over to placebo or toremifene for three additional cycles (Figure 1).

The medication was given in tablet form, and the participants were instructed to take one tablet daily from cycle day 15 until the next menstruation. The active (toremifene 20 mg) and control (placebo) tablets were identical, white and round in appearance, and supplied in identical bottles that were labelled with the patient number and the appropriate period of the study. A computerised block randomisation system was allocated to ensure an even distribution of toremifene and placebo. This system was independent of the investigators. The codes were kept confidential until the end of the study when the randomisation code was broken. All women and study investigator were blinded to the randomisation codes, and the screening and follow-up study assessments were completed by study personnel who were blinded to the randomisation code.

Double blinding and dispensing of medication were provided by Orion Pharma. The code was held by the investigator in a sealed envelope to be opened only in the event of an emergency, for example, allergic reaction or thromboembolic disease. However, there was no need to open the blinding code until the end of the study because no serious events occurred during the treatment period.

The participants underwent a careful clinical examination at baseline and at the end of the study, including a vaginal ultrasound examination. The first visit included a detailed history, and the exclusion and inclusion criteria were also checked.

Intensity of mastalgia was self-assessed with a numeric (0–10) visual analogue scale (VAS) by the participants. They completed a breast pain VAS at the late luteal phase of the baseline cycle and of each menstrual cycle until the end of the trial. The participants recorded the days of the menstruation during the study.

We used a modified 36-item Finnish Depression Scale,[24] with a score ranging from 0 to 108, to estimate the quality of life. The participants completed a quality-of-life questionnaire during the late luteal phase of the baseline cycle and also during the third and sixth treatment cycles.

It was estimated that the efficacy of placebo would be approximately 25% in relieving premenstrual mastalgia. To be able to show more than 33% units improvement in the efficacy of toremifene over placebo, 62 participants were needed ($\alpha = 0.05$, power = 80%). The statistical analyses were performed by SPSS for Windows, standard version 12.0.1, (SPSS Inc., Chicago, IL, USA). A *P* value less than 0.05 was considered as statistically significant. Normality of the distribution of the continuous variables studied was tested by Kolmogorov–Smirnov test. The distributions of all variables were skewed. Values of continuous variables were expressed as median, interquartile ranges, and ranges. Differences between run-in period, placebo and treatment groups were tested by Sign test due to different shape of distributions. Differences in quality-of-life scores in cycles between the placebo and treatment
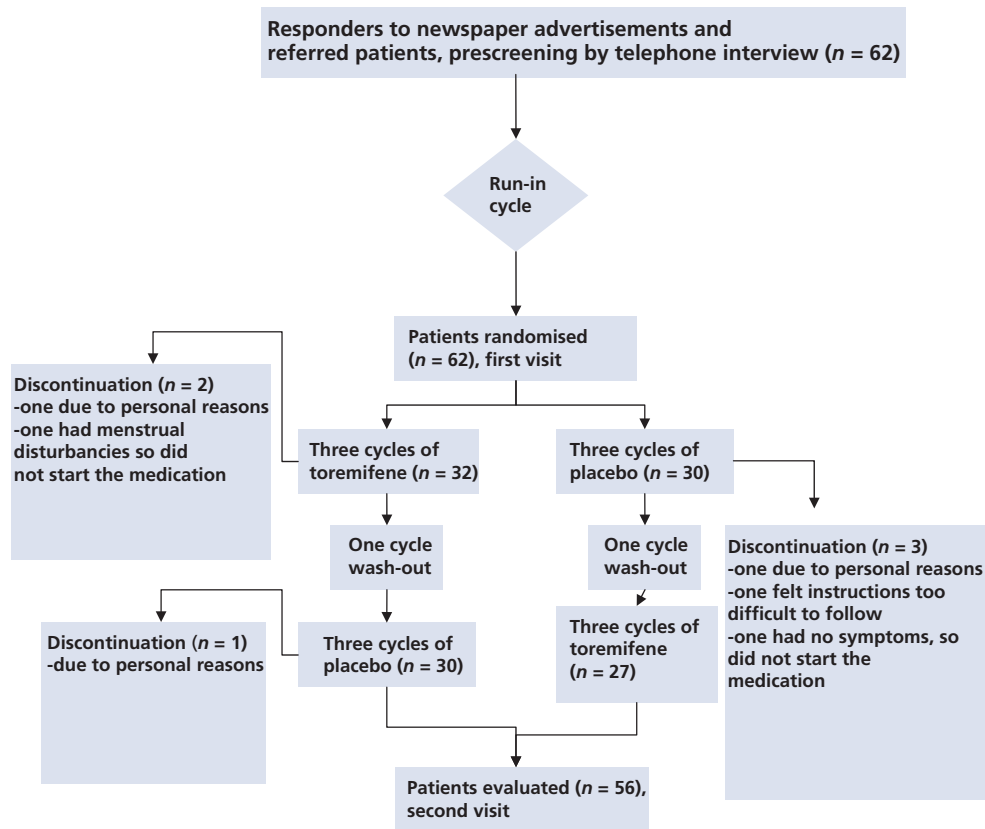
Figure 1. Flow of participants through the study.

groups were tested by Mann–Whitney U test. Treatment, carry-over, and period effects were calculated and differences between the treatment groups were tested by Mann–Whitney U test.

Results

The participants were recruited between April 2000 and December 2002. The clinical characteristics of the women are shown in Table 1. The mean follow-up time was approximately 8 months. The participants were defined as compliant with treatment, if they had adhered to the drug regimen and given complete diaries and questionnaires. The compliance was also assessed by counting the returned tablets at the end of the study. After a telephone prescreening, 62 women met the inclusion criteria and were followed up for the baseline cycle. Of them, 32 were randomised to receive toremifene, and 30 to receive placebo for the first three subsequent menstrual cycles. Six women (three from each group) were excluded from the analysis: three discontinued the trial for personal reasons (the women in the toremifene-first group, who withdrew after the wash-out period, lost all the study papers and medications); one had no symptoms any more; for one participant, the instructions were too difficult to

follow; and one had menstrual disturbances before starting the medication. The remaining 56 participants provided complete data for final evaluation (Figure 1).

Treatment, carry-over, and period effects of the VAS scores were calculated between the first and second treatment cycles, in view of the crossover design. The treatment effect between the treatment cycles was statistically significant ($P = 0.001$), with no significant carry-over effect ($P = 0.706$) and period effect ($P = 0.876$). The VAS pain scores and life quality scores from baseline cycles and treatment cycles are shown in Table 2 and Figure 2. In the group where toremifene was given first ($n = 29$), there was a significant reduction in mastalgia between the baseline cycle and the toremifene cycles ($P = 0.014$). During the placebo cycles, the breast pain reappeared (toremifene versus placebo, $P = 0.038$; baseline versus placebo, $P = 0.441$). On the contrary, in the placebo-first group ($n = 27$), the pain scores continued to decline throughout the study (baseline versus placebo, $P = 0.035$; toremifene versus placebo, $P = 0.078$; and baseline versus toremifene, $P < 0.001$).

When all the toremifene cycles were compared with all the placebo cycles and with the baseline cycle, the median VAS scores were 1.8, 3.7, and 5.0, respectively (baseline versus toremifene, $P < 0.001$; baseline versus placebo, $P = 0.034$;

Table 1. Patient characteristics, *n* = 62

	Placebo first (*n* = 30)	Toremifene first (*n* = 32)
Characteristics		
Mean age (year)	40.4	40.8
Mean weight (kg)	68.3	69.6
Mean age at menarche (year)	13	13.2
Mean length of menstrual cycle (days)	27.6	27.9
Median parity	2	2
Nulliparity	3	4
With one child	8	3
With two or more children	19	25
Contraception methods		
Sterilisation	20	17
Condom	8	13
Intrauterine device	2	2
Mammography	14	13
Median baseline pain scores	5 (*n* = 27)	5 (*n* = 29)
Median baseline quality-of-life scores	42 (*n* = 27)	45.5 (*n* = 29)

and toremifene versus placebo, *P* = 0.004). There was a 64% reduction in median pain scores in the toremifene-treated cycles compared with a 26% reduction in placebo-treated cycles.

Adverse reactions reported by the participants were mild and evenly distributed between the toremifene and placebo cycles. No participant discontinued her treatment because of adverse effects. Mild headache and nausea were the common adverse effects. Headache was reported by three women during toremifene cycles and by two women during the placebo cycles. The corresponding figures for nausea were two and

one, respectively. There were neither menstrual disturbances nor hot flushes during the treatment periods.

The overall quality-of-life scores remained unchanged, and there were no significant differences between the scores of the run-in, placebo and toremifene cycles (Table 2).

Discussion

The rationale for giving the treatment during luteal phase only came from earlier studies on pharmacological treatment of the PMS and mastalgia. Serotonin uptake inhibitors like sertraline are effective in alleviating the mood changes associated with PMS, given only during the later part of the menstrual cycle, although in the treatment of depression, it takes at least 2 or 3 weeks before their therapeutic effect starts to emerge.[25] Tamoxifen, given during the luteal phase only, exerts a luteotropic effect by increasing serum progesterone concentrations. It was speculated that this could be the mechanism underlying its ability to relieve premenstrual mastalgia: the compensation of progesterone deficiency could contribute to the antiestrogenic effect of tamoxifen.[13] Because toremifene and tamoxifen are related antiestrogenic compounds, it was reasonable to assume that toremifene would have similar antimastalgic effect as tamoxifen, when given during the luteal phase of the menstrual cycle.

In our study, the use of toremifene during the luteal phase was associated with a 64% reduction in the median VAS score, and its effect was significantly superior to that of placebo. The adverse effects were minimal and did not differ from those of placebo. We are aware of only one published study, where toremifene has been used to treat premenstrual mastalgia. That study differs from our study in that it was an open nonrandomised study, where toremifene at a higher

Table 2. The medians, quartile ranges, minimum and maximum of pain VAS scores and life quality scores in baseline cycles and two treatment cycles

	Pain VAS score	*P* value	Quality-of-life score	*P* value
First toremifene (*n* = 29)				
Baseline	5.0 (0–9.0)		46 (14–83)	
Toremifene	1.0 (0–8.0)	0.014*	38 (13–80)	0.700*
Placebo	3.3 (0–8.0)	0.441**	42 (12–75)	0.327**
		0.038***		0.700***
First placebo (*n* = 27)				
Baseline	5.0 (2.0–9.0)		42 (20–64)	
Placebo	4.3 (0–8.0)	0.035**	39 (8–62)	0.424**
Toremifene	2.3 (0–9.0)	<0.001*	40 (7–62)	1.000*
		0.078***		0.690***

Effects were calculated between treatment groups.

*Baseline compared with toremifene.

**Baseline compared with placebo.

***Toremifene compared with placebo.
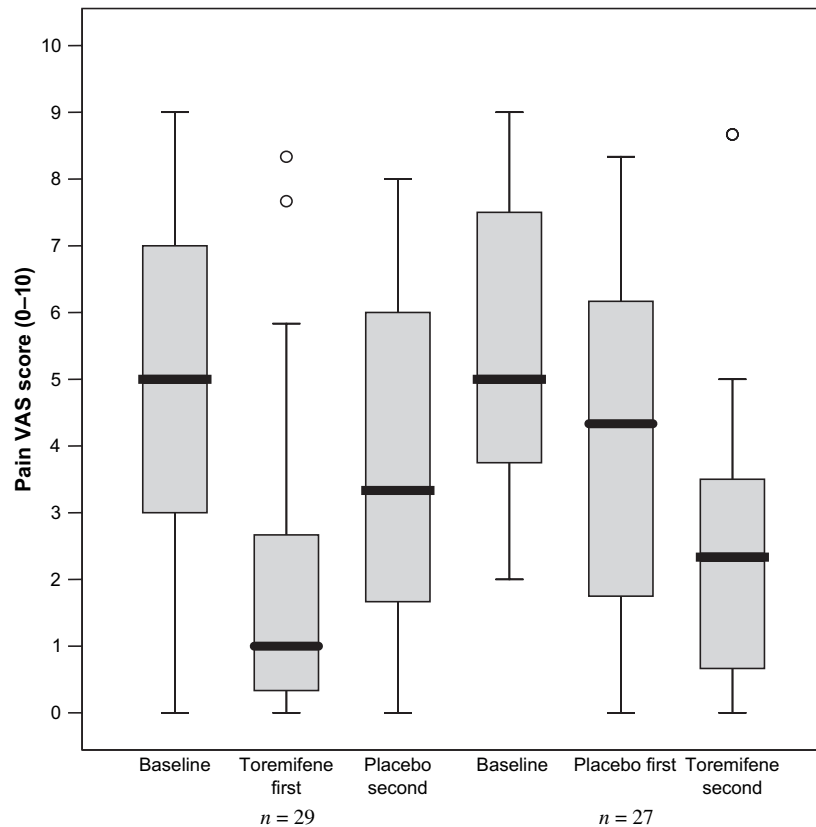
Figure 2. The medians, quartile ranges, and ranges of pain VAS scores from baseline cycles and treatment cycles.

dose or 60 mg daily was given continuously during the whole menstrual cycle. All subjects with cyclical mastalgia reported at least a 50% reduction in the pain score as compared with baseline, but 4 of 17 (24%) reported adverse effects like hot flushes, sweats, fatigue, nausea, menstrual disorders, dizziness, and chest pain.[26] Where tamoxifen has been used to treat premenstrual mastalgia, the adverse effects reported include hot flushes, menstrual disorders, nausea, and vaginal discharge or dryness.[18]

The crossover design of this study allowed for a successful differentiation of the effect of toremifene from that of placebo. The magnitude of the placebo effect (26%) was as great as expected. The six withdrawals did not significantly decrease the power of the study because they were evenly distributed among the groups; with the remaining 56 participants, the power was still 86%. The prestudy assumption was that toremifene would be 30% better than placebo. Actually, the final difference was even greater at 38%. The drop out rate of 9.7% can be considered low, taking into account that the study design demanded intensive and accurate cooperation from the patients. There was only one clinical investigator who coordinated all the contacts and performed all the assessments.

Our instrument for measuring quality of life or the modified Finnish Depression Scale seems to be less than ideal

because it failed to detect any differences in the global well-being between the untreated, placebo, and toremifene cycles, in spite of the differences between the pain scores. An alternative, although less plausible explanation, is that the reduction in breast pain was not marked enough to be reflected in the quality of life in general.

The baseline pain scores were based on the VAS measurements from only one menstrual cycle. The rationale was that we did not want to lengthen the untreated period in fear of increasing the number of withdrawals. Similar design has been used previously.[8] The present trial did not address the long-term safety of toremifene in premenopausal women.

Conclusions

Our finding that toremifene significantly alleviates premenstrual mastalgia over placebo with few mild adverse effects encourages us to further research on its long-term use for this indication. The research will focus on the long-term safety of toremifene and on the randomised comparison with other drugs such as tamoxifen or bromocriptine. Also, newer drugs like raloxifene warrant attention. Another important line of research would be the aetiology of premenstrual mastalgia that at present still remains inadequately addressed. ■

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THE ANTIOESTROGEN TOREMIFENE HAS LUTEOTROPIC EFFECTS IN WOMEN SUFFERING FROM PREMENSTRUAL MASTALGIA

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Abstract

Background. Prior studies examining cyclic mastalgia and sex hormones have failed to reveal any specific endocrine cause and their results of them are inconsistent.

Aim. To evaluate luteal hormonal levels in women with marked premenstrual mastalgia in response to toremifene.

Methods. In a double-blind crossover randomization procedure after one baseline cycle, 32 women were randomized to receive 20 mg toremifene, and 30 women placebo from cycle day 15 until the next menstruation for three menstrual cycles. After a wash-out cycle the women were crossed over to receive placebo and toremifene, respectively. The luteal hormonal levels were measured at baseline, and during the third cycle of toremifene and placebo. The study was setup in a general practice population from two Finnish hospital districts. Serum FSH, estradiol, progesterone, prolactin, androstenedione, total and free testosterone were measured.

Results. When all the toremifene-treated cycles were compared with all the placebo cycles and with the baseline, the median estradiol levels were 0.36, 0.27 and 0.31 nmol/L, respectively (baseline *versus* toremifene, P=0.005; baseline *versus* placebo P=0.095; and toremifene *versus* placebo P<0.001). The median progesterone levels were at baseline 32.5 nmol/L, during placebo 34.5 nmol/L and during toremifene 42.5 nmol/L (baseline *versus* toremifene P=0.002; baseline *versus* placebo P=0.802; and toremifene *versus* placebo P=0.002). The median prolactin level was significantly higher during the toremifene cycles (268 mU/L) as compared to the baseline (222 mU/L, P=0.046). There were no significant changes in other hormone concentrations evaluated.

Conclusion. Toremifene seems to have a luteotropic effect in women suffering from premenstrual mastalgia.

Key words: premenstrual syndrome, mastalgia, selective estrogen receptor modulators, toremifene, circulating estradiol and progesterone.

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INTRODUCTION

Benign breast pain is a common complaint of women in western countries. As many as 41-69% of women reported having mastalgia sufficient to interfere with their daily routines (1). Two thirds of breast pain is cyclic. Mild premenstrual breast discomfort lasting for 1 to 4 days can be considered "normal" (2). However, approximately 8-10% of premenopausal women suffer monthly from moderate to severe breast pain that lasts for more than five days (3, 4). Pain usually occurs during the luteal phase of the menstrual cycle and disappears with the onset of menstruation (3). Hormonal studies have failed to reveal any specific endocrine cause of cyclic mastalgia, despite the close relation to the menstrual cycle (2, 5, 6). However, premenstrual mastalgia is worsened by exogenous oestrogen and improved by suppression or elimination of the ovarian cycle (6). Several hormonal imbalances with potential causative roles in cyclic mastalgia have been investigated, and each has findings in support and opposition (7). These include absolute or relative hyperoestrogenism (8), luteal insufficiency (9), hyperprolactinaemia (10), and hyperandrogenism (11). Tamoxifen has previously been found to be effective in reducing premenstrual mastalgia (12). We showed that another triphenylethylene derivative, toremifene, significantly alleviated cyclic breast pain as compared to placebo (13). The present study was undertaken to investigate if there are any measurable changes in the hormonal profile of the luteal phase attributable to toremifene.

MATERIAL AND METHODS

Patients

The recruitment, randomization and study treatment procedures have been described previously (13). In brief, 62 women suffering from premenstrual mastalgia were randomly allocated to receive toremifene (20 mg) or placebo during the luteal phase for three consecutive cycles. The patients were then crossed over after a wash-out period to placebo or toremifene, respectively. The participants were instructed to take one tablet daily from cycle day 15 until the next menstruation. Venous blood samples were collected at baseline, and at the third of the toremifene and placebo cycles. All of the patients were premenopausal and ovulatory according to the baseline FSH and progesterone (P) values. Three of the patients had serum prolactin (PRL) values above normal at baseline and during all treatments. One patient had androstenedione (A) above normal values in all measuring points. Three of the study population had both total (total T) and free testosterone (free T) values higher than normal. The demographic data of the participants is given in Table 1.

The study protocol was approved by the Regional Ethics Committees of Pori Central Hospital and Tampere University Hospital, and all the women gave a written informed consent before enrollment.

Table 1. Characteristics of participants

Characteristics	All (n=62)	Followed (n=48)
Age years, Median [Range]	42 [25-45]	42 [25-45]
Weight (kg), Mean (Sd)	68.99 (12.62)	67.80 (13.68)
Age at menarche (yr), Mean (Sd)	13.18 (1.30)	13.10 (1.31)
Length of menstrual cycle (days), Mean (Sd)	27.77 (2.03)	27.81 (2.05)
Parity, Median [Range]	2 [0-5]	2 [0-3]
Nulliparity (n)	12	10
With 1 child (n)	12	10
With two or more children (n)	37	27
Not known	1	1
Contraception		
Sterilisation (n)	37 (60)	30
Condom (n)	21 (34)	15
Intrauterine device (n)	4 (6)	3
Mammography, yes, n (%)	27 (44)	21 (44)
Baseline pain scores, Median [Range]*	5 [0-9]	5 [0-9]
* n=56		

Assays

Venous blood samples for determination of serum hormone levels were obtained on three occasions during the luteal phase (cycle days 20-22) of the menstrual cycle: at baseline, and at the third toremifene and placebo cycle, respectively. Fasting blood samples were collected from each subject between 0800 and 1000 h, the serum was separated and stored at -20 C until assayed.

Serum estradiol (E) was measured by means of a competitive radioimmunological method (DiaSorin S.p.A, Saluggia, Italy) with a sensitivity of 0.020 nmol/L. The interassay coefficients of variation (CVs) were 10, 9, and 8 % for low-, medium and high-range values, respectively. The reference range of serum E for the luteal phase of the menstrual cycle was 0.25-0.90 nmol/L.

Serum FSH, PRL and P were measured using an automated time-resolved immunofluorometric assay (AutoDELFIA, PerkinElmer, Turku, Finland). The lower limits of detection of FSH, PRL and P were 0.1 IU/L, 9 mUI/L and 1 nmol/L, respectively. For low-, mid- and high-pooled values of FSH and PRL, the interassay CVs were 3, 3 and 2 %, and 4, 4, and 5 %, respectively. For low- and high-pooled values of P the interassay CVs were 6 and 2 %, respectively. The reference ranges were 2.0-8.0 IU/L for FSH, 70-500 mU/L for PRL, and 12-69 nmol/L for P, respectively. Serum A was measured after extraction by an in-house competitive 3H-radioimmunological method (antiserum by INC Biomedical Inc., CA, USA). The sensitivity of the assay was 0.8 nmol/L and the interassay CVs were 15, 13, and 10 %

for low-, medium and high-range values, respectively. The reference range of A was 3.0-11.0 nmol/L.

Serum total T was analysed using an automated competitive luminoimmunological method (ADVIACentaur, Bayer Coorporation, NY; USA). The sensitivity of the assay was 0.8 nmol/l, and the interassay CVs were 16, 9 and 8 % for low-, medium- and high- range values, respectively. The reference range of total T was < 2.8 nmol/L. Serum free T was measured by an in-house method after sample precipitation. The amount of free T was directly measured as percent (%) using radioactive steroid (5 α-Dihydroxy [1,2,4,5,6,7-^{3}H] testosterone, Pharmacia Biothec, England) . Free T as molar units (pmol/L) was calculated using the free T %-value and the total T concentration. The interassay CVs for low- and high- pooled values were 11 and 8 %, respectively. The reference range of serum free T was 2-20 pmol/L.

All the assays were performed at the Medix Laboratories Ltd, Espoo, Finland.

Statistical analysis

Normality of the distribution of the continuous variables studied was tested by Shapiro-Wilk's test. The distributions of all variables were skewed. Differences between baseline and placebo, between baseline and toremifene and between placebo and toremifene were tested by Wilcoxon Signed Ranks test. The statistical analyses were performed by SPSS version 14.0.2, for Windows (SPSS Inc., Chicago, IL, USA). A p-value less than 0.05 was considered as statistically significant.

RESULTS

The participants were recruited between April 2000 and December 2002. Sixty-two subjects were included and followed up for the baseline cycle after a telephone pre-screening. Thirty-two were randomized to receive toremifene and 30 to receive placebo for the first three subsequent menstrual cycles. Six patients (three from each group) were excluded from the pain score analysis: Three patients discontinued the trial for personal reasons, one patient had no symptoms any more, for one participant the instructions were too difficult to follow, and one patient had menstrual disturbances before starting the medication. For the present hormonal study, we included only the participants, who had given blood samples at all measuring points. Finally, 24 women randomized to toremifene first and 24 women randomized to placebo first gave a blood sample to become analysed.

The median serum luteal phase hormone levels of the patients with interquartile ranges are shown in Table 2. The baseline level was compared to all toremifene and placebo cycles, respectively. The median serum E and P concentrations were significantly higher in the toremifene-treated cycles (Table 2, Figs. 1 and 2). The median E level was 0.36 nmol/L during the toremifene cycles, while it was 0.31 nmol/L at baseline and 0.27 nmol/l during the placebo cycles, respectively (baseline *versus* toremifene, P=0.005; baseline *versus* placebo P=0.095; and toremifene versus placebo P<0.001). The median P level were 32.5 nmol/L at baseline, during placebo

Table 2. Median (Md) of luteal hormone levels of patients with interquartile ranges (Iq). Differences between baseline and placebo (p1), between baseline and toremifene (p2) and between placebo and toremifene (p3) were tested by Wilcoxon Signed Ranks test (N=48).

Hormone	Baseline Md (Iq)	Placebo Md (Iq)	Toremifene Md (Iq)	p_1	p_2	p_3
FSH IU/l	3.35 (2.20-4.30)	3.00 (2.15-4.25)	3.45 (2.75-4.20)	0.349	0.305	0.430
E nmol/l	0.31 (0.24-0.41)	0.27 (0.21-0.34)	0.36 (0.29-0.43)	0.095	0.005	<0.001
A nmol/l	5.50 (4.05-7.53)	5.95 (4.23-7.35)	5.95 (4.15-7.58)	0.996	0.121	0.479
PRL mU/l	222 (167-333)	242 (170-355)	268 (198-397)	0.157	0.046	0.583
PROG nmol/l	32.5 (21.3-45.0)	34.5 (24.8-46.5)	42.5 (29.5-65.0)	0.802	0.002	0.002
TOTAL T nmol/l	1.88 (1.59-2.26)	1.93 (1.50-2.31)	1.94 (1.62-2.39)	0.960	0.395	0.135
Free T pmol/l	16.0 (13.0-22.8)	15.9 (13.0-19.0)	15.5 (12.0-21.2)	0.100	0.078	0.852

34.5 nmol/L, and during toremifene 42.5 nmol/L, respectively (baseline *versus* toremifene P=0.002; baseline *versus* placebo P=0.802; and toremifene *versus* placebo P=0.002).
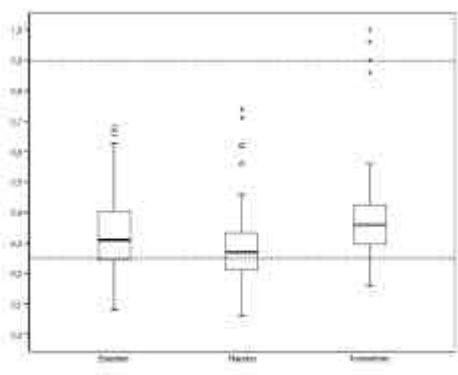

Figure 1. The medians, quartile ranges, and ranges of serum estradiol levels (nmol/L) with reference ranges.
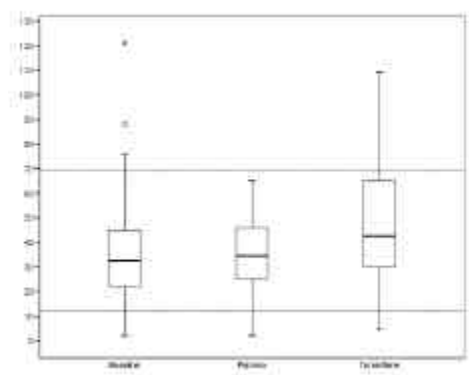
Figure 2. The medians, quartile ranges, and ranges of serum progesterone levels (nmol/L) with reference ranges.

The median PRL level of 268 mU/L was significantly higher during toremifene treatment when compared with the baseline (222 mU/L, P=0.046), but there was no significant difference between toremifene and placebo treated cycles (P=0.583), or between the baseline and placebo (P=0.157). The median concentrations of FSH, A, and total and free T were similar at baseline and during placebo or toremifene treatment.

DISCUSSION

The luteotropic effect of toremifene given 20 mg/day during the luteal phase of the menstrual cycle to women suffering from premenstrual mastalgia as shown in the present study was accompanied by a marked reduction in symptoms (13). Interestingly, tamoxifen 10 mg/day when given to healthy women during the luteal phase, also exerts a luteotropic effect by increasing serum P and E levels (14). These findings are contradictory to the mastalgogenic effect of both endo- and exogenous oestrogen (6, 8) but are in line with the connection between luteal insufficiency and mastalgia (9). Another somewhat puzzling phenomenon found in the present study is the significant albeit quite slight increase in PRL level during the toremifene cycles, when one would expect the opposite to happen with diminishing mastalgia. The latter finding may of course be biased with the fact that three or 6 % of our patients were in fact hyperprolactinaemic throughout the study period.

When toremifene is administered throughout the menstrual cycle to women with mastalgia, the endocrine response seems to be different from above. Gong *et al.* gave toremifene at a dose of 30 mg daily, and compared to the clinical response to the levels of E, P and PRL (15). Although they found a positive correlation between baseline breast pain score and serum estradiol level, there were no significant differences in the serum levels of E, P and PRL between patients receiving toremifene and those receiving placebo. Again toremifene turned out to be effective in alleviating cyclical mastalgia, but in this setting no correlation between the clinical response and the hormone levels was found.

The discrepancies in the hormonal and therapeutic effects of triphenylethylene antioestrogens mentioned above may be explained at least partly by their effects directly on the breast tissue on one hand and on the hypothalamo-hypophyseal axis on the other hand. It has been shown that tamoxifen, when administered only during the luteal phase of the menstrual cycle, significantly reduces the nuclear volume and mitotic activity of the normal breast tissue (16). Consequently, the local antioestrogenic effect may override the breast pain provoking effects of the elevated circulating estradiol levels associated with the use of tamoxifen or toremifene during the luteal phase of the menstrual cycle. The next phase in our research project on toremifene and mastalgia is indeed focusing on the effect of toremifene on the breast tissue as evaluated by aid of magnetic resonance imaging.

In conclusion, toremifene administered during the luteal phase of the menstrual cycle exerts a luteotropic effect and possibly also has prolactinogenic activity in women being successfully treated for premenstrual mastalgia. Further studies on the mechanism of action of toremifene in alleviating cyclic breast pain are warranted.

Abbreviations: CV: Coefficient of variation, A: Androstenedione, E: Estradiol, P: Progesterone , PRL: Prolactin, total T: Total testosterone, free T: Free testosterone

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CONTRACEPTION

Toremifene use does not alter serum inhibin A and B levels during mid-luteal phase in women with premenstrual mastalgia

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Abstract

Aims. To find out if there is any link between the therapeutic effect of toremifene on premenstrual mastalgia and luteal phase serum inhibin A and/or B levels.

Methods. Forty-eight patients participating in a randomized cross-over trial on toremifene *vs.* placebo for premenstrual mastalgia gave three blood samples during the luteal phase of the menstrual cycle: the first at baseline, the second during the third toremifene/placebo cycle, and the third during the third placebo/toremifene cycle, respectively. The blood samples were analyzed for inhibin A and B with respective specific two-site enzyme-linked immunosorbent assays. Toremifene (20 mg/d) and placebo were administered during the luteal phase only.

Results. When all the toremifene-treated cycles were compared with all the placebo cycles and with the baseline, the median inhibin A levels were 42, 38, and 40 pg/ml, respectively (baseline *versus* toremifene, $p = 0.638$; baseline *versus* placebo, $p = 0.468$; and toremifene *versus* placebo, $p = 0.365$). The median inhibin B levels were at baseline 19 ng/l, during placebo 20 ng/l, and during toremifene 17 ng/l (baseline *versus* toremifene, $p = 0.983$; baseline *versus* placebo, $p = 0.519$; and toremifene *versus* placebo, $p = 0.880$).

Conclusion. A luteal administration of toremifene does not seem to result in any changes in mid-luteal concentrations of inhibin A or B in serum.

Keywords: *Premenstrual syndrome, mastalgia, toremifene, SERM, breast, inhibin A, inhibin B*

Introduction

Benign breast pain is a common complaint of women. It has been reported that as many as 41–69% of women have mastalgia that interferes with their daily routines [1]. Two thirds of breast pain is cyclic. Discomfort lasting for 1 to 4 days can be considered 'normal' [2]. However, ∼8–10% of premenopausal women suffer monthly from moderate to severe breast pain that lasts for more than 5 days. Hormonal studies have failed to reveal any specific endocrine cause of cyclic mastalgia, despite its close relation to the menstrual cycle [2–4]. Several hormonal imbalances with potential causative roles in cyclic mastalgia have been investigated, with contradictory results [5]. Potential candidates include absolute or relative hyperestrogenism [6], luteal insufficiency [7], hyper-

prolactinemia [8], and hyperandrogenism [9]. However, premenstrual mastalgia is unequivocally worsened by exogenous estrogens and improved by suppression or elimination of the ovarian cycle [4].

Inhibins are heterodimeric glycoprotein hormones, which are built up from an α and one of two partially homologous β subunits, βA or βB, giving rise to the two isoforms inhibin A and inhibin B. The gonads are in both sexes the most important site of inhibin production. Both inhibin forms have the capacity specifically to suppress follicle-stimulating hormone (FSH) secretion by pituitary cells in culture, without affecting luteinizing hormone (LH) secretion [10]. Inhibin B rises from the late luteal phase to the mid-follicular phase in concert with FSH, whereas inhibin A increases in the late follicular phase along with LH and peaks in the mid-luteal phase [11].

Consequently, if it is assumed that a relative or absolute hyperestrogenism plays a role in the pathogenesis of premenstrual mastalgia one would expect that serum inhibin levels were higher in women with mastalgia, and vice versa that therapeutic interventions would in turn decrease the levels. However, we were not able to find any previous reports on inhibin levels associated with the premenstrual syndrome in general or premenstrual mastalgia in particular.

Tamoxifen, a specific estrogen receptor modulator (SERM) primarily used in the treatment of breast cancer has previously been found to be effective in reducing premenstrual mastalgia [12]. We have shown that another SERM or toremifene significantly alleviates cyclic breast pain as compared to placebo [13]. The mechanism by which these agents work in premenstrual mastalgia is quite poorly understood but both tamoxifen and toremifene seem, quite paradoxically, to be luteotropic when administered during the luteal phase only [14,15]. The luteotropic effect should theoretically lead to an increased production of inhibins. On the other hand, based on the discussion above on the potential role of inhibins on mastalgia, serum inhibin levels should rather decrease as a response to SERMs.

The aim of the present study was to investigate if there are any measurable changes in luteal phase serum inhibin levels attributable to toremifene in women suffering from premenstrual mastalgia.

Methods

Patients

The recruitment, randomization and study treatment procedures have been described previously [13]. In brief, 62 women suffering from marked premenstrual mastalgia were randomly allocated to receive toremifene (20 mg) or placebo during the luteal phase for three consecutive cycles. The patients were then crossed over after a wash-out period to placebo or toremifene, respectively. The participants were instructed to take one tablet daily from cycle day 15 until next menstruation. Venous blood samples were collected at baseline, and during the third of the toremifene and placebo cycles, respectively. The clinical characteristics of the participants are shown in Table I. The study protocol was approved by the Regional Ethics Committees of Pori Central Hospital and Tampere University Hospital, and all the women gave a written informed consent before enrolment.

Assays

Venous blood samples for determination of serum inhibin A and B were obtained at three occasions during the luteal phase (cycle day 20–22) of the

Table I. The clinical characteristics of the participants.

	All (*n* = 62)	Followed up (*n* = 48)
Demographics		
Age (yr), Median [Range]	42 [25–45]	42 [25–45]
Weight (kg), Mean (SD)	68.99 (12.62)	67.80 (13.68)
Age at menarche (yr), Mean (SD)	13.18 (1.30)	13.10 (1.31)
Length of menstrual cycle (d), Mean (SD)	27.77 (2.03)	27.81 (2.05)
Parity		
Median [Range]	2 [0–5]	2 [0–3]
Nulliparous (*n*)	12	10
With one child (*n*)	12	10
With two or more children (*n*)	37	27
Not known	1	1
Contraception		
Sterilization (*n*)	37	30
Barrier method (*n*)	21	15
Intrauterine device (*n*)	4	3

menstrual cycle: at baseline, and during the third cycle of toremifene and placebo treatment, respectively. Samples were collected from each subject while fasting, and serum was separated and frozen at −20°C until assayed. Serum inhibin B concentrations were determined using specific two-site enzyme-linked immunosorbent assay (ELISA) from Oxford Bio-Innovation, Oxford, UK. In the inhibin B assay, the detection limit was 16 ng/l and the intra- and inter- assay coefficients of variations (CVs) were less than 5% and 12%, respectively. The reference range of serum inhibin B for a normal menstrual cycle was 5–200 ng/l.

Serum inhibin A levels were measured by means of two-site ELISA from Diagnostic Systems Laboratories, Texas, USA. The detection limit of the inhibin A assay was 10 pg/ml and the interassay CVs for low-and high-pooled values were 3.3 and 4.6%, respectively. The reference range of serum inhibin A for the mid-luteal phase was 13–160 pg/ml. The inhibin A assays were performed at Limbach laboratories, Heidelberg, Germany and inhibin B assays at the laboratory of the University Hospital of Helsinki, Helsinki, Finland.

Statistical analysis

Normality of the distribution of the continuous variables studied was tested by Shapiro–Wilk's test. The distributions of all variables were skewed. Differences between baseline and placebo, between baseline and toremifene, and between placebo and toremifene were tested by Wilcoxon Signed Ranks test. The statistical analyses were performed by SPSS version 14.0.2, for Windows (SPSS, Chicago, IL). A *p*-value less than 0.05 was considered as statistically significant.

Results

Sixty-two subjects were eligible and followed up for the baseline cycle after a telephone pre-screening. Thirty-two were randomized to receive toremifene and 30 to receive placebo for the first three consecutive menstrual cycles. Three patients from each group were excluded from the pain score analysis: Three participants discontinued the trial for personal reasons, one patient had no pain symptoms any more, for one patient the instructions were too difficult to follow, and one participant had menstrual disturbances before starting the trial medication. Finally, for the present study, we included only the participants, who had given blood samples at all measuring points. A total of 48 women (24 in both randomized groups) gave all three blood samples. The median serum mid-luteal phase inhibin A and B levels and interquartile ranges are shown in Table II. All the inhibin A and B levels were between normal values. The baseline level was compared to all toremifene and placebo cycles, respectively. The median inhibin A concentration was 40 pg/ml during the toremifene cycles, while it was 42 pg/ml at baseline and 38 pg/ml during the placebo cycles, respectively (baseline *versus* toremifene, $p = 0.638$; baseline *versus* placebo, $p = 0.468$; and toremifene *versus* placebo, $p = 0.365$). The median inhibin B level was at baseline 19 ng/l, during placebo 20 ng/l and during toremifene 17 ng/l (baseline *versus* toremifene, $p = 0.983$; baseline *versus* placebo, $p = 0.519$; and toremifene *versus* placebo, $p = 0.880$).

Discussion

When given to healthy women during the luteal phase, tamoxifen 10 mg/day exerts a luteotropic effect as reflected in increasing serum progesterone and estradiol levels [14]. We have demonstrated earlier that also toremifene 20 mg/d during the luteal phase is luteotropic in women with premenstrual mastalgia [15]. Because inhibin A is a product of the periovulatory follicle and corpus luteum [16,17], one would assume that toremifene should have increased the mid-luteal serum levels of inhibin A. On the other hand, given the fact that opposing hyperestrogenism alleviates breast pain [4] as does also toremifene [13],

a decrease in inhibin levels would also have been possible. Neither of these, however, happened; toremifene seemed to have no effect on the production of inhibin A (or B). Consequently, we suggest that the mechanism by which toremifene has a therapeutic effect in premenstrual mastalgia does not involve stimulation or inhibition of ovarian inhibin production. Moreover, it is not likely that the luteotropic effect of both tamoxifen and toremifene would be related to their ability to reduce the cyclic breast pain.

During the menstrual cycle, the mammary gland goes through sequential waves of proliferation and apoptosis. The highest proliferative activity is during the luteal phase of the menstrual cycle, a time when both serum estradiol and progesterone concentrations are high [18]. The polypeptide hormone prolactin and the steroid hormones estrogen and progesterone act synergistically to increase the gland growth, development, and differentiation [19]. Therefore, another puzzling finding we made in our previous study was that toremifene is not only luteotropic but also somewhat prolactinogenic [13]. However, one should take into account that estrogen in the breast does not originate only from the ovaries. The breast itself produces a significant proportion of tissue estrogens [20]. In nipple aspirate fluid from a normal breast, estrogen levels have been found to be significantly higher than plasma levels are [21]. The decline in serum estradiol seen with the menopause is not seen to the same extent in nipple aspirate fluid [22]. Women with high plasma progesterone levels have been found to have significantly increased levels of estradiol in breast tissue, which suggest that progesterone may be a regulator in the local conversion of estrogen precursors into the potent estradiol in the normal breast tissue [23]. Because of this autocrine and paracrine activity of the mammary gland it is perhaps not reasonable to concentrate on plasma levels of sex hormones while studying the pathophysiology of cyclic mastalgia. Instead, our negative findings highlight the importance of focusing on local factors and phenomena in trying to understand the mechanism of action of SERMs on the premenopausal breast. Indeed, a group from Brazil has demonstrated that tamoxifen when administered only during the luteal phase of the menstrual

Table II. Median (Md) of inhibin levels of patients with interquartile ranges (Iq).

Hormone	Baseline Md (Iq)	Placebo Md (Iq)	Toremifene Md (Iq)	p_1	p_2	p_3
Inhibin A pg/ml	42 (27–53)	38 (15–67)	40 (19–62)	0.468	0.638	0.365
Inhibin B ng/l	19 (15–30)	20 (15–29)	17 (15–27)	0.519	0.983	0.880

Differences between baseline and placebo (p_1), between baseline and toremifene (p_2) and between placebo and toremifene (p_3) were tested by Wilcoxon Signed Ranks test ($n = 48$).

cycle, significantly reduces the number of lysosomes, the nuclear volume and mitotic activity in the mammary epithelium [24,25].

In conclusion, the findings of the present study imply that the therapeutic effect of toremifene in premenstrual mastalgia is not linked to any alterations in the inhibin production by the ovary.

Acknowledgements

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Breast Magnetic Resonance Imaging Findings in Women Treated with Toremifene for Premenstrual Mastalgia

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Oksa S, Parkkola R, Luukkaala T, Mäenpää J. Breast magnetic resonance imaging findings in women treated with toremifene for premenstrual mastalgia. Acta Radiol 2009;50:984–989.

Background: Toremifene, a selective estrogen receptor modulator, has been shown to be effective in alleviating premenstrual breast pain. However, the exact mechanism by which toremifene and related compounds work in premenstrual mastalgia is poorly understood.
Purpose: To find out if the effect of toremifene on breast would be detectable with dynamic magnetic resonance imaging (MRI).
Material and Methods: This randomized, double-blind crossover study was performed on women suffering from marked premenstrual mastalgia. Ten women were randomized to receive either toremifene (20 mg) or placebo from cycle day 15 until next menstruation for three menstrual cycles. After a washout period, the treatment was crossed over for three additional cycles. The MRI evaluations were performed premenstrually at the end of each treatment phase. Breast pain and quality-of-life scores were collected from one baseline cycle and from all the treatment cycles.
Results: Nine patients were evaluable for this analysis. Both the enhancement ratio and the maximum slope of enhancement tended to be smaller during the toremifene cycles as compared to placebo. On the left side, the difference in the maximum slope of enhancement between toremifene and placebo was statistically significant (median 5.150 [range 3.7–6.7] and 6.500 [range 4.9–9.5], respectively; $P = 0.047$). T2 relaxation times as well as breast pain and quality-of-life scores were inconsistent.
Conclusion: Use of toremifene is associated with measurable changes in dynamic breast MRI findings in women with cyclic breast pain.

Key words: breast; mastalgia; MRI; premenstrual syndrome; SERM; toremifene

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Premenstrual mastalgia is a common symptom that affects up to 80% of women during some time in their reproductive years. In about one-third of the women, mastalgia is severe enough to interfere with their usual sexual and physical activities (1). Cyclic breast pain has a temporal association with the menstrual cycle. Pain characteristically begins during the luteal phase of the cycle, builds in intensity as menstruation approaches, and then subsides once menstruation has started and often disappears after a few days (2).

Cyclic mastalgia is worsened by exogenous estrogens and is improved by suppression of the ovarian cycle (3). Also, selective estrogen receptor modulators (SERMs), such as tamoxifen (4) and toremifene, have activity in this setting. We have shown earlier that toremifene, when administrated during the luteal phase of the menstrual cycle, significantly reduces mastalgia as compared to placebo (5). The exact mechanism by which tamoxifen and toremifene alleviate premenstrual mastalgia is not clearly understood but both seem, somewhat paradoxically, to have luteotropic effects (6, 7).

Recently, gadolinium-enhanced magnetic resonance imaging (MRI) has emerged as a sensitive

method for finding subclinical premenopausal breast cancer (8). MRI also has potential in detecting efficacy of neoadjuvant chemotherapy prior to measurable changes in tumor size (9). The chronic administration of another SERM or raloxifene to premenopausal women seems to result in a decline in breast MRI volume (10). The present study was undertaken to find out if mammary effects of toremifene administered cyclically to women suffering from premenstrual mastalgia could be depicted by dynamic contrast-enhanced MRI, and even be correlated with a possible therapeutic effect.

Material and Methods

By means of newspaper advertisements or referral from their gynecologist or general practitioner, 10 women with marked cyclic mastalgia were recruited to this study between March and May 2007. The age of the subjects ranged from 37 to 45 years. A brief telephone interview had eliminated women using hormonal contraception or treatment; those reporting irregular menstrual cycles, history of breast or endometrial cancer, hysterectomy and/or bilateral oophorectomy, pregnancy, lactation, or a desire for pregnancy; and those having artificial cardiac pacemaker or metallic prostheses. Other exclusion criteria included history of thromboembolic disease or radiation therapy. The inclusion criteria were: 1) mastalgia lasting for more than 5 days during the luteal phase of the menstrual cycle, severe enough to interfere with social life and normal activities, and to cause desire to use medication against it; 2) age 20–45 years; and 3) reliable non-hormonal contraception. The participants underwent a careful clinical examination at baseline, including a vaginal ultrasound examination, breast palpation, and a detailed medical history.

After a single non-medicated baseline menstrual cycle, the women were randomly allocated to receive either toremifene (20 mg/day) or placebo during the luteal phase of the menstrual cycle for three consecutive cycles. After an untreated washout period lasting the length of one menstrual cycle, the participants were then crossed over to placebo or toremifene, respectively, for three additional cycles. The study medication was given in tablet form, and the women were instructed to take one tablet daily from cycle day 15 until the next menstruation. The active (toremifene 20 mg) and control (placebo) tablets were identical in appearance. A computerized block-randomization system was allocated to ensure an even distribution of toremifene and placebo. This system was independent of the investigators. The

codes were kept confidential until the end of the study. All participants and study investigators were blinded as to the randomization codes, and the screening and follow-up study assessments were completed by study personnel blinded to the randomization code. Orion Pharma (Espoo and Turku, Finland) provided the double blinding and the dispensing of medication. Adherence was assessed by a pill count.

The primary outcome variables of the study were changes in MRI contrast enhancement ratio, maximum slope of enhancement, and T2 relaxation times associated with toremifene and placebo treatment. The MRI investigations were performed on both breasts during the premenstrual period, within 5 days prior to menstruation, on two occasions, on the third toremifene or placebo cycle, respectively. The imaging was performed using Philips Gyroscan Intera 1.5T equipment (Philips Medical Imaging, Best, The Netherlands). The imaging sequences were as follows:

1. Axial T2-weighted spectral presaturation inversion recovery (SPIR) images, with the following parameters: repetition/echo time (TR/TE) 3000/120 ms, flip angle (FA) 90°, matrix 512 × 512, field of view (FOV) 350 × 350 mm, and slice thickness 4 mm.
2. Axial T1-weighted SPIR images, with the following parameters: TR/TE 550/11 ms, FA 90°, matrix 512 × 512, FOV 350 × 350 mm, and slice thickness 4 mm.
3. Axial dynamic T1-weighted images, with the following parameters; TR/TE 8.6/4.3 ms, FA 25°, matrix 512 × 512, FOV 350 × 350 mm, slice thickness 4 mm, and temporal resolution 1 min 3 s. During this sequence, contrast agent (Dotarem; Guerbet, Roissy, France) was administrated intravenously (0.1 mol/kg of body weight) after the first scan. The dynamic scan was repeated six times.
4. Axial T1-weighted SPIR images, with the following parameters and contrast enhancement: TR/TE 550/11 ms, FA 90°, matrix 512 × 512, FOV 350 × 350 mm, and slice thickness 4 mm.
5. Axial T2 calculation single-slice sequence with TR 1000 ms, matrix 256 × 256, FOV 380 × 380 mm, slice thickness 5 mm, and TE values of 20, 40, 60, 80, 100, 120, 140, and 160 ms at the level of the nipples.

All the sequences covered the whole breasts, except the T2 calculation single slice. After imaging,

subtraction of the dynamic images was performed, and dynamic enhancement curves of the retro-mamillar glandular tissue were determined with a GE workstation (GE AWS 4.3; GE Medical Imaging, Milwaukee, Wisc., USA) using a circular region-of-interest (ROI) tool. The T2 calculation of the retromamillar glandular tissue was performed from a single-slice sequence obtained at the level of the mamilla using the above-mentioned GE work-station. The clinical assessment of the images and dynamic scans, and enhancement curves was per-formed according to the BI-RADS lexicon for MR mammography, released in November 2003 (11). One experienced MR radiologist assessed the MR images blindly, without information regarding the symptoms or medication of the patient, and performed the postprocessing analysis. Breast MR imaging was performed in Turun TeslaVagus Oy, Turku, Finland. The radiologist responsible for the MR imaging was the author R.K.P.

Secondary endpoints included pain and quality-of-life scores. The participants were asked to rate intensity of mastalgia with a numeric (0–10) visual analogue scale (VAS). They completed a breast pain VAS at the luteal phase of the baseline cycle and of each menstrual cycle until the end of the study. The days of the menstruation were recorded during the study by the participants. To estimate the quality of life, we used a modified 36-item Finnish DEPS Scale (score range 0–108) (12). At the end of the study, the participants were interviewed individually, and the quality-of-life questionnaires and other study-related material were collected.

The study protocol was approved in advance by the regional ethics committee of Satakunta Central Hospital. All the women gave written informed consent before enrolment and also before MRI examination. The trial was registered at clinical-trials.gov (identifier NCT00534846).

Statistical analysis

Due to the small sample size, nonparametric exact tests were used. Differences between toremifene and placebo cycles were analyzed by the Wilcoxon signed-ranks test. Statistical analyses were per-formed by SPSS for Windows, version 16.0.2. (SPSS Inc., Chicago, Ill., USA). P values less than 0.05 were considered as statistically significant.

Results

One patient (patient 2) discontinued her participa-tion after the first appointment, and she never took the medication. The clinical characteristics of the remaining nine patients were as follows: mean age 42 years (range 37–45 years), and mean weight 70 kg (range 59–78 kg). Of the women, eight were parous, and one was nulliparous. Five were using the barrier method for birth control, while four had been surgically sterilized. The database is incomplete for two patients. Patient 6 withdrew her consent after the first MRI, because she felt claustrophobic during the examination. Consequently, only data on the base-line and toremifene cycles, respectively, are available for her. The MRI data on patient 4 during the placebo phase are missing due to technical problems with the MRI equipment during her appointment.

Six patients had normal MRI findings, while two patients (patients 1 and 3) were found to have small benign fibroadenomas, 7 and 6 mm in diameter, respectively.

The results of the dynamic contrast-enhanced MRI scans are given in Table 1. The median enhancement ratio of the right breast during the toremifene vs. placebo cycles was 8.50% (range 3–22%) vs. 12.50% (range 6–26%), respectively ($P = 0.235$), and for the left breast, 7.50% (range 3–22%) vs. 17.00% (range 5–32%), respectively ($P = 0.074$). Although, the differences are not sta-tistically significant, the enhancement ratio tended to be smaller during treatment with toremifene on the left side. The dynamic enhancement curves of patient 7 are given in Fig. 1. She had been given toremifene first (Table 1). During the latter or placebo cycle, the enhancement was more intense.

The median maximum slope of enhancement in the right breast during the toremifene versus placebo cycles was 5.9 (range 4.3–9.9) vs. 6.7 (range 6.1–7.6), respectively. The corresponding figures for the left breast were 5.2 (range 3.7–6.7) and 6.5 (range 4.9–9.5), respectively. For the left breast, the difference was statistically significant ($P = 0.047$). The median T2 relaxation times in both breasts varied between 55 and 79 ms, with no differences between the toremifene and placebo cycles.

The mastalgia VAS scores are shown in Fig. 2. Although the median VAS score during the placebo cycles was 6.33 and only 1.83 during the toremifene cycles, the difference is statistically only of border-line significance ($P = 0.078$), probably due to the small sample size. On the other hand, the median quality-of-life score appeared to be slightly lower (39.5, range 18–66) during the toremifene cycles as compared to the placebo cycles (41, range 31–68). Here, the difference was statistically significant, with a P value of 0.047.

Table 1. Dynamic MRI results

| Patient no.	Cycle	Enhancement ratio, right	Enhancement ratio, left	Mean max. slope of enhancement, right (SD)	Mean max. slope of enhancement, left (SD)	T2 relaxation time, right	T2 relaxation time, left
1	TOR	7%	9%	7.5 (3.8)	6.7 (3.9)	64 ms	63 ms
1	Placebo	10%	19%	6.6 (4.5)	6.6 (3.1)	69 ms	72 ms
3	TOR	12%	10%	5.9 (2.7)	4.7 (2.3)	68 ms	80 ms
3	Placebo	6%	5%	6.4 (2.6)	5.4 (2.8)	72 ms	79 ms
4	TOR.	14%	22%	7.2 (3.9)	7.9 (4.7)	58 ms	58 ms
4	Placebo	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5	TOR	22%	20%	5.7 (2.0)	5.5 (1.9)	55 ms	65 ms
5	Placebo	20%	22%	7.6 (4.5)	9.5 (6.3)	66 ms	59 ms
6	TOR	6%	6%	9.9 (5.6)	6.2 (2.2)	71 ms	72 ms
6	Placebo	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	TOR	14%	22%	5.8 (2.4)	5.8 (3.1)	60 ms	64 ms
7	Placebo	26%	32%	7.5 (2.9)	6.4 (2.0)	63 ms	70 ms
8	TOR	3%	3%	4.3 (1.5)	3.7 (1.2)	72 ms	72 ms
8	Placebo	6%	10%	6.6 (3.8)	7.0 (2.7)	72 ms	71 ms
9	TOR	6%	6%	4.6 (2.2)	4.6 (1.9)	62 ms	69 ms
9	Placebo	18%	15%	6.8 (3.9)	5.6 (2.5)	67 ms	73 ms
10	TOR	10%	6%	6.2 (3.3)	4.8 (2.2)	72 ms	73 ms
10	Placebo	11%	6%	6.1 (2.3)	4.9 (2.1)	70 ms	71 ms

TOR: toremifene; n.d.: no data; SD: standard deviation.
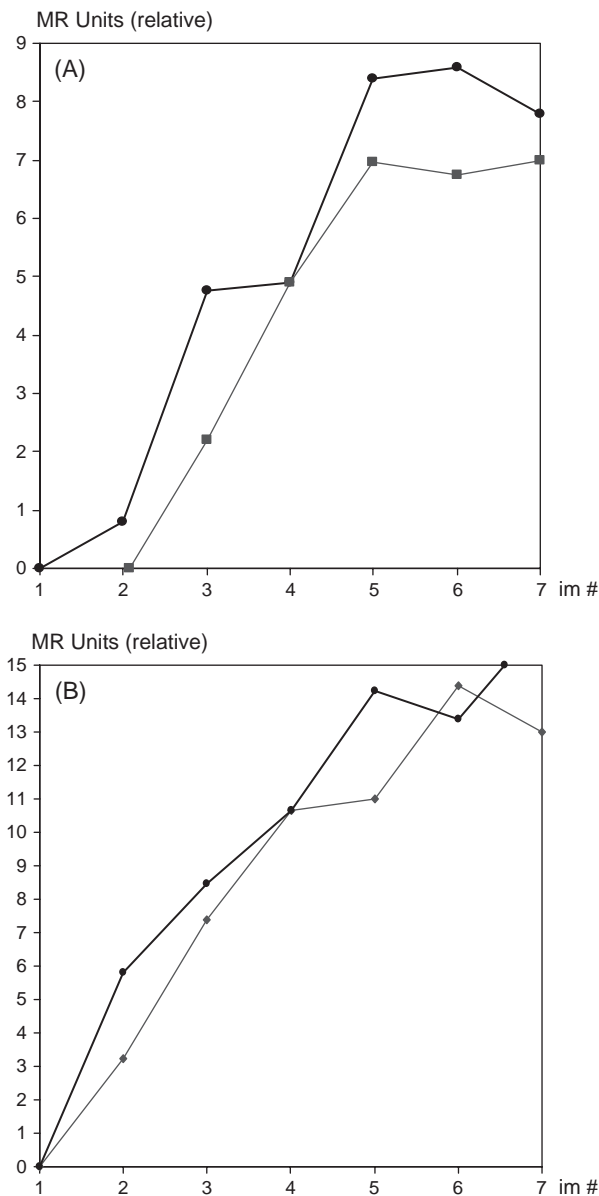
Fig. 1. Dynamic enhancement curves of the glandular tissue of both breasts during the first (A) and second (B) MRI.
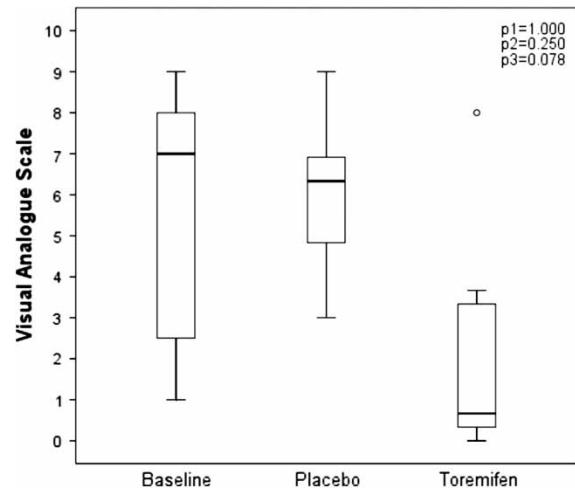

Fig. 2. Mastalgia VAS scores are shown by median (black line), interquartile range (box), and range (line bar) at baseline, and during the toremifene and placebo cycles. Outliers are expressed as dots. Differences between the baseline cycle and the placebo cycles (p1), between the baseline cycle and the toremifene cycles (p2), and between the placebo and toremifene cycles (p3) were tested by the Wilcoxon signed-ranks test.

Discussion

The hormonal changes associated with the menstrual cycle are reflected in both static and dynamic MRI findings of the premenopausal breast. Total breast volume and parenchymal volume have been reported to be larger in the luteal phase as compared to the follicular phase (13, 14). Similarly, there is a significant increase in enhancement and in the extraction flow product during the latter half of the menstrual cycle (15, 16). Hence, it has been recommended that, for premenopausal women, contrast-enhanced MRI should be performed between days 6 and 17 of the menstrual cycle (17). However, women with severe mastalgia experience maximum pain only a few days before menstruation. Consequently, MR imaging had to be performed at the end of the menstrual cycle in this study.

Toremifene seemed to reduce both the enhancement ratio and the maximum slope of enhancement as compared to placebo, while diminishing premenstrual pain scores. The reduction was more marked on the left side, where the difference in the maximum slope of enhancement between the toremifene and placebo cycles was statistically significant. The findings indicate that the therapeutic effect of toremifene in alleviating premenstrual breast pain may at least partly be mediated through diminished blood flow to the breast.

T2 relaxation times did not differ between the toremifene and placebo cycles. There have been conflicting reports with regard to how the menstrual cycle affects relaxation time. FOWLER et al. (13) reported that (T1) relaxation time increased during the luteal phase. In contrast, DELILLE et al. were not able to find any significant changes in (T1) relaxation times during the menstrual cycle (16).

We have previously demonstrated that the cyclic administration of toremifene 20 mg daily reduces premenstrual pain scores by 64%, with a statistically significant difference as compared both to the baseline and to the effect of placebo (5). Although toremifene apparently reduced the pain scores in the present study, the difference this time was statistically not quite significant, probably due to a much smaller sample size (nine vs. 56 patients,

respectively). In our previous study, toremifene also had luteotropic effects (7). The results of the present study, however, imply that a direct antiestrogenic effect of toremifene on the physiology of the breast, as reflected in the reversal of MRI findings typical of the luteal phase, is the mechanism by which toremifene alleviates premenopausal breast pain, rather than its systemic luteotropic effect. Previous reports on related SERMs, such as tamoxifen and raloxifene, reinforce the interpretation that, at least in the premenopausal setting, the effects of SERMs on the breast are mediated through a local rather than a systemic effect. Tamoxifen prevents premenopausal breast cancer (18) and is effective in reducing premenstrual breast pain (4), while decreasing mammographic breast density, especially in premenopausal women (19), and being luteotropic (6). Interestingly, raloxifene, the chemopreventive properties of which in the premenopausal setting are yet to be proven, seems to reduce the MRI volume of the premenopausal breast but does not seem to significantly affect the mammographic density (10).

In conclusion, the results of this study imply that toremifene reduces the MRI enhancement ratio and the maximum slope of enhancement in the breasts of patients with marked premenstrual mastalgia. This phenomenon may at least partially explain its therapeutic effect on cyclic breast pain.

Acknowledgements

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Effect of toremifene on premenopausal breast as evaluated by three dimensional power Doppler ultrasound. Preliminary findings.

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Short title: Toremifene and premenopausal breast on 3D US.

Manuscript category: Original research.

List of abbreviations:

Three dimensional ultrasound (3D US), selective estrogen receptor modulator (SERM), magnetic resonance imaging (MRI), two dimensional ultrasound (2D US), relative stopping power index (RSP), virtual organ computed aided analysis (VOCAL), vascularization index (VI), flow index (FI), vascularization flow index (VFI)

Abstract

Objective Toremifene, like another selective estrogen receptor modulator tamoxifen, has been found to alleviate cyclic mastalgia but their mechanism of action in this respect is unclear. The purpose of the study was to find out if toremifene causes in the premenopausal breast any vascular or volume changes that are measurable with three dimensional ultrasound (3D US).

Methods Twenty healthy premenopausal women were recruited to the study. Following a single non-medicated baseline menstrual cycle, the participants received toremifene 20 mg/d from cycle day 15 until a second examination. The 3D US evaluations were performed within 5 days prior to menstruation. The power Doppler setting was standardized with a 20 degree- volume angle and maximum quality. Four breast quadrants were assessed.

Results There were no significant differences between the assessed volumes during the baseline cycle or during the toremifene cycle in either breast (p=0.126 on the right side, p=0.748 on the left side, respectively). Neither were the differences in the vascularization indices VI, VFI, and FI measured from the four quadrants of each breast significant between the baseline cycle and toremifene treated cycle.

Conclusion Toremifene did not have any marked effects on the breast volume and vasculature in healthy premenopausal volunteers.

Introduction

Premenstrual mastalgia is a common symptom experienced by up to 80% of women during some time in their reproductive years. In about one third of the women mastalgia is severe enough to interfere with their usual sexual and physical activities (1). Cyclic breast pain has a temporal association with the menstrual cycle. Pain characteristically begins during the luteal phase of the cycle, builds in intensity as menstruation approaches, and then subsides once menstruation has started and often disappears after few days (2).

Cyclic mastalgia is worsened by exogenous estrogens and is improved by suppression of the ovarian cycle (3). Also selective estrogen receptor modulators (SERMs), such as tamoxifen (4) and toremifene, have activity in this setting. We have shown earlier that toremifene, when administrated during the luteal phase of the menstrual cycle, significantly reduces mastalgia as compared to placebo (5). The exact mechanism by which tamoxifen and toremifene alleviate premenstrual mastalgia is not clearly understood but both seem, somewhat paradoxically, to have luteotropic effects (6, 7). Mammary effects of toremifene administered cyclically to women suffering from premenstrual mastalgia, could be depicted by dynamic contrast enhanced magnetic resonance imaging (MRI). Toremifene seemed to reduce the enhancement ratio and especially the maximum slope of enhancement as compared to placebo, while diminishing premenstrual pain scores (8). This indicates that the therapeutic effect of toremifene in alleviating premenstrual breast pain may at least partly be mediated through a diminished blood flow to the breast. The chronic administration of another SERM or raloxifene to premenopausal women seems to result in a decline in breast volume as measured with MRI (9).

The potential of combining volume and vascular data that 3D US can offer has made it popular in various clinical settings. The 3D US has been used to estimate the placental volume and vascular flow indices in the prediction of adverse pregnancy outcomes (10, 11). In gynecology the evaluation of vascularization by 3D power Doppler sonography may increase the diagnostic accuracy in the work-up of adnexal masses (12), endometrial (13, 14) and cervical (15) cancer. The method has also been used in predicting pregnancy and ovarian response during infertility treatments (16, 17). In breast diseases the conventional 2D US is very useful for distinguishing breast cysts (18), but 3D US has the capacity to delineate lesion margins and topography, thereby helping to differentiate benign tumors from malignant ones (19,20,21). In addition, 3D US can help in facilitating the

needle localization and guidance during a breast biopsy, thereby reducing the number of core samples needed to achieve a reliable histological diagnosis (22).

To our knowledge, 3D power Doppler sonography has not previously been applied to benign breast diseases. However, based on the experience with other organs, 3D power Doppler imaging may have potential to detect even minimal blood flow changes in breast tissue, and to assess changes in flow and vascularity that occur in response to therapeutic efforts. At least measurements of the placental volume and vascularization obtained by the 3D power Doppler US have been shown to have a good reproducibility (23, 24). These placental studies have been performed dividing the organ into a few quadrants, to overcome the problem that the entire placenta cannot be scanned and measured simultaneously (25, 26).

The aim of the present study was to explore if toremifene causes any differences in mammary blood flow and/or volume, which are detectable with the 3D power Doppler US.

Materials and Methods

Twenty healthy premenopausal women volunteered to the study. The age of the subjects ranged from 25 to 45 years. The inclusion criteria were as follows: age 20-45 years and reliable non-hormonal contraception. At the beginning a brief telephone interview eliminated women using hormonal contraception or treatment, and those reporting irregular menstrual cycles, history of breast or endometrial cancer, hysterectomy and/or bilateral oophorectomy, pregnancy, lactation, or a desire for pregnancy. Other exclusion criteria comprised of a history of thromboembolic disease or radiation therapy.

Following a single non-medicated baseline menstrual cycle the participants received toremifene at a dose 20 mg/day orally from cycle day 15 until the second examination. At baseline a detailed medical history was taken followed by a careful clinical examination including breast palpation, pelvic examination, and vaginal 2D US using Voluson 730 PRO device equipped with a transvaginal transducer 3.3-10 MHz (GE Medical Systems Kretztechnik GmbH & co, Zipf, Austria). The breast 3D US scans were performed within 5 days prior to menstruation, on two occasions, one during the untreated baseline cycle and the other during the toremifene cycle using Voluson i device with a relative stopping power index (RSP) 3D transducer RSP 6-16 frequency of 5.6-18.4 MHz and rotational virtual organ computed aided analysis (VOCAL) (GE Medical Systems Kretztechnik GmbH & co, Zipf, Austria). The probe was held still and the patients were asked to stop breathing for a few seconds to allow the US unit to generate the 3D data. All imaging was performed with the patient lying in the supine position with one arm extended overhead. The measurements were made using a customized breast program preset (GE Medical Systems Kretztechnik GmbH & co, Zipf, Austria). The same pre-established instrument power settings were used in all cases. The power Doppler setting was standardized with twenty degrees volume angle and maximum quality. From the both breasts four quadrants were measured as shown in (Figure 1). One side of the probe was placed on the edge of the areola and the other side was pointed towards eleven, two, five and seven o'clock, respectively. The frame of the region of interest was adjusted by a manual tracing to include skin at the top and fascia of the pectoral muscle at the bottom. The 3D volume mode was switched on. Manual drawing of the contour of each quadrant was performed in plane A. At the end of the 180° rotation, the built-in software calculated the volume, and the three vascularity indices or the vascularization index (VI), the flow index (FI) and the vascularization flow index (VFI) automatically. VI, expressed as a percentage, is the proportion of color voxels in the studied volume, representing the proportion of blood vessels within the tissue;

FI, expressed as a scale of 0–100, is the average value of all color voxels, representing the average power Doppler amplitude within blood vessels; VFI, expressed as a scale of 0–100, is the average color value of all grey and color voxels, a product of the number of color voxels as a percentage and the relative amplitude of these voxels. All collected data was saved on the hard disc of the equipment for further analysis. The compliance with study medication was assessed by counting the returned tablets at the end of the study. The investigations, all performed by one investigator (SO) took place at a private clinic (Porin Lääkäritalo), in Pori, Finland from August to December, 2010. SO is an experienced gynecologist with 12-year long experience in 2D US. The study protocol was approved by the Regional Ethics Committee of Satakunta Central Hospital, and all women gave a written informed consent before enrollment. The trial was registered at ClinicalTrials.gov (identifier NCT01417754).

Statistical analyses

Due to the small sample size, nonparametric exact tests were used. Differences between toremifene and baseline cycles and between right and left sides were analyzed by the related samples Wilcoxon signed-ranks test. Statistical analyses were performed by SPSS for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). P values less than 0.05 were considered as statistically significant.

Results

All 20 women were included in the final analysis. The median age of the participants was 39.5 years (range 25-45 years) and the median body mass index 23.9 (range 18.6-35.9). Of the women, 16 were parous, and four were nulliparous. Thirteen women were using the barrier method for birth control, while seven had been surgically sterilized. Nineteen women had normal vaginal US findings, but one patient was found to have a small submucosal leiomyoma, 25 mm in diameter. All participants had normal breasts on palpation, and neither were any abnormal 2D or 3D US breast findings.

The combined volumes of the four breast quadrants of both breasts are shown in (Figure 2). As compared to the baseline, the breast volumes remained essentially same during the toremifene treatment. The median volumes of the both breasts tended to be higher during treatment with toremifene. However, the differences between the assessed volumes from the baseline cycle and from the toremifene cycle were not significant on either side (p=0.126 on the right breast, and

p=0.748 on the left breast, respectively). The difference between the volumes of right and left breast was significant (p=0.009) during the toremifene but not during the baseline cycle (p=0.135).

The distributions of the VI, FI, and VFI from the four sectors of the right and left breast in baseline and during toremifene medication are shown in Table 1. The differences of the vascularization indices VI, VFI, and FI from each four quadrants of the both breasts were not significant between the baseline cycle and toremifene treated cycle.

Discussion

Many trials have documented antiestrogenic effects of SERMs on premenopausal breast. Follow-up data from the phase III prevention trials have confirmed that tamoxifen 20 mg/day has a net benefit in the prevention of estrogen receptor positive breast cancer, particularly in women younger than 50 year of age. This benefit was continuing for years even after the cessation of medication. (27,28). The breast volume measured with MRI decreased while the mammographic density remained unchanged after treatment with another SERM or raloxifene at a dose of 60 mg daily in premenopausal women at an increased risk for breast cancer (9). In our previous study applying MRI, toremifene seemed to diminish the mammary blood flow in women with severe premenstrual mastalgia (8). Tamoxifen treatment has also been demonstrated to reduce the incidence of clinically detected benign breast diseases. The antiestrogenic effects were more pronounced especially among premenopausal women and they also underwent fewer breast biopsies (29). Fibroadenoma is a benign hormone-dependent neoplasm which contains higher estrogen receptor levels than found in the mammary lobules. Tamoxifen 20 mg/day has been shown to reduce the expression of a proliferative cell nuclear antigen in both the epithelium and the stroma of the fibroadenoma in premenopausal women (30). Both breast fibroglandular tissue volume and density has been reported to decrease following treatment with tamoxifen 20 mg/day in premenopausal breast cancer patients. The reduction of fibroglandular tissue volume was correlated with baseline volume and the duration of the treatment. The changes were evaluated by 3D MRI (31).

The 2D power Doppler US seems to be able to depict differences in breast blood flow. In one study venous impedance indices from upper-outer quadrant veins of the breast in patients with cyclic mastalgia and in healthy volunteers were compared by 2D Doppler US. The measurements were performed in both premenstrual and postmenstrual period. Significantly higher venous impedance indices were found during the premenstrual period in patients with cyclic mastalgia. After the menstruation the differences vanished (26). The 3D power Doppler imaging gives an opportunity to assess quantitatively macroscopic blood flow and microscopic vascularity at the same time inside the organs, and to evaluate changes that occur in response to therapeutic efforts. However, most studies of the breast 3D Power Doppler ultrasound have concentrated to analyze the diagnostic possibilities of the equipment.

All studies cited above have shown antiestrogenic effects of SERMs on the premenopausal breast. Against that background, it is quite surprising that in the present study, toremifene had very little if any effect on the breast volume or the breast circulation, as investigated with the 3D power Doppler US. A possible explanation to our negative results may be the fact that we studied healthy volunteers rather than women suffering from mastalgia. The basal flow values may have been too low to allow for an antiestrogenic effect of toremifene to become evident in a normal breast. Madjar et al. found out using 2D power Doppler that the basal breast circulation flow values were much higher in women suffering from severe breast pain than in asymptomatic women. A significant decrease was detected as response to luteal progesterone in women with cyclic mastalgia (27).

The negative result of this trial should not discourage investigators from applying 3D power Doppler US in the study of breast vasculature. However, the importance of the proper selection of study participants is to be emphasized. Future studies should aim at a direct comparison between the breast dynamic MRI and 3D power Doppler US in mastalgia patients.

In conclusion, 3D power Doppler US failed to demonstrate any significant changes in the breast volume or circulation as a response to toremifene in healthy volunteers.

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Legends for the tables and figures:

Figure.1 A demonstration of the measurement of the upper lateral quadrant of the right breast, with the probe at 11 o'clock position. Probe (P), Areola (A), Nipple (N).

Figure. 2 The combined volumes (cm^3) of the four breast quadrants from both sides are shown by median (black line), interquartile range (box), and range (line bar) at baseline cycle and toremifene cycles, respectively. The differences were tested by Wilcoxon signed rank test.

Table 1. Distributions of the vascularization index (VI), Flow index (FI), and vascularization flow Index (VFI) measurements shown by medians with ranges (n=20). Differences between the right and left side at baseline (p_b) and during the toremifene medication (p_t), and at baseline vs. the toremifene cycle in the right (p_r) and left sides (p_l), respectively, were tested by the Related-Samples Wilcoxon Signed rank Test.

Table 1.

	Baseline			Toremifene			Baseline vs. Toremifene	
	Right Median (Range)	Left Median (Range)	pb	Right Median (Range)	Left Median (Range)	pt	Right p_r	Left p_l
Upper lateral								
VI	0.041 (<0.001-3.25)	0.243 (0.002-1.12)	n.s	0.145 (<0.001-2.16)	0.191 (0.001-3.59)	n.s	n.s	n.s
FI	27.3 (20.5-50.0)	30.3 (21.5-47.4)	n.s	29.6 (20.3-48.8)	30.6 (21.6-40.5)	n.s	n.s	n.s
VFI	0.010 (<0.001-1.038)	0.074 (<0.001-0.510)	*0.062*	0.042 (<0.001-0.831)	0.059 (<0.001-1.075)	n.s	n.s	n.s
Upper medial								
VI	0.202 (0.010-14.4)	0.096 (0.001-4.09)	n.s	0.219 (<0.001-5.10)	0.275 (0.004-2.80)	n.s	n.s	n.s
FI	31.7 (21.8-50.1)	28.0 (22.7-45.3)	n.s	29.7 (18.0-45.8)	29.9 (22.5-48.1)	n.s	n.s	n.s
VFI	0.070 (0.002-4.61)	0.029 (<0.001-1.020)	n.s	0.074 (<0.001-1.51)	0.091 (0.001-0.876)	n.s	n.s	n.s
Lower medial								
VI	0.136 (0.005-6.02)	0.104 (<0.001-4.74)	n.s	0.108 (<0.001-4.70)	0.261 (<0.001-3.74)	n.s	n.s	n.s
FI	27.9 (21.7-56.0)	26.7 (19.0-45.7)	n.s	26.9 (21.5-49.4)	26.5 (19.0-39.8)	n.s	n.s	n.s

VFI	0.042 (0.001-1.72)	0.027 (<0.001-1.307)	n.s	0.032 (<0.001-1.88)	0.068 (<0.001-1.42)	n.s	n.s	n.s

<u>Lower lateral</u>

VI	0.255 (<0.001-3.91)	0.087 (0.002-3.42)	n.s	0.124 (<0.001-5.45)	0.258 (0.002-3.66)	n.s	n.s	n.s
FI	29.5 (20.3-48.0)	26.9 (21.2-45.2)	n.s	28.6 (23.8-38.9)	28.2 (21.4-38.0)	n.s	n.s	n.s
VFI	0.085 (<0.001-1.102)	0.024 (<0.001-0.965)	n.s	0.040 (0.001-2.06)	0.049 (0.001-1.39)	n.s	n.s	n.s

Nearly statistical significant values (0.050<p<0.099) were shown by italic font, n.s (not significant).
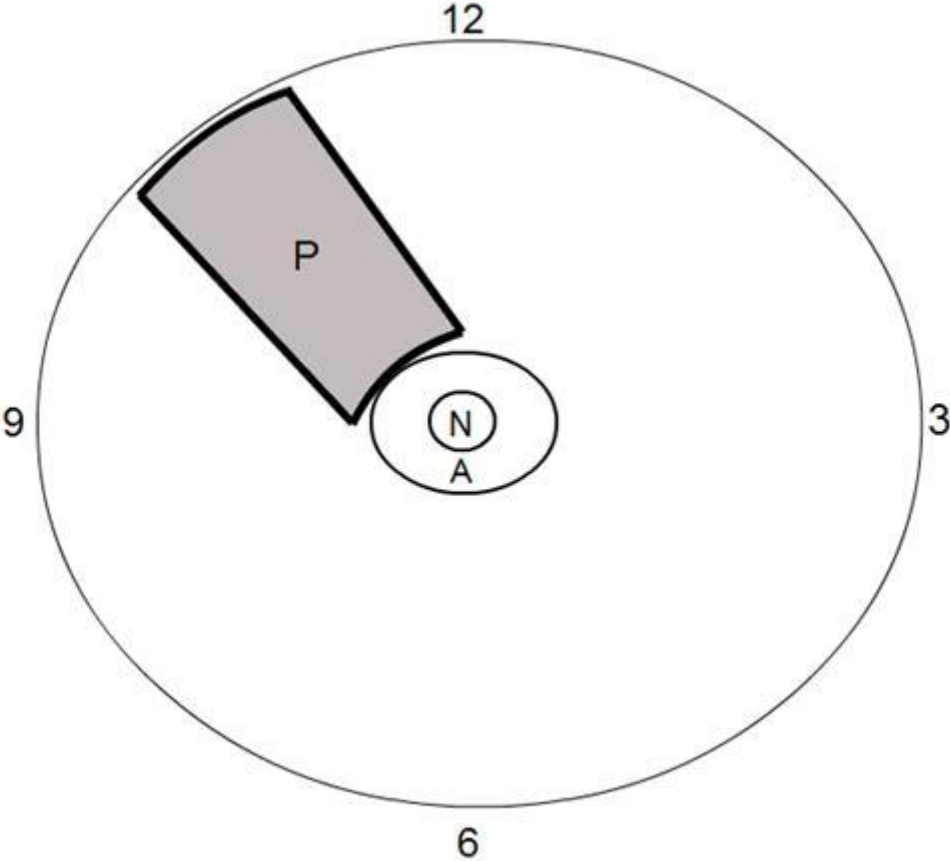
Figure.1

Figure. 2

| Volume right breast baseline | Volume right breast toremifene | Volume left breast baseline | Volume left breast toremifene |