RECENT STUDIES & ADVANCES IN BREAST CANCER

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Recent Studies &
Advances in Breast Cancer

Chapter 1

Breast reconstruction – current practice and future directions

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Abstract

Rates of mastectomy are increasing internationally due to phenomena such as contralateral and bilateral prophylactic mastectomies and women eligible for breast conserving surgery opting for mastectomy. Breast reconstruction has been demonstrated to improve psychosocial and quality of life outcomes in this patient cohort, and has become the standard of care in the treatment of breast cancer. With an ever increasing emphasis being placed on this aspect of care, there have been significant advances within the field over recent decades. The development of skin and nipple sparing mastectomy has done much to enhance cosmetic outcomes. Refinement of breast implants to reduce complications and development of free autologous flaps have revolutionised patient outcomes. Results are still heavily influenced by adjuvant breast cancer therapies such as radiation and chemotherapy, and much has been accomplished in making breast reconstruction more compatible with these treatment modalities. However, breast reconstruction is still evolving and novel technologies such as tissue engineering hold promise for the development of superior techniques of breast reconstruction post-mastectomy.

1. Introduction

Breast cancer is the most commonly diagnosed cancer in females, with approximately 1.7 million women diagnosed and treated worldwide annually [1]. While significant progress has been made in the multimodality management of breast cancer, complete surgical resection with disease free margins remains the cornerstone of effective therapy. In order to achieve adequate locoregional control approximately 40% of patients will undergo a total mastectomy [2,3]. In recent years there has been an increase in the number of patients undergoing mastec

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tomy; this is explained by an increase in prophylactic risk-reducing surgery in patients with cancer predisposing genetic mutations and increasing numbers of patients with breast cancer opting for contralateral prophylactic mastectomy (CPM) [4-10]. Furthermore, a trend has also been reported of women who are eligible for breast conserving surgery opting to undergo mastectomy [3,9,11,12]. For patients who undergo mastectomy, breast reconstruction is known to improve psychosocial and aesthetic outcomes [13]. Recent guidelines recommend that breast reconstruction should be discussed and offered as an option for the majority of women undergoing mastectomy [14,15]. Post-mastectomy breast reconstruction (PMBR) has thus been incorporated into the contemporary surgical treatment of breast cancer patients, resulting in increasing reconstruction rates as reported in audits of both the US and UK populations. Rates of breast reconstruction post-mastectomy are increasing by 5% per annum [16]. As a consequence of both the increasing number of mastectomies being performed and improved survival of breast cancer patients, surgical techniques have evolved in an effort to maximise aesthetic and quality-of-life outcomes. Refinement of the mastectomy technique itself has included the development of skin-sparing and nipple-sparing mastectomies which preserve the skin envelope +/- the nipple-areolar-complex (NAC). These procedures are increasingly performed for patients with breast cancer and those with genetic predisposition. Correspondingly, the range of reconstructive techniques on offer for patients undergoing PMBR is expanding due to the innovation of breast and plastic surgeons. Recent advances have seen the addition of novel autologous reconstructive approaches in addition to the expansion of indications for prosthetic reconstruction facilitated by the use of Acellular Dermal Matrices (ADM). Advances in the fields of tissue engineering and regenerative medicine hold enormous potential for novel reconstructive approaches and recent efforts have focused on stem cell-based regeneration of adipose tissue.

This chapter provides an overview of the current/contemporary approaches for post-mastectomy breast reconstruction and the challenges that must be overcome in the development of future novel reconstructive techniques.

2. Historical perspective / evolution of breast reconstructive techniques

The primary goal of surgery for breast cancer is to achieve local disease control. Historically this was achieved with extensive surgery in the form of the Halstead radical mastectomy, which achieved a 6% rate of local recurrence, albeit at the expense of significant associated physical and psychosocial morbidity [17]. The development of adjuvant therapies which effectively reduce both distant and loco-regional recurrence [18-20], and the recognition that tumour biology also impacts local control [21] have contributed to a paradigm shift towards increasingly conservative therapeutic surgical approaches [22]. Despite this, approximately 40% of women still require mastectomy to achieve locoregional control. Mastectomy is proven to have adverse psychosocial effects on breast cancer patients including anxiety, depression and

negative body image, all of which impact negatively on quality of life in a cohort of patients who are already dealing with cancer diagnosis, treatment and the fear of disease recurrence [23]. The practice of breast reconstruction has evolved to afford clearly defined psychosocial and aesthetic benefits for women undergoing mastectomy [23-25] and it is for this reason that PMBR has become an important component of multidisciplinary breast cancer care. The evolution in breast reconstructive approaches over time is outlined in figure 1.

The first post-mastectomy breast reconstruction was successfully carried out in 1895 by Vincent Czerny by transplanting a lipoma from the patient's flank to the chest wall, "the left breast was well formed, perhaps somewhat smaller than and firmer than the right but the disparity in any case was far less than with the usual mastectomy" [26]. The pectoral muscle was first used as a mound to reconstruct the breast in 1905 by Ombredanne [27]. In 1906, Tanzini was cited as the first to utilise a musculocutaneous flap for the purposes of breast reconstruction when he developed a pedicled flap of latissimus dorsi muscle and overlying skin paddle. However, as a result of Halsted's beliefs that breast reconstruction was a risk factor for disease recurrence, Tanzini's LD flap breast reconstruction technique was not utilised and forgotten [28]. Different forms of pedicled flaps were subsequently developed over the 20th century with limited success, mainly due to the requirement for multiple operations to complete the reconstructive process. These included use of the opposite breast as a donor site and a thoracoepigastric flap with prosthesis pioneered by German surgeons Hohler and Bohmert [29] (figure 1).

Autologous flap reconstructions were popularised with the reintroduction of the Latissimus Dorsi flap for breast reconstruction in 1977 by Schneider, Hill and Brown [30], and Muhlbauer and Olbrisch [31]. These were also used in conjunction with an implant as they often did not produce adequate breast volume alone. An extended LD flap (harvesting of the LD muscle and accompanying lumbar fat without the use of an implant [32] was developed with positive aesthetic outcomes; however, donor site morbidity was a significant problem with this procedure. The Transverse Rectus Abdominis (TRAM) flap was first described in 1982 which allowed for a more aesthetic donor site than that of the LD, leading it to become widely used as a method of breast reconstruction post-mastectomy.

Free microvascular tissue transfer was first described in 1973 for the primary closure of a compound leg injury, a development which broadened the horizons of breast reconstruction [33, 34]. Microvascular free flaps have increased in popularity in recent years, particularly in the case of immediate breast reconstruction and have been associated with lower rates of flap necrosis. Free TRAM and Deep Inferior Epigastric Artery Perforator (DIEP) flaps are the most commonly utilised free flaps for breast reconstruction, though other donor sites are also utilised including deep circumflex iliac artery flaps, lateral thigh (tensor fascia latae) flaps, superior and inferior gluteal musculocutaneous flaps, gracilis flaps and triceps flaps [29].

Perforator flaps were developed from the principles of free microvascular tissue transfer which have further minimised donor site morbidity associated with harvesting the musculocutaneous flap. Deep inferior epigastric perforator (DIEP), latissimus dorsi perforator and gluteal artery perforator (GAP) flaps have had successful outcomes [29]. The DIEP flap is the most commonly performed for breast reconstruction and relies on microdissection of the branches of the deep inferior epigastric vessels that perforate the rectus abdominis and its fascia. The internal mammary vessels are currently the most commonly used recipient vessels on the chest wall for microvascular anastomosis after transfer of the flap to the chest wall [35].

Prostheticbreast reconstruction began with the introduction of the silicone breast implant in 1963, which was originally performed as a delayed reconstruction but then became more commonly used in immediate reconstruction [28]. This trend changed in the 1980's when Radovan published on the use of immediate-delayed reconstruction using tissue expander implants in 1982 [36]. This was a popular breast reconstructive option as it was deemed to have superior outcomes in the case of postmastectomy radio therapy. Over time, modifications have been made to the shape, texture, site of ports and integrated valves of expander implants, lowering complication rates and increasing their effectiveness. Permanent implants and their design have also evolved over time with modifications being made to their shape, silicone shell thickness, gel viscosity and texture. When the safety of silicone implants was questioned, and they were eventually withdrawn from the market in 1992, there was an increased use of saline-filled implants which were shown to be superior with regard to implant rupture, capsular contracture, ease of revision surgery and cost [37]. However, they can be associated with a "rippling" effect which significantly reduces patient satisfaction and cosmetic outcome [38]. Textured implants, which have a rough external surface giving traction once implanted,are currently widely utilised as they have been shown to have lower rates of capsular contracture than smooth implants. Polyurethane-coated implants have also been used to prevent capsular contracture, which is effective until the breakdown of the polyurethane coating after years in situ [39]. The introduction of Acellular Dermal Matrices (ADMs) in 1994 has helped to overcome limitations of prosthetic breast reconstruction such as inadequate infra-mammary fold support, reduced expansion of the inferior pole and inadequate soft-tissue coverage of the implant [40]. It also allows for direct-to-implant reconstructions without the need for insertion of a tissue expander, speeding up the reconstructive process [41]. ADMs are soft tissue matrix grafts produced by a process of tissue decellularisation while leaving the extracellular matrix (ECM) intact. They were first used in breast implant reconstruction in 2005 [42] and later used in conjunction with tissue expander breast implants in 2007 [43].

The evolution in mastectomy technique has also influenced PMBR. The advent of the "skin sparing mastectomy", first reported in 1991, has had a significant impact on the improvements seen in contemporary breast reconstruction techniques [44,45]. The skin envelope is

preserved with this technique as it involves the removal of only the nipple-areola complex and skin involved with or in close proximity to the tumour. Preserving the skin results in superior symmetry due to matching skin colour and texture. It also aids the surgeon in shaping the breast mound in reconstruction. Skin sparing mastectomy is suitable in most breast cancer patients, though it is contraindicated in inflammatory carcinoma, locally advanced breast cancers, and is relatively contraindicated in smokers. Necrosis of the mastectomy flaps must be avoided and in patients who are also undergoing placement of expanders, it is crucial to ensure complete coverage of the implant, either with a complete muscular pocket or an ADM. Despite the lack of a randomised controlled trial, SSM is as safe oncologically as simple mastectomy, with similar rates of local recurrence, as shown in a meta-analysis, in 2010, of 3739 patients (1104 SSM and 2635 non-skin sparing mastectomy) [46].

Nipple reconstruction has become an accepted part of the breast reconstruction process, with tattooing the reconstructed nipple areola being commonly carried out. Nipple sparing mastectomy, first described in 1962 [47], is also becoming popularised, obviating the need for this reconstructive step and improving aesthetic outcomes [28]. Preservation of the nipple-areolar complex (NAC) has been shown to be oncologically safe with no increased risk of breast cancer recurrence in women with sporadic breast cancer. There have been some concerns raised regarding its safety in BRCA gene mutation positive patients as this procedure requires a small amount of tissue to be left behind the NAC to maintain an adequate blood supply [48]. However, the procedure has been deemed oncologically safe by a meta-analysis of 5594 patients with a follow up of greater than 5 years [49]. Nipple sparing mastectomy is becoming more widely performed and has a central role in improving patient satisfaction outcomes [50].

3. Contemporary reconstructive approaches

There are two primary decisions involved when planning breast reconstruction in post-mastectomy patients; (a) Timing i.e. immediate vs. delayed reconstruction and (b) Type i.e. implant vs. autologous [51].

4. Timing of breast reconstruction

The rate of immediate breast reconstruction (IBR) has risen dramatically in the last two decades, with one study reporting a 78% increase from 1998 to 2008, an average of 5% per year [16]. IBR results in better aesthetic outcomes in those patients who do not require post-mastectomy radiation therapy (PMRT), superior psychosocial and patient satisfaction outcomes than delayed breast reconstruction (DBR). Breast reconstruction can also be achieved in fewer surgical procedures with IBR. IBR is oncologically safe, with no increased risk of locoregional disease recurrence or in the ability to detect recurrence [51]. The National Institute for Clinical Excellence (NICE) guidelines state that IBR should be offered to all suit-

able patients undergoing mastectomy, however there is a decreased likelihood of this in older patients, those of African-American race, patients who are married or from rural locations and those with increased comorbidities [52]. Despite the aesthetic advantages of IBR, its cosmetic outcomes are said to deteriorate over time independent of radiotherapy, type and volume of implant, patient age or mastectomy incision [53]. There are no clear indications with regard to timing or technique of PMRT administration in IBR, thus, capsular contracture is the most common limitation, with a rate of 40.4% in IBR compared to 17% in DBR. PMRT negatively influences outcomes of both implant and autologous reconstructions. The challenge lies in being able to predict the need for PMRT when deciding about reconstruction timing. Therefore, in cases where the need for PMRT is ambiguous, the patient should be offered an immediate-delayed or delayed procedure in order to ensure optimal aesthetic results [51].

4.1 Type of breast reconstruction

4.1.1 Prosthetic reconstruction

There has been a change in the trends of breast reconstruction most commonly carried out in recent years. Autologous methods of breast reconstruction were most popular early in the breast reconstruction era. However, this has been surpassed by the use of implant based reconstructions. This trend is also evident in those patients undergoing PMRT [54]. Breast reconstruction utilising implants can be carried out either as (a) single stage, direct to permanent implant (DTI) procedures or (b) two-stage procedure with the insertion of a tissue expander, which is inflated with saline over time and then replaced by a permanent implant. Several advantages such as shorter operation times, lack of a donor site and the associated morbidity, and quicker return to normal activities make this an attractive reconstructive option to both patients and surgeons. The FDA and WHO have recently confirmed an association between breast implants, particularly those with textured surfaces, and anaplastic large cell lymphoma (ALCL). This is a rare T-cell lymphoma requiring surgical management. It usually presents as a peri-prosthetic fluid collection 8-10 years after breast implant insertion. It is imperative that patients are counselled about the risk of breast implant associated anaplastic large cell lymphoma (BIA-ALCL) prior to breast reconstruction [55].

Direct to implant (DTI) reconstructions, carried out in one procedure were commonly associated with problems such as pectoralis muscle retraction, implant malposition and capsular contracture. More modern DTI procedures make use of Acellular Dermal Matrices (ADMs) which overcome these issues by fixing the pectoral is muscle and forming a complete pocket around the inferior pole of the implant in the required position. This also decreases the stress on the inferior skin envelope, resulting in lower rates of contracture [56]. Traditionally, DTI with total muscle coverage of the implant was only possible in small-breasted women as it was limited by the degree of expansion of the overlying pectoral muscles. This limitation

has also been overcome by ADMs as they obviate the need for total muscle coverage [57]. DTI reconstruction is suitable for women with small to moderate sized breasts who wish to remain a similar breast size (figure 2). Those patients who wish to be a significantly larger size should undergo a two-stage procedure with a tissue expander [56]. During their early use, there was concern that ADMs were associated with a higher risk of infections and complications such as seroma [58-61]. "Red breast syndrome" (RBS) was a phenomenon synonymous with ADM use, first described in 2010 [62,63]. It was described as a non-infectious erythema, appearing days to weeks after ADM implantation, localised to the areas of ADM placement, typically along the inferior pole of the breast [64]. However, more recent research has shown that there is no increase in complication rates [65-67]. It is postulated that the reason for this incongruity in data is due to the learning curve associated with the introduction of a new product or technique [56,68]. The cost of ADMs are offset by completing the reconstructive process in a single procedure [56].

For those patients for whom there is a possible need for post-mastectomy radiation therapy (PMRT), which has deleterious effects on implant reconstructions, an immediate-delayed reconstruction is an option where a tissue expander is inserted at time of mastectomy and inflated over time. This can then be replaced by a permanent implant after completion of PMRT. This approach allows for the preservation of the skin envelope and matching of skin colour and texture. Preservation of the breast skin envelope allows for immediate placement of a permanent implant post-PMRT, reduces the need for the use of autologous flaps and lessens the size of the skin paddle required from an autologous flap [53].

4.1.2 Complications of prosthetic reconstruction

Capsular contracture, haematoma and infection are the most commonly cited complications of prosthetic breast reconstruction, and rates of these complications have been shown to be higher than in those patients who undergo autologous reconstruction, especially post-radiation therapy [69]. Reconstructive failure is associated with patient factors factors such as smoking, obesity type 2 diabetes, tumours of a higher grade, nodal disease involvement and tamoxifen use, and technical factors including incomplete muscle coverage of the implant, large implant volume (>400ml), thin mastectomy flaps [53].

Capsular contracture is a significant complication of prosthetic reconstruction, with risk factors such as bacterial colonisation, type of implant used (smooth), implant placement, smoking, haematoma, and most significantly, delivery of PMRT [70]. Staphylococcus epidermidis is the bacteria most commonly implicated in capsular contracture, and forms a biofilm around the silicone implant [71]. Higher rates of capsular contracture exist in IBR (20% - 40.4%) than in DBR (17% - 26.4%). Radiotherapy is the greatest predictor of capsular contracture with rates of 87% being reported compared to 13% in patients who did not undergo radiotherapy.

The risk of capsular contracture is seen to decrease when an autologous flap is used in conjunction with the implant [53].

A controversial relationship exists between implant breast reconstruction and PMRT. The delivery of ionising radiation has direct toxic effects on both malignant cells and healthy tissue. It's mechanism for tissue damage includes direct tissue cellular damage with chromosomal alteration, ischaemia as a result of microvascular occlusion and prevention of fibroblast activity [72]. The deleterious effects of radiotherapy on breast reconstruction are unpredictable and tend to be biphasic in nature, with acute changes occurring in the days to weeks post-PMRT (e.g. desquamation or necrosis of tissue), and changes also occurring at a later stage, months to years' post-PMRT(atrophy, fibrosis, obstructed wound healing) [73]. A systematic review by Berber et al investigating complications after radiotherapy and reconstruction in general reported a rate of 37% which varied widely from 8.7% to 70% [74]. As previously discussed, radiotherapy is the greatest predictor of capsular contracture, a complication often requiring another operation to excise the capsule and replace the implant. However, some radiation oncologists are of the opinion that breast reconstruction interferes with the delivery of effective PMRT through alteration of the chest wall anatomy and therefore the radiation field, resulting in under/over-dosing the targeted tissues unpredictably [75,76]. Surveyed radiation oncologists report differing preferences in the degree of inflation of tissue expanders at time of radiotherapy delivery: 60% moderately inflated (150-250 CC); 13% completely deflated and 28% completely inflated [77]. Higher grades of capsular contracture (Baker III or IV) are more common with radiotherapy delivery [78,79]. Capsular contracture secondary to PMRT is also a risk factor for persistent pain post-operatively up to 2 years after reconstruction [80]. Radiotherapy is associated with a higher rate of complications overall in patients receiving both IBR (0-64%) and DBR (22-55%) compared to those patients not in receipt of PMRT, both IBR (0-12%) and DBR (13-34%) [81]. Overall, patients who undergo radiotherapy with implant reconstruction have worse psychosocial outcomes and lower satisfaction in comparison to non-irradiated reconstructed patients [82-84].

4.2 Autologous reconstruction

Autologous breast reconstruction remains an important option in post-mastectomy breast reconstruction, particularly in patients who have poor skin quality of the mastectomy flaps or for whom delayed reconstruction is preferred [85]. Some authors predict an increase in the need for autologous reconstructions secondary to the increasing number of indications for radiotherapy, and thus an unacceptably high rate of capsular contracture and radio-dermatitis in implant-based reconstructive procedures [86]. Autologous reconstructions are more cosmetically natural in shape and texture than implants. They provide skin coverage in cases of poor quality of the mastectomy flaps or delayed reconstruction. It is believed that DIEP reconstruction is more suitable in patients who will require PMRT. Conversely, the effect of

radiotherapy on an LD reconstruction can be catastrophic secondary to muscular atrophy [87]. Although initial complication rates may be higher, autologous reconstructions provide a more consistent and durable reconstruction over time [88]. This approach however is not without its unique set of complications; autologous reconstruction is associated with morbidity at the donor and reconstruction site. Tissue flap necrosis and loss may occur secondary to ischaemia of transferred tissue. Complications may arise from the donor site in the form of, for example, an incisional hernia in the case of a TRAM flap. These operations have a longer operative time, require longer admissions and recovery times [52]. Complex patient selection and requirement for pre-operative CT angiography to detect the perforator vessel supplying the skin flap (in DIEP flaps) make autologous reconstruction a less attractive reconstructive technique [51]. Autologous flap procedures are longer and more technically challenging, particularly in the case of free DIEP and TRAM flaps which require the formation of a microvascular anastomosis [89]. As surgical techniques have evolved, there has been a progression from pedicled and free musculocutaneous flaps to muscle-sparing perforator flaps [29]. Currently, the abdominal wall is the most commonly used donor site.

4.2.1 Transverse rectus abdominis (TRAM) flap

The TRAM flap was pioneered in 1982 by Hartrampf, Scheflan and Black [90]. The technique has since been refined, with improvements in blood supply. It has evolved from a pedicled flap with a necrosis rate of approx. 10% to a free flap with a possible success rate of 98%, producing a breast reconstruction potentially superior to any other technique. TRAM flaps make up approx. 20% of breast reconstructive procedures carried out in the US. Originally, the pedicled TRAM flap took its blood supply from the superior epigastric vessels via a series of vessels within the rectus abdominis. The more modern use of the inferior epigastric vessels in the free TRAM flap allows larger amounts of abdominal tissue to be removed completely from the body and transplanted to the chest wall with minimal risk of fat necrosis. In addition, limiting the muscle harvest to the portion of muscle containing the medial and lateral rows of perforating vessels reduces the risk of donor site morbidity by minimising violation of the abdominal wall [91]. The anterior rectus sheath is usually sutured closed, however, in cases of difficult closure, particularly if both rectus muscles are used, a synthetic mesh may be required to achieve closure [92].

4.2.2 Deep inferior epigastric perforator/superficial inferior epigastric perforator (DIEP/SIEP) flap

It is possible to preserve all of the rectus abdominis muscle when raising a TRAM flap. In this case, only the perforating vessels are taken with the flap and the inferior or superior epigastric vessels are left intact. A deep inferior epigastric perforator (DIEP) flap results if the primary vessels are the deep inferior gastric artery and vein, which was described for use in

breast reconstruction in 1994 [93]. If the primary vessels are the superior epigastric gastric vessels, the procedure is known as a superficial inferior epigastric perforator (SIEP) flap [94]. They are anastomosed to the internal mammary vessels preferably, though they may also be anastomosed to the circumflex scapular vessels [95]. Donor site morbidity is minimised even further with this technique, however, increased dissection and longer operative times are required for this method of reconstruction. Due to the minimal breach of the rectus sheath, DIEP or SIEP flaps are associated with minimal loss of function, reduced risk of hernia, less post-op pain and shorter length of stay. Reduced abdominal wall disruption makes a tension free closure possible without requirement of a synthetic mesh [91]. DIEP flaps are indicated in young healthy women, those undergoing prophylactic mastectomy and patients who do not require PMRT; and contraindicated in patients of ASA Grade 3, collagen vascular disease, previous abdominoplasty or radiation to the abdomen that may have damaged perforating vessels, patients with severe haematological disorders or contraindications to anticoagulation. Relative contraindications include obesity, older age (<70 years) or smoking [96,97].

4.2.3 Latissimus dorsi (LD) flap

Alternative donor sites to the abdominal wall are required occasionally, specifically in patients who have had previous abdominal surgery. Although its use has been surpassed by that of the TRAM and DIEP flap in recent years, the LD flap is still a widely used method of breast reconstruction. This flap produces a ptotic breast with projection and texture similar to that of native breast tissue. It may be used alone or in conjunction with an implant in order to recreate the breast mound depending on the volume required to achieve symmetry. LD flaps are useful in the case of failed expander/implant reconstructions. LD flaps have evolved over time, particularly in the late 1970's when a greater understanding of the vascular connections to the skin allowed for a skin paddle to be transferred along with the muscle, improving the skin coverage and replacement of the breast mound contour [98]. As a result, superior breast symmetry and cosmetic outcomes were achieved. Although the transfer of abdominal tissue is preferable in the setting of breast reconstruction, it is not suited to all patients. Indications suggested for LD reconstruction include: previous abdominal operations; a preferred dorsal donor site; failed implant or TRAM flap; patients who wish to become pregnant at a later stage. LD is suitable for use in the immediate and delayed setting. The latissimus dorsi, a large triangular muscle on the upper back, is dissected along with a "paddle" of muscle, vascularised by the thoracodorsal artery and vein, and the overlying skin and fat (musculocutaneous flap). Once raised, the muscle is tunnelled below the axilla and implanted subcutaneously under the axilla, into the breast pocket and then sutured in place. The LD is often augmented by implants or fat grafting to provide symmetry and cosmesis [99]. An "extended LD flap" allows for greater volume generation without the use of an implant by harvesting lumbar adipose tissue along with the muscle flap in order to reconstruct the breast mound.

4.2.4 Transverse upper gracilis (TUG) Flap

The TUG flap is a less commonly performed method of breast reconstruction suitable for those in whom the abdomen in unsuitable as a donor site. The TUG flap is harvested from the medial aspect of the thigh and is associated with advantages such as a relatively consistent anatomy, a reasonably inconspicuous donor site scar and relatively little functional morbidity. However, potential limitations include medial thigh paraesthesia, chronic lower limb lymphoedema and contour deformities of the medial thigh. The flap is supplied by the medial circumflex artery. For patients with large breast volumes, the volume requirement of the flap can result in severe donor site morbidity with large contour deformities, widened and lowered donor scars, impaired wound healing and higher rates of lower leg lymphoedema. This has led to the use of a bilateral TUG flap for unilateral breast reconstruction in selected cases. Harvest of tissue anterior or beyond the femoral axis should be avoided to prevent flap necrosis. In addition, the preservation of the saphenous vein preserves lymphatics that lie below [100].

4.2.5 Thoracodorsal artery perforator (TDAP) flap

The TDAP flap is a de-epithelialised flap taken from the lateral thoracic wall and the back that can be transplanted to the anterior thorax for breast mound reconstruction. It was first described for breast reconstruction in 2004 [101]. This method of breast reconstruction has sufficient volume to recreate a B cup-sized breast using a totally or partially de-epithelialised flap. The TDAP flap allows for harvesting of the same skin and subcutaneous tissue as that in an LD flap, without the muscle, thus avoiding the possible associated complications. TDAP flaps have a very low incidence of seroma, no impairment of shoulder motion and have a satisfactory aesthetic outcome. Distal tissue necrosis is the most commonly occurring complication [102].

4.2.6 Superior gluteal artery flap

The SGAP flap was first described in 1973 as part of a multistage procedure. It was refined to a one stage procedure in 1975 [29]. This is considered to be superior to the inferior gluteal artery flap as the IGAP flap requires exposure of the sciatic nerve. This flap utilises only fat and skin from the gluteal region, which creates good projection and volume of the reconstructed breast. The pedicle is anastomosed to the internal mammary vessels. The long pedicle of the GAP flap minimises the need for venous grafts at the site of anastomosis and it has been shown that an S-GAP flap can survive successfully on a single perforator [103].

5. Autologous fat grafting

Autologous fat grafting involves liposuction of adipose tissue from the abdomen, thighs or buttocks and subsequent reinjection of the lipoaspirate into an area in which there is a defect

for the purposes of reconstruction. Autologous fat grafting has been successful in small volume breast augmentation, filling small volume defects post-breast conserving surgery [104-108] and adds value in implant based reconstructions [109,110]. Although positive outcomes have been demonstrated in this setting, the larger volume of adipose tissue required to carry out breast reconstruction post-mastectomy has proven beyond its capabilities thus far [111]. Autologous fat transfer is limited by resorption, with rates ranging from 25-80% and complications such as fat necrosis, oil cyst formation and microcalcifications in patients receiving autologous fat transfer in addition to primary reconstructive procedure e.g. LD flap [112] or as a filler for small volume defects post breast-conserving surgery (BCS) [106,113].

Cell-assisted lipotransfer, first described by Matsumoto et al in 2006, involves enrichment of autologous lipoaspirates with ADSCs harvested from half of the lipoaspirate prior to reinjection [114]. Enrichment of autologous fat lipoaspirates with ADSCs, which have been expanded ex-vivo has had more successful outcomes in terms of volume retention, likely as a result of superior graft maintenance due to increased vascularisation and collagen synthesis within the graft [115]. Kolle et al demonstrated fat residual volume of >80% in 10 patients over 121 days utilising abdominal lipoaspirate enriched with ADSCs that had been expanded ex-vivo for 14 days prior to reimplantation into the upper posterior arm. Compared to controls, without ADSCs, there were higher amounts of adipose tissue, less necrotic tissue and newly formed connective tissue [116]. Yoshimura et al conducted a study in 40 healthy patients undergoing cosmetic breast augmentation, where a mean volume of 270ml ADSC-enriched fat was injected into the breast. There was minimal post-op atrophy of the injected fat which did not change significantly over 2 months. Small cystic formations and microcalcifications were observed in some cases; however the microcalcifications were readily distinguished from those associated with breast cancer. Post-op CT and MRI images showed that transplanted fat tissue survived and formed a substantial thickness of the fatty layer subcutaneously on and around the mammary glands and also between the mammary glands and the pectoralis muscle. Breast volume stabilised 2-3 months post-op. This data indicates that cell-assisted lipotransfer is suitable for repair of smaller breast defects [117].

There have been concerns regarding the oncological safetyof autologous fat grafting. This issue has been addressed by several clinical studies. A small, retrospective series showed an increased risk of breast cancer recurrence in patients with intraepithelial neoplasia undergoing autologous fat grafting. Only patients with intraepithelial neoplasia (n=37) who underwent autologous fat grafting in this series demonstrated an increase rate of local recurrence (10.8%) [118]. A follow-up matched cohort study investigating fat grafting in 59 patients with intraepithelial neoplasia concluded that there is a higher risk of local recurrence in this patient cohort compared to age and stage matched controls (n=118) [119]. While these results are concerning, it must be noted that they are from a single centre retrospective study with small

numbers. More encouraging results are observed in larger studies with no increase in locoregional or systemic recurrence[120-122]. Delay et alretrospectively analysed outcomes in 880 patients who underwent fat grafting. They demonstrated , no increased risk of cancer recurrence or new cancer development after 10 years of follow up [113]. They also reported that the radiological appearance of the breasts post-lipofillingdid not negatively influence the ability to identity a neoplastic process. To date, the largest retrospective carried out was by Kronowitz et al. where 719 patients underwent autologous fat grafting post-tumour resection. There was no increase in locoregional or systemic recurrence or of a second breast cancer [120]. The RESTORE-2 trial assessed the oncological safety of ADSC-enriched fat grafting in patients undergoing BCS with defects up to 150ml. 67 patients reported high levels of satisfaction with the cosmetic outcomes. No incidences of local recurrence were reported within 12 months of the procedure. While these results are encouraging, longer follow up is required to accurately investigate the oncological safety of this procedure [123]. Systematic reviews conclude that autologous fat grafting appears to be oncologically safe with low rates of complications and good patient and surgeon satisfaction [124-126]. However, all authors suggest that there is an urgent need for randomised controlled trials with adequate follow up to confirm this opinion, and to exercise caution in carrying out these procedures at high risk patients.

6. Breast reconstruction and neoadjuvant/adjuvant therapy

6.1 Chemotherapy and breast reconstruction

There is some concern over the relationship between breast reconstruction and the delivery of chemotherapy in the treatment of breast cancer. No clear evidence exists for the optimal time for initiation of adjuvant chemotherapy, however most guidelines state that chemotherapy can be safely initiated within 4 weeks of mastectomy. It has been previously suggested that breast reconstruction is responsible for delays in the delivery of adjuvant chemotherapy, therefore compromising oncological treatment and outcomes [127]. This has been disproven by several studies and it is now widely accepted that breast reconstruction does not pose a risk for delayed delivery of adjuvant therapies [128,129]. There have been reports of increased surgical complications post-breast reconstruction (e.g. wound healing, tissue necrosis and infection) in patients also in receipt of chemotherapy, secondary to its myelosuppressive and cytotoxic effects [72]. A limited number of studies have examined this; however, the largest of these studies did not find significantly higher complication rates in this patient cohort undergoing reconstruction and chemotherapy. There is a paucity of data relating to neoadjuvant chemotherapy and breast reconstruction, though it is accepted that neoadjuvant chemotherapy results in similar outcomes to adjuvant chemotherapy post-breast reconstruction [72].

6.2 Radiotherapy and breast reconstruction

A controversial relationship exists between post-mastectomy radiation therapy (PMRT)

and breast reconstruction, particularly in the case of implant only reconstructions. PMRT has deleterious effects on aesthetic outcomes and complication rates in implant-based reconstructions as it can affect the symmetry, volume and projection achieved at the time of initial reconstruction [130]. Implant-based reconstructions have a significantly higher rate of complications than autologous reconstructions in the setting of PMRT: infection (13.5% vs. 5.8%), mastectomy flap necrosis (10.5% vs. 5%), and reoperation secondary to complication (37.0% v 16.6%)(131). There is a reconstructive failure rate of 16.8% in implant reconstructions in the presence of PMRT [131]. The timing of PMRT is an important consideration in the avoidance of complications. For those patients who have already received PMRT, insertion of implants and tissue expansion techniques can troublesome, with increased rates of infection, implant extrusion and capsular contracture. Autologous reconstruction gives a more predictable aesthetic outcome in those patients previously treated with PMRT. The reconstructive procedure itself is less complicated in those patients who have not received PMRT but exposure of the reconstruction to ionising radiation creates its own issues, both for implant and autologous reconstructions. In patients in whom PMRT is expected to be required, oncoplastic surgeons will insert a tissue expander implant which will be inflated over time and replaced by a permanent implant prior to delivery of PMRT [92]. In the case of autologous reconstructions, there is no difference in complication rates, flap failure or rates of revision surgery depending on the timing of PMRT. A systematic reviewof breast reconstruction before and after PMRT by Berbers et al recommend that definitive implant reconstruction be carried out before PMRT and autologous reconstruction be carried out post-PMRT to avoid radiation-induced fibrosis and compared cosmesis [74].

6.3 Hormonal therapy

A paucity of evidence exists in the literature regarding the effects of hormonal therapy on breast reconstruction. The principle consideration in this regard appears to be the increased risk of thromboembolic events associated with tamoxifen therapy in those patients who have undergone a breast reconstruction procedure involving a microvascular anastomosis (e.g. DIEP) according to a systematic review by Parikh et al [132].

7. Future Directions

Despite the clear aesthetic and psychosocial benefits of breast reconstruction [133], currently available techniques, including synthetic implants and autologous tissue grafts are limited by morbidity risks at both the reconstruction and donor sites. Increasing patient expectations for cosmetic/aesthetic outcomes means that surgeons are persistently attempting to optimise reconstruction methods through innovative development of a functional tissue substitute for postmastectomy reconstruction. The rapidly advancing fields of tissue engineering and regenerative medicine hold enormous potential in this regard and recent years have seen

key innovations in vascular, osseus, cutaneous and soft tissue regeneration [134]. For breast cancer patients, the ability to generate living functional tissue to fill disfiguring defects following tumour resection will have enormous implications for future quality of life. Recent efforts have focused on cell-based regeneration of adipose tissue to fill the defect following BCS or mastectomy [135]. Adipose-derived stem cells (ADSCs) offer the advantage of an abundant autologous source, a minimally invasive method of harvesting, significant proliferative capacity, and secretion of growth and angiogenic factors to stimulate tissue regeneration [136]. For these reasons,

ADSCs) have become the gold standard as a cell source for tissue engineering [137]. ADSCs can be easily isolated from lipoaspirates obtained at liposuction procedures, of which, approximately 400,000 are carried out in the US annually. Each procedure yields 100ml-3L of lipoaspirate, in which 90% of ADSCs are viable, which is usually discarded post-operatively [138]. ADSCs can be used as autologous and allogenic grafts. It has been determined that passaged ADSCs, as opposed to freshly isolated SVF cells, reduce histocompatibility surface antigen expression and no longer induce a lymphocytic reaction when cocultured with allogenic peripheral blood monocytes. Immunoreactions are suppressed by ADSCs, indicating that ADSCs may not elicit a cytotoxic T cell response in vivo though this hypothesis has yet to be tested comprehensively [137,139].

As discussed above, the use of autologous adipose tissue via fat-grafting is in widespread clinical use for breast augmentation and correction of small volume defects following breast conserving surgery [140]. The use of fat grafts supplemented with ASCs in “cell-assisted lipotransfer” has been reported to result in more durable outcomes than conventional fat grafting [141,142].

However, in order to regenerate sufficient tissue volume to fill a larger mastectomy defect it is likely that a de-novo adipose tissue engineering approach will be required; combining living cells (ADSCs), a biocompatible scaffold, and a microenvironment that will provide the appropriate cues to support cell growth, differentiation and long-term volume retention to promote tissue regeneration.

A scaffold acts as a template for new tissue formation. Correct scaffold material and design selection will be paramount in overcoming the obstacles of volume retention and vascularisation. A variety of synthetic and natural scaffold materials have been studied for this purpose [135,143-149].

Patrick et al was one of the first groups to investigate scaffolds in adipose tissue regeneration. Preadipocytes were isolated and cultured on a polymeric scaffold which was then implanted into a murine model. Good adipose tissue formation was evident at 2 months; however a decrease was noted at 3 months, with complete disappearance of all engineered adipose

tissue and the PLGA scaffold at 12 months [150-152].

Von Heimburg et al. investigated freeze-dried collagen sponges seeded with preadipocytes. These were implanted into immunodeficient mice and preadipocytes differentiated to mature adipocytes in vivo. The constructs were explanted at 3 and 8 weeks and histology revealed adipose tissue with rich vascularisation attached to the scaffold beneath a thin capsule layer of fibrovascular tissue [153]. A study on HYAFF11 sponges, a derivative of hyaluronic acid, concluded that these were superior to collagen sponges with regard to cellularity achieved in adipose tissue engineering [154]. This has been found to be a suitable scaffold material for the culture and in vivo differentiation of ADSCs [155,156].

Pati et al successfully bioprinted a 3D cell laden construct with decellularised extracellular matrix (dECM) that showed high cell viability and functionality [157]. A similar biomaterial adipose tissue construct was implanted into a mouse model, which demonstrated positive tissue infiltration, constructive tissue remodelling and adipose tissue formation.

One study 3D-printed patient-specific breast scaffolds with a poly-lactide polymer cultured for 6 weeks. The constructs were seeded with human umbilical vein endothelial cells and subcutaneously implanted in athymic nude mice for 24 weeks. Explanted samples were well-vascularised constructs of adipose tissue without necrosis, inflammation or cysts. There was an increase in adipose tissue produced from 37.17% to 81.2% 15 weeks [158].

One study seeded ADSCs onto decellularised adipose tissue (DAT) bioscaffolds and implanted them into female Wistar rats. At explantation at 12 weeks, 56.1 +/- 9.2% of the ADSC-seeded DAT had been remodelled into mature adipose tissue with a higher density of blood vessels within the areas of the implant that had been remodelled into mature adipose tissue [159].

The largest volumes of sustained regeneration of adipose tissue have been achieved by “additive biomanufacturing” utilised delayed fat injection into a custom-made scaffold implanted in minipigs for 24 weeks after a period of prevascularisation. The prevascularisation + lipoaspirate group had the highest relative area of adipose tissue upon explantation (47.32 +/- 4.12%) which was similar to native breast tissue (44.97 +/- 14.12%)[160]. Morrison et al are the first group to engineer clinically relevant volumes of adipose tissue in humans through the use of a porous chamber and an arterio-venous loop, producing 80ml of adipose tissue [161].

Although these results are promising from a technical and tissue regeneration perspective, a critical question is that of oncological safety; there is a recurrence rate of 20% at 10 years for breast cancer patients, indicating the persistence of dormant cancer cells even in the setting of contemporary multimodality therapy [162]. A major concern is the risk of stimulating tumour recurrence by the use of stem cells for breast tissue regeneration. There is conflicting

data regarding the possible interplay between breast tumour cells and transplanted ASCs; the ASC secretome has been shown variably to promote [163-165] and suppress tumour growth *in-vitro* [166,167]. Our knowledge of ASC behaviour in-vivo is limited [168] and the concept of a detrimental interaction between transplanted ASCs and residual/dormant cancer cells requires further clarification through *in-vivo* and clinical studies which should aim to clarify how ASCs can be exploited for their regenerative function in this setting without influencing tumorigenesis. If this can be achieved, translation to the clinical setting will offer the exciting potential to engineer a reconstruction, generated from autologous cells which may be surgically implanted without requiring tissue transfer, thereby eliminating or reducing donor site morbidity, and answering a clinical need for breast cancer patients.

8. Conclusion

Post-mastectomy breast reconstruction is an integral component of optimal multimodality breast cancer care. It is an ever-evolving field, partly due to increasing patient expectations with regard to aesthetic outcomes and due to the need to adapt to new oncological and radiation-based treatments. While historically, breast reconstruction has been composed of implant-based and autologous tissue techniques, research into the field of tissue engineering has yielded promising results, suggesting that this may be the future solution to the many limitations of current approaches and will maximise aesthetic and quality of life outcomes for breast cancer patients.

9. Figures

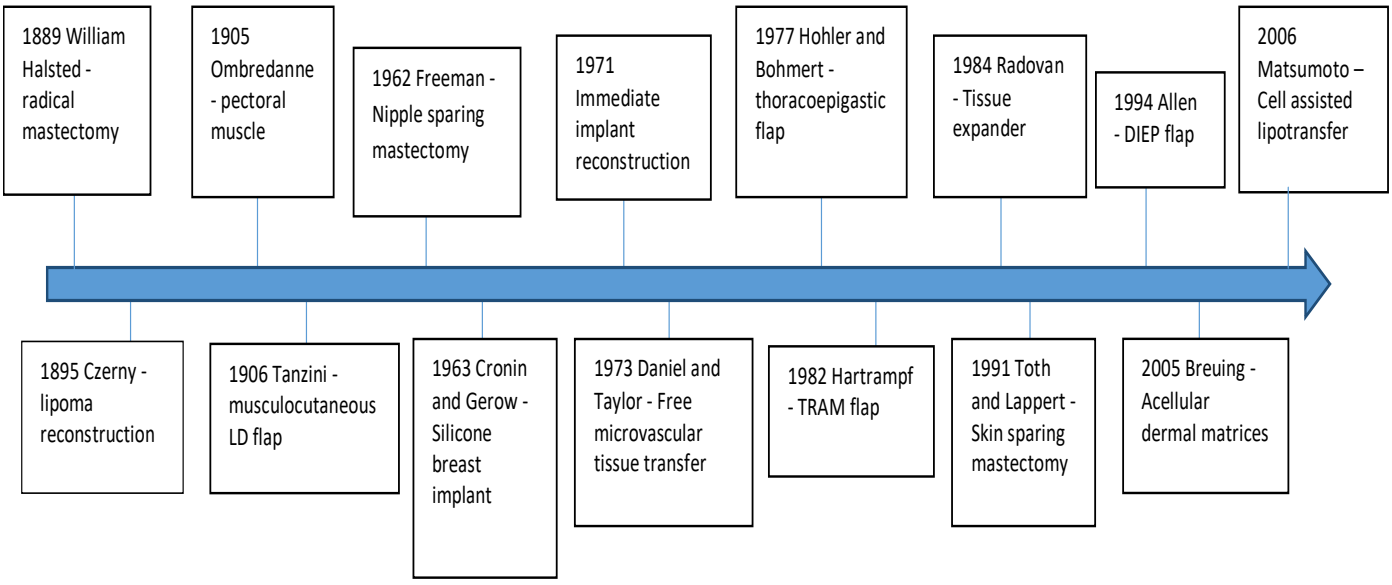

Figure 1: Evolution of Post-Mastectomy Breast Reconstruction

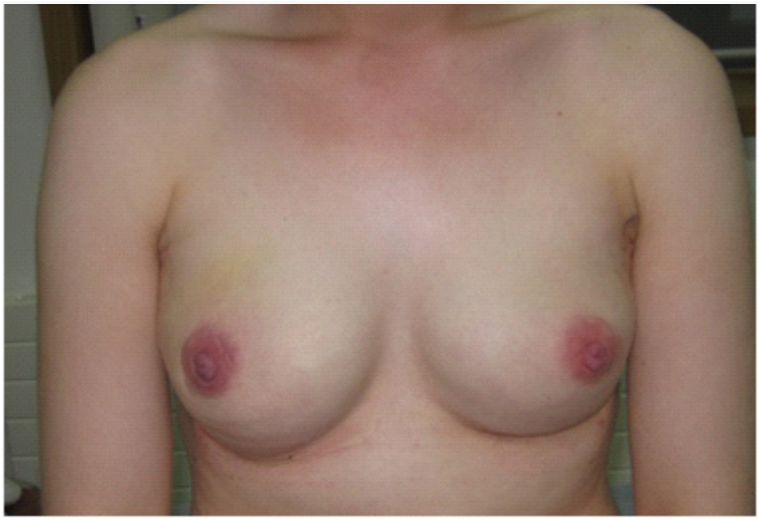

Figure 2: Bilateral implant reconstruction

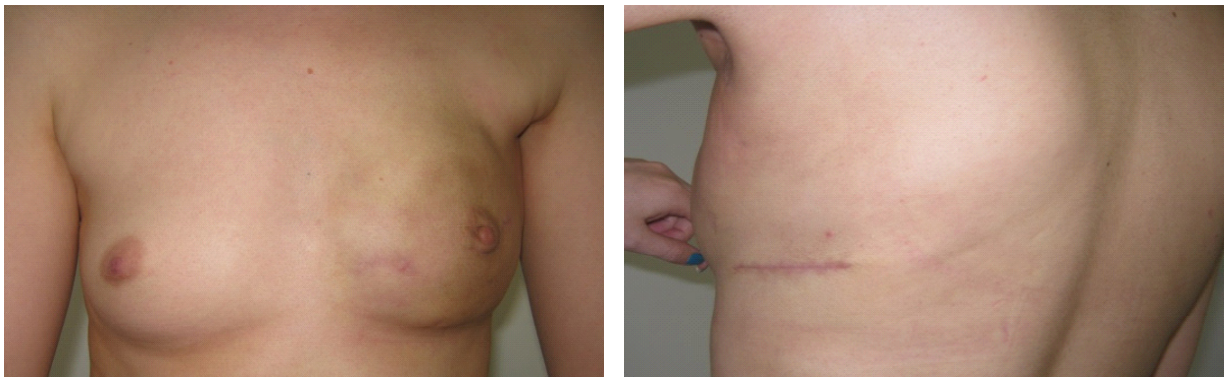

Figure 3: LD flap reconstruction

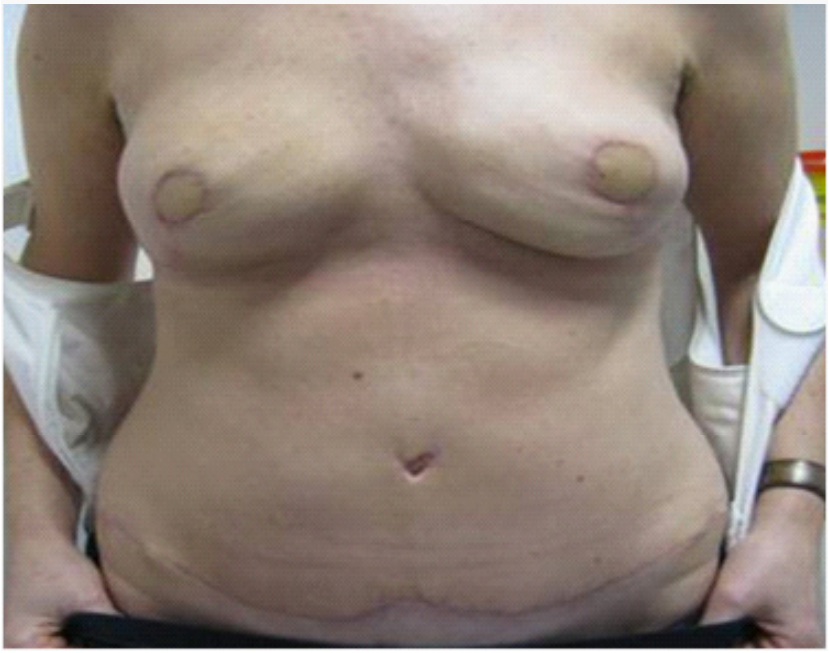

Figure 4: TRAM flap reconstruction

10. Tables

Table 1: Commercially available Acellular Dermal Matrices (ADMs) used in direct-to-implant reconstructions or in conjunction with a tissue expander. ADMs are created by a decellularisation process that leaves the extracellular matrix of the original tissue intact.

Acellular Dermal Matrix (Trade Names)	Company	Tissue Source	Sterile	Advantages
Flex HD	Ethicon	Human allograft skin	No	• Little elasticity • Prehydrated
AlloDerm	Life Cell	Human cadaveric skin	No	• Can be irradiated • Widely used, extensive studies carried out • Rapid revascularisation • Allows lymphocyte migration
DermaMatrix	Synthes	Human skin	Yes	• Bacterially inactivated • Rapid rehydration • No refrigeration required
Permacol	Covidien	Porcine dermis	Yes	• Cross-linked for greater durability • No refrigeration or rehydration required • Available in larger sizes
Strattice	LifeCell	Porcine dermis	Yes	• Good biomechanical strength • Prevents adhesions • Allows revascularisation • Allows lymphocyte migration and cell ingrowth
SurgiMend	Polytech	Foetal bovine dermis	Yes	• Rapid rehydration • Easy to suture • Fenestration to allow fluid drainage
AlloMax	Bard Davol	Human dermis	Yes	• Virally inactivated • Hydrates rapidly • Little elasticity • Early cellular infiltration and neovascularisation 7 days post-implant

Table 2: Biomaterials used as scaffolds in adipose tissue engineering. A scaffold acts as a template for new tissue formation. They can be naturally occurring materials or synthetic, each with their own properties, advantages and limitations.

Natural Scaffolds	Advantages	Limitations
Collagen	Can be modified e.g. addition of growth factors Supports adipogenesis, vascularisation and ECM deposition Licensed for clinical use	Limited mechanical strength Short degradation time

HYAFF11	Longer degradation time than collagen Licensed for clinical use Good mechanical stability Supports vascularisation and ECM deposition	Decreased differentiation of ADSCs
Silk	Low immunogenicity Slow, controlled degradation Licensed for clinical use Supports adipogenesis in vitro and in vivo	No data on stability of degradation products Limited surface modification
Synthetic Scaffolds	**Advantages**	**Limitations**
PLGA	Biodegradable Easily mass produced Adipogenic differentiation of ADSC in vivo Surface modification improves tissue growth	Foreign body capsule formation Short degradation time Requires surface modification
PEG	Licensed for medical use Low toxicity Water soluble Biodegradable	Hydrogel Poor mechanical strength Rapid degradation
PLA	Easy surface modification Good mechanical strength Allows vascularisation Adipogenic differentiation of BMSCs	Rapid degradation

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Recent Studies & Advances in Breast Cancer

Chapter 2

Magic Sized Quantum Dots as a Theranostic Tool for Breast Cancer

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Abstract

The discovery of quantum dots has sparked the research on biological imaging with many *in vitro* and *in vivo* applications, due to their incredible photostability and greater luminescence. Recently, the new magic sized quantum dots (MSQD) have surpassed most of the problems related to labeling, especially because of several important characteristics: the ultra-small size (<2 nm) that allows passive absorption into cells, the variable functional surface engineering that permits conjugation to different molecules for selectivity improvement, the highly biocompatible nature due to its colloidal synthesis, the luminescence tuning capability in different wave lengths, and the low complexity of the system, which have led us to a new level of bioimaging. In this review, we demonstrate the potential applications of MSQD conjugated to three novel molecules as theranostic tools for both diagnosis and treatment of triple negative breast cancer.

Keywords: Magic Sized Quantum Dots; Bioconjugation; Biocompatibility; Specific probe; Breast Cancer; PhospolipasesA2; Pepstatin A.

1. Introduction

Quantum dots have revolutionized biological imaging, but an important pitfall preventing its use for *in vivo* imaging and as therapeutic tools is their cytotoxicity effects. Recently, a new class of ultra-small quantum dots with very high stability and low toxicity has been developed by our group, which has been successfully exploited as a theranostic tool. This chapter presents the proof-of-concept with novel applications of MSQD in breast cancer diagnostics and therapeutics, which will be explored here in.

1.1. Quantum dots as specific probes

In the area of biological labeling, the great applicability of the quantum dots (QDs) occurs because they present several advantages over the traditional organic fluorophores, such as, long fluorescence life, high photo-resistance and chroma-degradation [1,2]. However, its cytotoxicity is a highly studied subject because these QDs show high cytotoxicity.

Magic sized quantum dots (MSQDs) are a category of quantum dots that have extremely small sizes (≤ 2 nm), stability of size and luminescence in function of time, present a broad emission spectrum, that allows their detection in the most diverse channels in the fluorescence microscopes [3]. In addition to these several advantages over traditional quantum dots, we have demonstrated that is possible control their biocompatibility and specificity during the synthesis [4]. Therefore, the use of these MSQDs is extremely important in monitoring applications of biological assays as a function of time.

The dispersion of QDs in biological fluids and conjugation with biological molecules consists of coating the surface of the QDs molecules containing specific chemical groups aiming at their use as luminescent probes. In our recent work, we performed functionalization process in which we demonstrated the formation of the CdS shell around CdSe QDs as a function of the synthesis temperature [3], and the concentration of stabilizer with external thiol group [5]. These methodologies were developed to increase the luminescence and biocompatibility of the QDs, since we will show that the cytotoxicity is related to the amount of $Cd2^{+}$ ions adsorbed on the surface of the CdSe QDs.

The use of QDs as specific probes in biological and biomedical applications is important because is possible to attach on the your surface a variety of biomolecules, including nucleic acids, proteins (avidin/streptavidin, albumin and antibodies), polysaccharides, and peptides. Several methods of bioconjugation have been used; however, the characteristics of biological molecules must be taking into consideration for specific coupling strategies. Biomolecules are generally conjugated on QDs' surface by the following bioconjugation methodologies: 1) cross-linked covalent bond: bonds the carboxyl groups on the surface of the PQs to the amine moieties present in the biomolecule using 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide

(EDC) [6] or N-hydroxysulfosuccinimide (NHS) [7]; 2) adsorption or non-covalent self-assembly, using protein engineering (polyhistidine(His) [8-11]. Further more, due to the high ratio surface volume is possible to couple several different kinds of biomolecules in a single QD where each has a specific function that ensures the multifunctionality of QDs [12].

1.2. Carcinogenesis and Cancer

The functioning of the human body can be considered as a society, whose members are cells, which reproduce themselves through cellular divisions and are organized in sets (tissues) that collaborate with each other. Thus, to coordinate this behavior the cells send, receive, and interpret a sophisticated set of signals. Each cell behaves in a way, being able to divide, differentiate or die. Any molecular change that disrupts this harmonious behavior can result in problems for the body. In the human body, many cells constantly mutate, and sometimes such a mutation can result in a certain selective advantage to a cell, allowing it to grow and divide more vigorously and survive more easily than other cells, becoming the founder of a mutant clone that grows out of the normal context [13].

Carcinogenesis is a complex process, in which normal cells progress to cells with neoplastic phenotypes. This is a multi step process describes like somatic evolution conducted by mutations and alterations of DNA. The tumor cells confers interactions where cell-cell contact is lost, followed by inadequate growth, hypoxia, ischemia, senescence, resistance to apoptosis, and self-sustaining growth. Innumerable mechanisms for invasion and metastasis have been shown elsewhere [14,15].

1.2.1. Breast Cancer

Breast cancer is generally a malignant tumor that has developed from cells in the breast. This cancer is genetically and clinically heterogeneous and is always caused by genetic mutations on the DNA: although only 5-10% of these mutations are hereditary, the majority (90-95%) is caused by abnormalities originating of lifestyle and environmental factors [16-19].

In this context, breast cancer classification systems have been developed to organize this heterogeneity. The advances in genomics have led to elucidation of the subtypes of breast cancer based on the molecular profile of three markers: estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor type 2 (HER2). This classification is critical for choosing the appropriate treatment of patients, and for the development of new therapeutic strategies [17,20,21].

The most aggressive subtype of breast cancer, called triple negative breast cancer (TNBC) is characterized by lack of the three markers expression: ER, PR and HER2. As a consequence, TNBC cells are insensitive to hormone or HER2-targeted therapies and the

treatment options for these patients is chemotherapy, surgery and radiation therapy. Thus, the investigation of TNBC-targeted therapy is imperative and the cell culture model has become a useful tool for understanding the cancer functionality and provides interesting information about the biology of the disease becoming a good candidate for development of specific targeted therapy. Here, we investigated the efficacy of Pepstatin A and Phospholipases BnSP-6 and BThtX-II as a TNBC treatment.

1.2.1.1. Pepstatin A

Pepstatin A is an aspartic protease inhibitor that acts specifically in the inhibition of Cathepsin D catalytic activity in triple negative breast cancer [22]. Cathepsin D is a protease that is currently investigated as a biomarker among triple negative breast cancers. In addition, Cathepsin D inhibition is essential to reduce breast cancer cells aggressiveness, since it is involved in proteolytic events responsible for breast cancer progression and metastasis [23].

Evidences suggest that Pepstatin A induced apoptosis and autophagy processes in triple negative breast cancer cell, while reducing proliferation, migration and invasion [24]. Cathepsin D inhibition is also crucial to increase sensitivity of breast cancer cells to chemotherapy, because it may protect cancer cells from chemotherapeutic agents [25]. So, it is important to investigate the bioconjugation of Pepstatin A with MSQD to allow its tracking within the cell and, in addition, to verify the specificity of this treatment for triple negative breast cancer cells.

1.2.1.2. Snake venom phospolipase A2

Snake venoms constitute a mixture of bioactive components that are involved not only in envenomation pathophysiology but also in the development of new drugs. Different enzymatic and non-enzymatic proteins such as phospholipases A2 (PLA_2) are present in the venom composition, with PLA_2 being responsible for specifically catalyzing the hydrolysis of the sn-2 acyl groups of membrane phospholipids to release arachidonic acid and lysophospholipids [26,27]. The replacement of the amino acid aspartate by a lysine in position 49 provokes loss of enzymatic activity of these PLA_2, and therefore these variants have been referred to as Lys49 PLA_2 homologues capable of disrupting the integrity of membranes and provoking many pharmacological effects [28-31]. These proteins have been studied for a long time and this last decade many studies have demonstrated their therapeutic potential as promise models for therapeutic agents design, since numerous studies have demonstrated the action microbicides, antitumor, antiplatelet and antiangiogenesis activities, although many advances have been made in cancer therapy, the search for new drugs from natural resources is one important topic of biomedical research [32-34].

1.2.1.2.1. BnSP-6 – Lys49 PLA_2 from *Bothrops pauloensis*

PLA2-BnSP-6 has a molecular mass of 13,420 Da, with 122 amino acid residues in its-dimeric form. It has a high content of basic and hydrophobic amino acids and isoelectric point (pI)of 8.6. This phospholipase also has no phospholipase or coagulant activity [35,36].

In this context, recently our group published a work showing the in vitro antitumor effect of BnSP-6, a Lys 49 PLA_2 isolated from Bothropspauloensis venom on human breast cancer MDA-MB-231 cells [34]. In this work, we demonstrated that BnSP-6 caused a dose-dependent cytotoxicity and inhibited cell adhesion of human breast cancer MDA-MB-231 cells. Interestingly, cytotoxic activity of BnSP-6 was significantly lower against MCF10A, a non-tumorigenic breast cell line. BnSP-6 stimulated the autophagy process and induced both early and late apoptosis. Apoptosis of MDA-MB-231 cells were also confirmed by up-regulation of different genes related to the apoptosis pathway, such as TNF, TNFRSF10B, TNFRSF1A and CASP8 and decreased expression of anti-apoptotic genes (BCL2 and BCL2L). In addition, BnSP-6 caused a remarkable increase in gene expression of BRCA2 and TP53 tumor suppressors. Finally, BnSP-6 induced down-regulation of Angiopoietin 1 gene, a potent pro-angiogenic factor, and inhibited adhesion and migration of MDA-MB-231 cells, suggesting pharmaceutical applications of this PLA_2 as an anti-angiogenic and anti-metastatic agent [34].

1.2.1.2.2 BthTX-II- Phospolipase A2-Asp49 of *Bothrops jararacussu*

Several constituents of Bothropsjararacussu venom have already been isolated, and biochemically and functionally characterized. Among them, two PLA_2s named Bothropstoxin I (BthTx-I) and Bothropstoxin II (BthTx-II) [37] have been intensively explored. The BthTx-II is a basic Asp49 phospholipase A2 with 13,976 Da and 120 amino acid residues [38-40]. This toxin has several biological effects, including myotoxicity, edematogenic activity, low phospholipase activity, induces platelet aggregation and presents hypotensive activity [39-41] PLA2 BthTx-I has already been explored for its antitumor potential by our research group [42]. We are currently investigating its action on breast cancer cells.

2. Results and Discussions

2.1. Pepstatin A

CdSe/CdS MSQDs were incubated with MCF-10A and MDA-MB-231. The internalization of MSQDs was visualized under a confocal fluorescence microscopy.

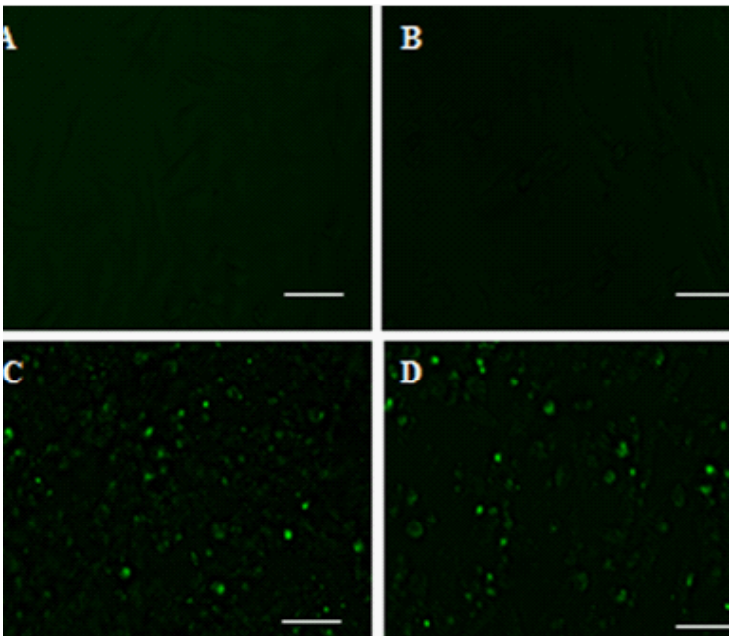

Figure 1: Pepstatin A effect in breast cells. A) MCF-10 A normal breast cells with quantum dots; B) MDA-MB-231 triple negative breast cancer cells treated only with MSQDs; C) MDA-MB-231 triple negative breast cancer cells treated with 1μm of MSQDs bioconjugated with Pepstatin A. D) MDA-MB-231 triple negative breast cancer cells treated with 10μM of MSQDs bioconjugated with Pepstatin A. The bar corresponds to 200 μm.

In the **Figure 1** observed a green fluorescence of the MSQDs bioconjugated to Pepstatin A in two doses (1μm. - **Figure 1C**, and 10μm - **Figure 1D**) was detected inside the cytosolic compartment of MDA-MB-231 cells in 6h after treatment. Notably, we verified that CdSe/CdS MSQDs alone is not internalized both in MCF-10A (**Figure 1A**) and MDA-MB-231 (**Figure 2B**).

Our present study suggests that Pepstatin A bioconjugated with MSQDs could be a novel and specific treatment for triple negative breast cancer since it was able to penetrate specifically at MDA-MB-231 in a short period (6 hours). However, for clinical use, we suggestan additional investigation with *in vivo* models to better understand the pharmacokinetics, toxicity, tissue distribution and MSQD elimination.

2.2 Phospholipases

2.2.1 BnSP-6

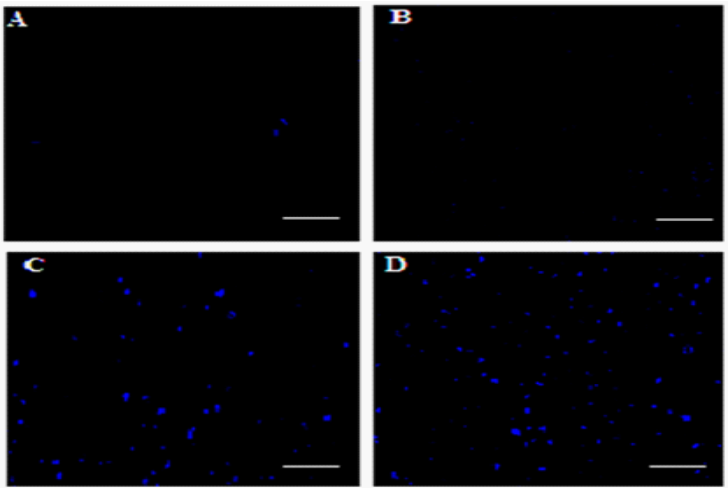

Figure 2: BnSP-6 effect in breast cells. A) MCF-10 A normal breast cells with quantum dots; B) MDA-MB-231 triple negative breast cancer cells treated only with MSQDs; C) MDA-MB-231 triple negative breast cancer cells treated with 1µg/ml of MSQDs bioconjugated with BnSP-6. D) MDA-MB-231 triple negative breast cancer cells treated with 50µg/ml of MSQDs bioconjugated with BnSP-6. The bar corresponds to 200 µm..

As shown in Figure 2, the blue fluorescence of the MSQDs bioconjugated with BnSP-6 in two doses (1µg/ml - Figure 2C,and 50µg/ml - Figure 2D) was detected inside the cytosolic compartment of MDA-MB-231 cells after3h treatment. Notably, we verified that MSQDs alone is not internalized both in MCF-10A (Figure 1A) and MDA-MB-231 (Figure 1B).

Our present study suggests that BnSP-6 coupled to the MSQD could be a recent and specific treatment for triple negative breast cancer since it was able to penetrate specifically at MDA-MB-231immediately after treatment. However, more studies must show itsclinical use. We also suggestin vivo models for additional investigation todemonstrate its pharmacokinetics, toxicity, tissue distribution and MSQD elimination.

2.2.2. BThtX-II

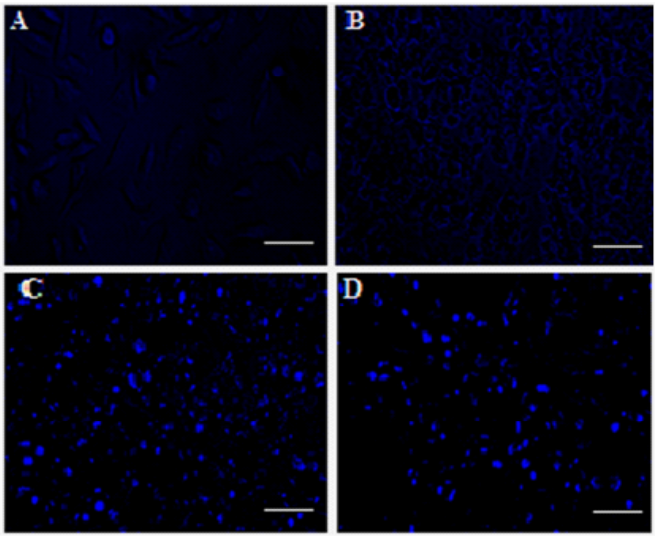

Figure 3: BThtX-II effect in breast cells. A) MCF-10 A normal breast cells with quantum dots; B) MDA-MB-231 triple negative breast cancer cells treated only with MSQDs; C) MDA-MB-231 triple negative breast cancer cells treated with 1µg/ml of MSQDs bioconjugated with BThtX-II. D) MDA-MB-231 triple negative breast cancer cells treated with 50µg/ml of MSQDs bioconjugated with BThtX-II. The bar corresponds to 200 µm..

Similarly to the BnSP-6 effect shown in **Figure 2**, the blue fluorescence from MSQD bioconjugated to BthTx-II in two doses (1µg/ml - **Figure 3C**,and 50µg/ml - **Figure 3D**) was detected inside the cells'nucleicompartment of MDA-MB-231 cells 3h after treatment. Notably, we verified that the MSQD alone was not internalized in both MCF-10A (**Figure 2A**) and MDA-MB-231 (**Figure 2B**).

Our present study suggests that BthTX-II bioconjugation to the MSQD could be a noveland specific treatment for triple negative breast cancer, since it was able to specifically penetrate MDA-MB-231 cells immediately after treatment. However, new studies are under way in order to demonstrate the PLA2 applications as promising drug against triple negative breast cancer.

3. Conclusion

In conclusion, our review has successfully demonstrated the exploitation of possible therapies for triple negative breast cancers with unexplored theranostic tools, specifically by tracking three major tumor targets coupled with CdSe/CdS MSQDs in tumor cells, Pepstatin A, BnSP-6 and BthTx-II, which may become potential therapeutic strategies in the near future.

4. Acknowledgments

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Chapter 3

Depression in Female Breast Cancer Patients

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1. Introduction

The knowledge of the diagnosis of cancer often leads to anxiety and depression in the affected person. A pessimistic interpretation of one's disease and perspectives on life in the future is almost always present even in those who seeminly continue with their professional and other everyday activities. The studies in the area of psychooncology have shown that five years after the mastectomy in female breast cancer patients the disease outcome depends on the mode of mental reaction in the first three postoperative months.

Visits to one's oncologist tend to focus primarily on the treatment of bodily disease and its adverse effects, and secondarily on the management of pain and other symptoms. Emotional symptoms can be underestimated and even completely overlooked and neglected as an expected companion of cancer. It is quite logical that a psychiatrist should be involved in the treatment of a cancer patient, especially the women with breast cancer. It is a fact that such a treatment approach is not institutionally introduced and therapeutic teams in the management of breast cancer patients do not have psychiatrists.

The patients themselves are reluctant to ask their doctors for help regarding emotional complaints, fearing it could draw the doctor's attention away from cancer treatment, or they are afraid of negative cultural attitudes towards depression [1].

The emotional response of an individual with cancer is determined by the following three factors:

a) attitude towards the diagnosis (eg. as a challenge or a threat);

b) perception of control (partial or none), and

c) view of prognosis (good or poor) [2].

A holistic orientation itself requires that the approach in the treatment of individuals with most serious malignant diseases should be multidisciplinary, involving a collaboration of experts in different areas (various medical specialists, nurses, technicians, psychologists, psychotherapists, spiritual counselors, ethicists, physical therapists, and volunteers) [3].

The success of treatment in oncology is influenced not only by the disease stage, tumor histology, degree of malignancy, and mode of treatment, but also by numerous psychic factors. All the theories concerning psychosomatic background of malignant diseases can be categorized into permissive and causal ones [4].

The permissive theory is a more moderate one and assumes that psychogenic factors involved do not act as direct cancerogens, but that other immediate causal agents make possible that malignant injuries to the tissues and organs appear openly.

The causal theories are numerous, but the one should be mentioned that claims that unconscious unresolved conflicts find their expression in the somatic plane.

Psychological factors that could possibly influence the onset of a cancer would be as follows [5]:

1) stressful life events

2) social relationships and support by the environment

3) personal traits (personas)

4) facing the disease

5) negative emotional reactions

6) psychiatric disorders

7) suppression of emotions

1) Stressful life events

The research efforts concerning this factor are mostly associated with breast tumors and elements preceding the disease onset linked to frequent stressful events (twice as prevalent compared to controls); these investigations lack prospectiveness.

2) Social relationships and support

The most relevant study followed 224 women with breast cancer for 7 years, showing significantly poorer survival rates in those without any social support in their immediate social environment, compared to those who clearly received and felt such support in their families and social environment.

3) Personal traits

Numerous authors have been concerned with the question whether oncologic patients share a similar psychic structure; many of them have concluded that cancer patients often have difficulties in expressing their emotions, inability to express aggression openly, and suppression of depressive moods; in short, these individuals are well adapted to others, but they are alienated from themselves.

4) Facing the disease

It has always been known that an active stance against the disease significantly improves treatment success; an active stance in facing the tumor means better survival.

5) Negative emotional reactions

Anxiety, grief, and depression are significant psychiatric symptoms associated with the onset of cancer, but the studies have failed to demonstrate any causal relationship between the malignancy and these symptoms, but it has been shown that they markedly influence the treatment outcome.

6) Psychiatric diseases

Studies investigating the association of psychiatric diseases with the onset of oncological diseases have not been consistent-only one such study showed that a psychiatric disease was the protective factor in cancer survival; a negative relationship was found in four studies, and five studies were not able to find any association between the two.

7) Suppression

It has been found that cancer patients often have difficulties in expressing their emotions, which has been confirmed in the studies measuring the degree of suppression and length of survival – the more a person shows emotions, the better the chance of longer survival.

In all the phases of breast cancer treatment various psychiatric problems can be present.

Posttraumatic disorders can be associated with the diagnosis of cancer, mastectomy, or chemotherapy. Depressive disorders are commonly linked to the development of metastases.

The use of adjuvant chemotherapy is associated with possible development of psychiatric disorders, and such a treatment is used in even 80% of affected women. Around 20-38% of women with breast cancer diagnosis have a high level of psychological distress in the first year after the diagnosis [6].

In some women, a high level of intrusive symptoms and avoidance coping can last for years after the initial diagnosis. The prevalence rate of depression in women with breast cancer varies from 1.4% to 46%, depending on a number of factors, including stage and size of the tumor, patient age, availability of social support, socioeconomic background, type of surgery, ability to make one's own choices and to be involved in one's treatment, type of therapy (radiation therapy, chemotherapy), and familial and personal history of depression [7].

The treatment and cancer itself have an impact on the anxiety, sexual satisfaction, sleeping, quality of life, perception of one's own body (body image) and self-confidence. Some patients feel traumatized and mutilated, and may have the reactions of grief after surgical interventions, especially after prophylactic bilateral mastectomy. The psychological impact of breast cancer is present too in the spouse, partner, children, and parents of women with breast cancer.

In an analysis of the possible conditions preceding psychological distress, the importance of avoidance coping has been suggested, although possible positive effects of such a behavior have been considered as well, especially in the active treatment phase.

Numerous adverse treatment effects produce some long-lasting consequences, from the distorted body image (mutilation surgery, radiation therapy) to disturbed self-esteem, and inflict significant sexual problems as well. Psychiatric diseases that may accompany this phase of the disease are also an important problem; this particularly refers to the onset of posttraumatic stress disorder, full-blown in as high as 10% of survivors, and in over 48% of patients reflected in some other elements, primarily in the symptoms of reliving the trauma and in avoidance coping [8].

With active palliative management in recent years a step forward has been made from the hospital to out-patient framework, reflected especially in the control of symptoms (pain) in home environments. More adequate supporting psychosocial elements have thus been provided, assuring better quality of life, shifting the disease management burden towards the family, but assuring instead better family relationships with appropriate support from well trained associates in the process.

2. Investigation

The investigation described here took place at the Clinic of Oncology, Clinical Cen-

ter Niš, in the period from May 6, 2014 to December 2016. Two groups of examinees were formed for the purpose of the study. The first group consisted of 120 women with the diagnosis of breast cancer, actively treated, aged 27 to 65 years. In the period of 6 months four patients died and were excluded from the analysis. There were 13 patients over 65 years of age. The patients were tested on the day of their enrollment in the study and again after 6 months of treatment. The second group consisted of 50 depresion women no breast cancer aged 22 to 65 years, accompanying breast cancer patients to the oncology clinic. The sample was stratified (the studied and control groups) to a degree by the factor of age of the examinees.

The socio-demographic questionnaire, designed for the purpose of this study, involved questions related to the patient age, marital status, level of education, employment and economic status, high risk behaviors and habits, and the disease, breast cancer.

The rights of the examinees were not in any way compromised during the study. The data obtained was protected and accessible only to the researcher. The examinees were first informed in detail about the study itself, its purpose and scientific rationale, confidentiality of the collected data, and were asked to sign their informed consent to participate in the study.

The study was approved by the Decision of the Ethics Committee of the Clinical Center Niš, No. 12613/57 of May 6, 2014.

The study enrolled 153 examinees, among which 103 (67.3%) breast cancer patients comprising the study group, and 50 (32%) healthy women – patient companions – comprising the control group. The average age of the whole studied population was 48.5±11.4 years (ranging from 22 to 65 years). Age structure of the examinees in different groups was not statistically significantly different, i.e. the groups were homogenous (studied group: 49.4±10.8 vs. control group: 46.5±11.5 years; t=1.471; p=0.143).

Table 1: Socio-demographic characteristics, by groups

		Studied group n (%)	Control group n (%)	$\chi 2$ / t*	p
Employment status	**Unemployed**	39(37,9)	16(32,0)		
	Employed	41(39,8)	25(50,0)		
	Retired	23(22,3)	8(18,0)	1,435	0,488
Educational status	**Incomplete primary school**	3(2,9)	6(12,0)	24,671	<0,001
	Primary school	12(11,7)	12(24,0)		
	High school	17(16,5)	17(34,0)		
	College	22(21,4)	9(18,0)		
	Higher education	49(47,6)	6(12,0)		
Marital status	**Married**	11(10,7)	12(24,0)	7,685	0,104

	Common-law marriage	14(13,6)	7(14,0)		
	Single	67(65,0)	25(50,0)		
	Widow	5(4,9)	5(10,0)		
	Divorced	6(5,8)	1(2,0)		
Children	**Yes**	74(71,8)	46(92,0)	8,083*	0,004
	No	29(28,2)	4(8,0)		
Age at birth of the first child	**±SD**				
25,1 ± 3,0	**23,8 ± 6,9**	1,286	0,201		
Number of children	**Without children[a]**	29(28,2)	6(12,0)	9,959	0,007
	One child	16(15,5)	1(2,0)		
	Two children	56(54,4)	43(86,0)		
	Three and more	2(1,9)	0(0,0)		

Educational status was statistically significantly different between the two groups ($\chi2=24.671$; $p<0.001$). It was further established that a significantly larger number of control group examinees had incomplete primary school ($\chi2=26.184$; $p<0.001$), completed primary school ($\chi2=5.960$; $p=0.014$), and high school ($\chi2=3.881$; $p=0.048$). Higher education was significantly more common among the women with breast cancer ($\chi2=18.986$; $p<0.001$).

Table 2: Personal and family history, by groups

		Studied group n (%)	**Control group n (%)**	**χ2**	**p**
Psychic complaints before the disease	**yes**	4(3,8)	45(91,8)	0,887	0,346
	no	100(96,2)	4(8,2)		
Stressful life events	**yes**	102(99,0)	13(26,0)	96,163	<0,001
	no	1(1,0)	37(74,0)		
Psychiatric drugs	**yes**	8(7,8)	5(10,0)	0,216	0,642
	no	95(92,2)	45(90,0)		
Tumors in the family	**yes**	24(23,3)	3(6,0)	6,933	0,008
	no	79(76,6)	47(94,0)		
Psychiatric diseases in the family	**yes**	7(6,8)	1(2,0)	1,563	0,211
	no	96(93,2)	49(98,0)		

The presence of psychic complaints did not differ significantly between the examined populations ($\chi2=0.887$; $p=0.346$). Stress was present at a high percentage in the patients before the onset of their disease ($\chi2=96.163$; $p<0.001$). Use of psychiatric drugs ($\chi2=0.216$; $p=0.642$), as well as the familial presence of psychiatric diseases ($\chi2=1.563$; $p=0.211$) did not differ sig-

nificantly between the groups. There were significantly more diagnosed tumors in the family histories of breast cancer patients ($\chi2=6.933$; $p=0.008$).

Table 3: Personal habits and needs in the studied groups

		Studied group n (%)	Control group n (%)	χ2	p
Alcohol intake	**yes**	11(10,7)	10(20,0)	2,469	0,116
	no	92(89,3)	40(80,0)		
Cigarette smoking	**yes**	39(37,9)	45(90,0)	36,953	<0,001
	no	64(62,1)	5(10,0)		
Communication avoidance	**yes**	82(79,6)	38(76,0)	0,260	0,610
	no	21(20,4)	12(24,0)		
Sexual desire	**yes**	80(77,7)	49(98,0)		
	no	23(22,3)	1(2,0)	10,219	0,001

Alcohol intake did not differ significantly between the studied groups ($\chi2=2.469$; $p=0.116$), while cigarette smoking was significantly more common in control group women ($\chi2=36.953$; $p<0.001$).

The number of friends and acquaintances did not differ significantly between the cancer patients and healthy women ($\chi2=0.260$; $p=0.610$). Sexual desire was significantly less common in breast cancer patients ($\chi2=10.219$; $p=0.001$).

Table 4: Depression score according to the HAMD scale by groups at the beginning of the study

	$\bar{x}$	SD	Med	Iq	Min	Max	Z	p
Studied group	16,03	5,16	17,00	7,00	5,00	37,00	8,508	<0,001
Control group	7,42	2,86	7,00	4,00	2,00	17,00		

Table 5: Depression score according to the HAMD scale by groups after 6 months

	$\bar{x}$	SD	Med	Iq	Min	Max	Z	p
Studied group	14,43	4,48	16,00	4,00	5,00	33,00	1,363	0,173
Control group	15,36	2,99	16,00	14,0	7,00	21,00		

Statistically significant changes in the score values were found in both groups: in the group of patients the score value decreased significantly after 6 months ($Z=5.031$; $p<0.001$), while the score values in controls increased significantly after the same period of time ($Z=6.058$; $p<0.001$).

Table 6: Components of the HAMD score by groups at the beginning of the study

HAMD components	Studied group		Control group		
	$\bar{x}$	SD	$\bar{x}$	SD	p
H1 DEPRESSIVE MOOD	0,10	0,37	0,26	0,44	0,006
H2 FEELING OF GUILT	0,18	0,46	0,28	0,45	0,090
H3 SUICIDE	0,05	0,21	0,26	0,44	<0,001
H4 INSOMNIA EARLY	1,08	0,47	0,32	0,47	<0,001
H5 DIFFICULTIES STAYING ASLEEP	1,34	0,67	0,38	0,49	<0,001
H6 INSOMNIA LATE	1,09	0,65	0,46	0,50	<0,001
H7 WORK AND ACTIVITIES	1,42	0,98	0,42	0,53	<0,001
H8 RETARDATION	0,66	0,57	0,48	0,50	0,076
H9 AGITATION	0,92	0,52	0,26	0,44	<0,001
H10 ANXIETY	1,24	0,68	0,50	0,51	<0,001
H11 ANXIETY, SOMATIC	1,02	0,60	0,34	0,49	<0,001
H12 GASTROINTESTINAL SOMATIC SYMPTOMS	0,77	0,48	0,48	0,50	0,001
H13 GENERAL SOMATIC SYMPTOMS	0,87	0,41	0,38	0,53	<0,001
H14 GENITAL SYMPTOMS	0,83	0,56	0,38	0,53	<0,001
H15 HYPOCHONDRIA	0,83	0,48	0,64	0,48	0,019
H16 LOSS OF WEIGHT	0,72	0,49	0,46	0,50	0,003
H17 INSIGHT INTO THE DISEASE	0,89	0,56	0,24	0,43	<0,001
H18 DIURNAL MOOD VARIATION	0,94	0,34	0,20	0,40	<0,001
H19 DEPERSONALIZATION AND DEREALIZATION	0,46	0,54	0,44	0,50	0,950
H20 PARANOID SYMTOMS	0,30	0,46	0,24	0,43	0,433
H21 OBSESSIVE-COMPULSIVE SYMPTOMS	0,25	0,46	0,18	0,39	0,370

The comparison of individual 21 components of the HAMD score measured at the beginning of the study between the examined groups are shown in Table 6. Statistically significant differences between the groups were established for 16 HAMD score components as follows: depressive mood ($Z=2.759$; $p=0.006$), suicide ($Z=3.795$; $p<0.001$), insomnia early ($Z=7.744$; $p<0.001$), difficulty staying asleep ($Z=7.310$; $p<0.001$), late insomnia ($Z=5.492$; $p<0.001$), work and activities ($Z=6.058$; $p<0.001$), agitation ($Z=6.771$; $p<0.001$), anxiety ($Z=6.051$; $p<0.001$), somatic anxiety ($Z=6.29$; $p<0.001$), somatic gastrointestinal symptoms ($Z=3.376$; $p=0.001$),

general somatic symptoms (Z=5.839; p<0.001), genital symptoms (Z=4.601; p<0.001), hypochondria (Z=2.341; p=0.019), loss of weight (Z=2.925; p=0.003), insight into the disease (Z=6.465; p<0.001), diurnal mood variation (Z=8.730; p<0.001).

Table 7: HAMD score components by groups after 6 months of study

HAMD components	Studied group		Control group		p*
	$\bar{x}$	SD	$\bar{x}$	SD	
H1 DEPRESSIVE MOOD	0,04	0,24	0,00	0,00	0,224
H2 FEELING OF GUILT	0,02	0,14	0,00	0,00	0,323
H3 SUICIDE	0,02	0,14	0,02	0,14	0,981
H4 INSOMNIA EARLY	1,16	0,54	0,94	0,42	0,010
H5 DIFFICULTIES STAYING ASLEEP	1,11	0,49	1,08	0,34	0,575
H6 INSOMNIA LATE	1,00	0,39	1,08	0,44	0,258
H7 WORK AND ACTIVITIES	1,14	0,74	0,96	0,19	0,336
H8 RETARDATION	0,79	0,62	1,00	0,35	0,010
H9 AGITATION	0,66	0,53	0,96	0,35	<0,001
H10 ANXIETY	1,16	0,65	1,12	0,39	0,626
H11 ANXIETY, SOMATIC	1,08	0,59	1,06	0,24	0,734
H12 GASTROINTESTINAL SOMATIC SYMPTOMS	0,64	0,50	0,98	0,25	<0,001
H13 GENERAL SOMATIC SYMPTOMS	0,89	0,34	0,94	0,24	0,371
H14 GENITAL SYMPTOMS	0,60	0,49	0,90	0,30	<0,001
H15 HYPOCHONDRIA	0,78	0,42	0,92	0,27	0,030
H16 LOSS OF WEIGHT	0,61	0,49	0,92	0,27	<0,001
H17 INSIGHT INTO THE DISEASE	1,14	1,77	0,90	0,30	0,408
H18 DIURNAL MOOD VARIATION	0,84	0,37	0,88	0,34	0,466
H19 DEPERSONALIZATION AND DEREALIZATION	0,58	0,49	0,60	0,49	0,750
H20 PARANOID SYMTOMS	0,12	0,32	0,04	0,19	0,125
H21 OBSESSIVE-COMPULSIVE SYMPTOMS	0,08	0,30	0,00	0,00	0,060

After 6 months, comparing the individual components of the HAMD score between the studied and control groups, it was found that there was a statistically significant difference in 6 HAMD components: insomnia early (Z=2.570; p=0.010), retardation (Z=2.590; p=0.010), agitation (Z=3.597; p<0.001), somatic gastrointestinal symptoms (Z=3.579; p=0.019), geni-

tal symptoms (Z=3.759; p<0.001), hypochondria (Z=2.174; p=0.030), and loss of weight (Z=3.939; p<0.001).

Table 8: Changes in the HAMD score components by groups after 6 months of study

HAMD components	Studied group		Control group	
	Z	p	Z	p
H1 DEPRESSIVE MOOD	1,748	0,080	3,606	<0,001
H2 FEELING OF GUILT	3,900	<0,001	3,742	0,001
H3 SUICIDE	1,342	0,180	3,207	<0,001
H4 INSOMNIA EARLY	0,956	0,339	5,240	<0,001
H5 DIFFICULTIES STAYING ASLEEP	3,101	0,002	5,296	<0,001
H6 INSOMNIA LATE	1,508	0,132	4,943	<0,001
H7 WORK AND ACTIVITIES	2,997	0,003	5,014	<0,001
H8 RETARDATION	1,899	0,058	4,747	<0,001
H9 AGITATION	3,856	<0,001	5,596	<0,001
H10 ANXIETY	1,192	0,233	4,625	<0,001
H11 ANXIETY, SOMATIC	0,732	0,464	5,684	<0,001
H12 GASTROINTESTINAL SOMATIC SYMPTOMS	2,271	0,023	4,642	<0,001
H13 GENERAL SOMATIC SYMPTOMS	0,447	0,655	5,292	<0,001
H14 GENITAL SYMPTOMS	3,333	0,001	4,459	<0,001
H15 HYPOCHONDRIA	1,177	0,239	3,300	0,001
H16 LOSS OF WEIGHT	1,808	0,071	4,796	<0,001
H17 INSIGHT INTO THE DISEASE	1,470	0,142	5,578	<0,001
H18 DIURNAL MOOD VARIATION	2,294	0,022	5,516	<0,001
H19 DEPERSONALIZATION AND DEREALIZATION	1,852	0,064	1,789	0,074
H20 PARANOID SYMTOMS	3,212	0,001	2,887	0,004
H21 OBSESSIVE-COMPULSIVE SYMPTOMS	3,402	0,001	3,000	0,003

Table 9: Univariate linear regression analysis of the factors related to the degree of depression according to the Hamilton scale at the beginning of the study

	Beta	95% CI		p
		lower limit	upper limit	
Age	0,010	-0,080	0,090	0,904
Groups	-0,667	-10,165	-7,072	<0,001
Employment status	-0,092	-2,065	0,560	0,259
Educational status	0,230	0,351	1,844	0,004
Marital status	0,028	-0,845	1,199	0,733
Children	0,227	1,042	5,655	0,005
Age at birth of the 1st child	0,098	-0,127	0,386	0,318
Number of children	-0,307	-3,260	-1,090	<0,001
Psychic complaints before the disease	-0,258	8,680	-2,152	0,001
Stressful life events	-0,526	-9,286	-5,450	<0,001
Psychiatric drugs	-0,070	-5,007	1,962	0,389
Tumors in the family	-0,218	-5,957	-0,969	0,007
Psychiatric diseases in the family	-0,219	-10,234	-1,696	0,006
Alcohol intake	-0,235	-3,234	2,425	0,778
Cigarette smoking	0,236	0,966	4,774	0,003
Communication avoidance	0,025	-1,994	2,741	0,756
Sexual desire	0,207	0,822	6,063	0,010

Table 9 presents the results of univariate linear regression analysis of individual socio-demographic factors, factors in personal and family histories, habits and needs, related to the onset of depression according to the Hamilton scale measured at the beginning of the study. Statistically significant risk factors for depression were as follows: women with breast cancer (Beta=-0.667; p<0.001), higher educational status (Beta=0.230; p=0.004), childlessness (Beta=0.227; p=0.005), as well as a lower number of children (Beta=-0.307; p<0.001), presence of psychic complaints before the disease (Beta=-0.258; p=0.001), stress before the disease (Beta=-0.526; p=0.004), tumors in the family (Beta=-0.218; p=0.007), presence of psychiatric diseases in the family (Beta=-0.219; p=0.006), absence of smoking (Beta=0.236; p=0.003), and lack of sexual desire (Beta=0.207 p=0.010).

Table 10: Multivariate linear regression analysis of the factors related to the degree of depression according to the Hamilton scale at the beginning of the study

	Beta	95% CI		p
		lower limit	upper limit	
Groups	-0,678	-11,339	-6,167	<0,001
Educational status	-0,021	-0,684	0,489	0,742
Children	0,224	-6,996	0,399	0,080
Number of children	-0,389	-4,527	-0,980	0,003
Psychic complaints before the disease	-0,171	-6,017	-1,157	0,004
Stress before the disease	0,041	-2,120	3,264	0,675
Tumors in the family	-0,079	-3,139	0,615	0,186
Psychiatric diseases in the family	-0,093	-5,732	0,693	0,123
Cigarette use	-0,144	-3,406	-0,099	0,038
Sexual desire	0,074	-0,793	3,243	0,232

Multivariate linear regression analysis was performed to evaluate depression according to the Hamilton scale in relation to the individual factors that stood out as statistically most significant. Ten factors could be included into the multivariate model: groups, educational status, children and number of children, psychic complaints before the disease, stress, tumors and psychiatric diseases in the family, cigarette use, and sexual desire. The model as a whole was highly statistically significant – $F(10, n=103) = 17.878$, $p<0.001$. The model as a whole explains 52.6% of variance of depression score according to the Hamilton scale. The following factors gave a statistically significant contribution to the model: patient group (Beta=-0.678; $p<0.001$), number of children (Beta=-0.389; $p=0.003$), psychic complaints before the disease (Beta=-0.171; $p=0.004$), and cigarette use (Beta=-0.144; $p=0.038$).

Table 11: Univariate linear regression analysis of the factors related to the degree of depression according to the Hamilton scale after 6 months of study

	Beta	95% CI		p
		lower limit	upper limit	
Age	0,097	-0,092	0,022	0,232
Groups	0,107	-0,459	2,306	0,189
Employment status	-0,039	-1,098	0,666	0,630
Educational status	-0,003	-0,552	0,505	0,974
Marital status	0,178	-1,431	0,084	0,028
Children	0,124	-0,359	2,796	0,127
Age at birth of the 1st child	-0,012	-0,183	0,162	0,902
Number of children	-0,144	-1,437	-0,073	0,076

Psychic complaints before the disease	0,088	-3,485	1,022	0,282
Stress before the disease	0,082	0,737	2,272	0,315
Psychiatric drugs	-0,043	-2,959	1,715	0,600
Tumors in the family	-0,029	-1,398	2,022	0,719
Psychiatric diseases in the family	-0,051	-3,861	-1,991	0,529
Alcohol use	-0,035	-2,308	1,481	0,667
Cigarette use	-0,184	-2,792	-0,215	0,022
Communication avoidance	0,038	-1,213	1,957	0,643
Sexual desire	0,103	-0,634	2,934	0,205

Table 11 presents the results of univariate linear regression analysis of inidividual socio-demographic factors, factors in the personal and familial history, and habits and needs of impact on depression according to the Hamilton scale measured 6 months after the beginning of the study. Statistically significant risk factors for depression were marital status (Beta=0.178; p=0.028) (meaning that a higher risk was established for the divorced, widows and single women), and cigarette use (Beta=0.236; p=0.003).

Table 12: Multivariate linear regression analysis of the factors related to the degree of depression according to the Hamilton scale after 6 months of study

	Beta	**95% CI**		**p**
		lower limit	**upper limit**	
Marital status	0,093	-1,154	0,360	0,302
Cigarette use	-0,180	-2,790	-0,142	0,030
Number of children	-0,123	-1,418	0,250	0,168

The following two factors, shown to be statistically significant, were entered into the multivariate model to evaluate depression according to the Hamilton scale: marital status and cigarette use, as well as the third factor, number of children (fulfilled the requirements to be entered into the model, p<0.010). The whole model was statistically significant – F (3, n=103)= 3.593, p= 0.015. The model as a whole was able to account for 4.9% of variance of depression score according to the Hamilton scale. The factor of cigarette use was the only one with a statistically significant contribution to the model (Beta=-0,180; p=0,030).

Table 13: Univariate linear regression analysis of the type of therapy in relation to the degree of depression according to the Hamilton scale at the beginning of the study

Type of therapy	**Beta**	**95% CI**		**p**
		lower limit	**upper limit**	
Cytostatic therapy	0,057	-8,600	15,705	0,563
Biological therapy	0,228	0,444	5,042	0,020
Radiation therapy	0,008	-2,972	2,752	0,939
Hormonal therapy	-0,260	-6,060	-0,943	0,008

At the beginning of the study, it was found that the patients on biological therapy were exposed to a higher risk for depression (Beta=0.228; p=0.020), while those on hormonal therapy had a lower risk for the onset of depression (Beta=-0.260; p=0.008).

Table 14: Univariate linear regression analysis of the type of therapy in relation to the degree of depression according to the Hamilton scale after 6 months of study

Type of therapy	Beta	95% CI		p
		lower limit	upper limit	
Cytostatic therapy	0,012	-8,410	9,410	0,900
Biological therapy	0,231	0,352	3,780	0,019
Radiation therapy	0,036	-1,724	2,502	0,716
Hormonal therapy	-0,294	-4,783	-1,041	0,003

The type of therapy as a factor of impact in the onset of depression after 6 months of study is presented in Table 14. It was established that the patients on biological therapy were at a higher risk for depression (Beta=0.231; p=0.019), while those on hormonal therapy had a lower risk for depression (Beta=-0.294; p=0.003).

3. Discussion

Impelled by the ample literature material on the topic and a lot of conflicting data, this study was performed aiming to establish the prevalence of depression in women with breast cancer.

The study, as well as the clinical practical experience acquired so far, has indicated that

30-50% of oncologic patients, in addition to their malignant disease, are affected by various psychiatric-psychological disorders that require a timely and appropriate diagnosis and therapy. Numerous predisposing factors could be held responsible for the development of psychiatric disorders in oncologic patients, such as various organic factors, the nature of their disease, treatment-related influences, reduced fertility, prior stress and psychiatric disorders, disturbed communication with the family, etc. [10,11].

Depression is one of the most widespread diseases in the 21st century. Its prevalence is ever increasing, mostly due to modern stressful and hectic lifestyles. It is thought that nowadays one in every ten people is at the risk of developing depression. However, it is not a rare case that depression is accompanied by other somatic or psychic diseases, e.g. various cardiovascular, endocrine, neurological, dermatological, malignant diseases, anxiety, addictions, and others. In most cases, a cause-and-effect relationship can be identified: depression may occur as the consequence of another phenomenon, when it often remains unrecognized or is even willfully neglected as „less important“, which is utterly wrong. There is also the possibility that depression and a comorbid condition have a common cause. In any case, numerous studies have shown that depression and comorbidities reduce quality of life of a patient, especially in

those in terminal phases of their somatic disease. Moreover, there are multifold adverse

effects of depression on the course and prognosis of another disease; it has been demonstrated that depression concurrent with another morbidity increases the risk of fatal outcome. It is therefore extremely important to focus attention on the prevention of depression, its timely recognition, and not to underestimate depression and possible comorbidities, so that adequate therapeutic support can be instituted not only in the form of pharmacological measures, but also as a cognitive-behavioral therapy and other psychotherapeutic approaches.

This study enrolled 103 patients with breast cancer planned to receive some of the anticancer therapies, aged 48.5±11.4 years on the average. Breast cancer incidence rises with advancing age, peaking in the age group from 50 to 60 years. The data in our study agreed with the literature data, where the average age of examinees ranged from 50 to 60 years.

Age distibution of the patients in our group corresponded to the breast cancer age distribution epidemiological information for the countries with high incidence rates of this tumor [12].

In most developed countries the number of breast cancer patients is on a constant rise, with the incidence peak shifting towards younger women, which could perhaps be explained by lifestyle changes. A number of studies have shown that among the breast cancer patients there are only 23% of those below 50 years of age [13].

With advancing age, the frequency of malignant diseases in general rises [14,15]. A number of studies have suggested that the onset of the disease in an advanced age is related to a more favorable biological profile of breast cancer [16]. A study by *Nixon et al.* has confirmed that women below 35 years of age have a poorer prognosis compared to the elderly patients. It has been shown that with advancing age the prevalence of markers of poor prognosis decreases significantly [17].

The women with a larger number of menstrual cycles during their reproductive age due to an earlier menarche (before 12 years of age) or those with late-onset menopause (after their 55 years of life) are exposed to a higher risk due to a prolonged exposure to the action of estrogen and progesteron.

The rates of incidence and mortality of breast cancer increase with advancing age. Approximately 95% of new cases and 97% of cancer deaths are reported in those over 40 years of age [18, 19, 20, 21]. In the USA, in the period 2004-2008, the lowest incidence rates were reported in the age group 20-24 years, and the highest incidence rates in the age group 75-79 years (421/100.000). In Central Serbia, the highest breast cancer incidence rates are reported in the age group 60-69 years [22].

Related to the marital status, most of the examinees in this study were single (65.0%), and in a common-law marriage (13.6%). These are followed by widows (4.9%), married women (10,7%), while the lowest percentage was reported for divorced women (5.8%). The data confirmed the trends present in the current literature on the issue [23,24].

The study by from the Norwegian Cancer Registry has shown that married persons affected by cancer stand a greater chance of defeating their disease than those who are not married. For instance, a married man is 35% less susceptible to cancer-related death compared to a bachelor. Women who have never married are 17% more susceptible to cancer-related death compared to their married counterparts (22%) [25].

Married women have longer overall survival and lower mortality compared to the singles, widows, or divorced women [25].

Some other studies [26] have suggested that the women with a lower educational level, even if married or living with a partner, have a higher risk of developing depression. Studies have shown that the women with a lower educational level have fewer affirmative (positive) social interactions, emotional support, and are therefore more prone to depression [27]. On the other hand, the fact that female patients are married or that they are living with a partner should imply that they have more of the social support compared to the singles, divorced or widows, and therefore are not expected to have more of the depression symptoms.

One of the more important aspects of breast cancer are its physical consequences, i.e. the scars that could impair the physical appearance of women and could therefore produce discontent and problems with their sexual life, having a general adverse influence on their sexual relationship with the partner. In intimate relationships, the issue of the disease is the one with the priority.

The belief that men desert women with breast cancer is not unusual and can be a source of stress for women faced with the disease. The notions such as this can have an adverse impact on the psychic status of women with the diagnosis of breast cancer. In contrast, various studies and partial reports suggest that after the diagnosis of breast cancer a marriage collapses most commonly as the consequence of already present marital problems.

Regarding the level of education, there was a statistically significant difference between the groups. In Turkey, for instance, 36.2% had primary education, 41.9% high school education, and 21.9% had higher education, while in Iran there were slightly more illiterate and women with primary education (47.3%), 33.9% had high school education, and 18.8% had higher education [28]. Compared to these data, the number of examinees in our study with college or higher education was twice as high. It was still considerably below the European standards, but still below the percentage of prevalence of highly educated women reported in some of

the studies. The level of education of women is important in the onset of breast cancer since it possibly influences other risk factors: educated women are better informed about the adverse effects of some of the agents and are able to perform better the proper measures of prevention and abide by the expert advice regarding proper nutrition, alcohol intake, cigarette smoking, and so on. Better education also provides a better socioeconomic background and high quality living conditions (nutrition, dwelling, etc.).

The literature reports a lower percentage of retired women (10-20%), a higher percentage of employed women (30-60%), while the number of housewives ranges from 16% to 60% [29].

Depression among employed women was significantly reduced after the surgical treatment, while the prevalence of depression among retired and unemployed women remained the same. Among married women, the prevalence of depression was significantly reduced, in contrast to widows and divorced women. A week after their operation, the percentage of depression among married women was lower than in widows or divorced women. A similar effect was observed when employed women were compared to unemployed or retired women [29].

It is believed today that a positive family history of breast cancer is an important factor in the onset of the disease. It has been shown in the literature that a patient with breast cancer has at least one first-degree relative with the disease, although the results of numerous studies can hardly be interpreted adequately due to a lack of such cases [30,31]. It is thought that 5-10% of women with breast cancer have an autosomal inherited *BRCA1* mutation, and in a smaller number of cases a *BRCA2* gene mutation.

A woman with a cancer in one portion of the breast is at a 3-4 higher risk of developing cancer in the same breast. Such cancers are not considered a recurrent disease, but as *de novo* lesions.

Introduction of the concept of adjuvant chemotherapy has markedly increased the survival of breast cancer patients [32]. Chemotherapy acts systemically. If distant metastases are present at a site, still clinically silent and diagnostically unconfirmed, the use of adjuvant chemotherapy prevents the development of metastatic foci. With time, the type of chemotherapy has changed and evolved since the middle of the XX century when CMF protocol was administered, to FAC combination regimen in the 1970s with a considerably better therapeutic effect. Nowadays, AC chemotherapy in combination with Taxanes is the treatment administered to breast cancer patients at a higher risk for disease relapse. In the elderly, CMF is still considered a gold standard.

Together with chemotherapy, adjuvant hormonal therapy is used as well. It is used if

the tumor in question is hormone dependent. The use of tamoxifen in the period of five years represents the treatment modality able to prolong survival markedly. In patients with favorable primary tumor-related factors with free lymph nodes, adjuvant chemotherapy is often the only required therapeutic modality. In our study, systemic chemotherapy was used in 51% of women with breast cancer.

In addition to the above, immunotherapy is also used in breast cancer treatment, with some key advantages compared to the above modalities. The effects of targeted therapy depend on specific signaling pathways and oncogene changes in the cells. However, it is known that cells may activate compensatory signaling pathways that could make them resistant to this type of treatment. The studies designed to examine the combination of tyrosine-kinase inhibitors for multiple receptors are under way, but the potential toxicity of such combinations may limit their usefulness [33].

In many countries with different cultural backgrounds, an association of psychological distress with somatic symptoms has been confirmed. In the analysis and understanding of the association of stress with somatic diseases, a multidimensional approach is the one most appropriate. Clinical and research models based on the theoretical assumptions of the biopsychosocial model require ample theoretical knowledge and tolerance of cognitive dissonance, while the researchers and therapists are educated mostly to work with specific methods, and most of the studies assess only inidividual risk factors.

The exciting research in psychoneuroimmunology in the last fifteen years or so has shown that stress-provoked biopsychosocial reaction may be the cause or may trigger numerous psychic and somatic disorders. A significant issue in that regard is whether stress is able to influence health compromising the immune system [34,35]. According to the study, the diseased faced stressful life situations prior to the onset of their disease statistically significantly more often than control examinees.

The leading researcher, a professor at the Breast Cancer Research Institute in Great Britain, tried to examine whether psychological stress or adverse life events had an impact on breast cancer risk [36]. Most of the examinees enrolled in the period 2003-2010 were asked how often they had experienced some stressful event in the past 5 years. They were also asked if they lost one of the parents till their 20th year of life. The researchers excluded the influence of other risk factors such as obesity, physical activity, alcohol intake, family history of breast cancer, age at menarche, age at menopause, number of children, age of their mothers at birth, length of breast feeding, and so on. The results showed that 34% of women reported frequent stressful events or a continuing stress in the period of 5 years prior to the study, and 74% reported at least one adverse life event, such as grief (loss or disease of a close person) or a divorce. Of 106.612 women, in 1783 breast cancer was diagnosed. After a careful consideration of other-

risk factors, the authors found that there was not any statistically significant association of the frequency of stress with overall breast cancer risk. Further studies are warranted, but other possible factors of risk for breast cancer should be taken into account as well.

A weak association could be found for only one of eight characteristic types of stressful events. A divorce can be associated with negative breast cancer estrogen receptors in premenopausal women, but this occurred in 25 cases only and has only a statistical significance. Such a result was not substantiated by an increased risk with other similar stressful events such as grief.

The results showed that women who often or continuously experienced stressful events had a risk for breast cancer similar to those who never experienced such stresses or experienced them only occasionally.

Dr Minouk Schoemaker reported: "Stressful life events are common and many women will have experienced them in the run-up to being diagnosed with breast cancer, but our results suggest that those stressful events are unlikely to be the cause of the disease."

The analysis showed a sightly higher risk for breast cancer in women who had lost their mothers while still young, but not if they had lost their fathers. Genetic predisposition is a much more probable breast cancer factor of risk than stress.

The Center for the Biology of Chronic Disease (Valley Cottage, New York, USA) performed the research and demonstrated that stress increased cortisol binding by the glucocorticoid receptor (GR) [37]. Glucocorticoid receptor (GR) interacts with GABP-β to produce a *BRCA1* promoter. This in turn activates *BRCA1*. The study also shows that the addition of hydrocortisone, which binds to GR, eliminates the interaction of GR with GABP-β, which induces a lack of GABP transcription factor in BRCA1 gene, decreasing its value and increasing the risk of breast cancer. Another pathway via which stress causes a lack of GABP transcription factor has been suggested, involving the presence of certain latent viruses in the cell. This event was described in 2003 in a book [38].

Many studies have confirmed that our psychic state has an impact on our health. A cancer appears when the defense system is defeated and cannot cope with various threats. A significant accent in the treatment of cancer is put on the human will, eagerness and decision of the affected to be treated.

Our immune system is suppressed by various psychic factors, out of which the most important are long-standing grief, feeling of failing in life, anger, and often mentioned stress. Chronic exposure to conflict situations, especially when there is a conflict between the needs, wishes and opportunities, produces chronic frustration as well. Since the usual de-

fense mechanisms (rationalizations, projections, sublimations) are no longer able to deal-with the problem, a physical disease occurs. Cancer most frequently affects depressive people, too rational, afraid of emotions, with a poor life of imagination, psychically constrained and passive [39,40,41,42].

David Kissen studied the patients with breast cancer and their ability to express emotions. He concluded that poor emotional outlet combined with narcissistic omnipotency of a person with the idea that all of the problems she should and can resolve alone, is the key characteristic of a person with breast malignancy. The study by S. Levi corroborates these findings, reporting that women with breast cancer who clearly show their fear, depression, anger, stand a better chance to survive their disease compared to those who are „good patients“ or „stoics“. The results of these studies show that the probability of survival is higher in patients in whom psychotherapy and chemotherapy is applied, than in those treated with only one of these modalities.

Numerous studies show that stressogenic life events have an impact on the course of depression, quality of remission, and relapse rates [39]. Etiological significance of these facts, however, has not been fully elucidated [43]. For instance, although most of the depressive patients experience stressful life events before the onset of the episode of depression, only a small portion of the individuals exposed to such stressors in fact become depressive.

A prevailing opinion of today’s authors is that a wide spectrum of biological, social, and psychological etiological factors have a role in the genesis of depression. They act in variable proportions in each individual person. In addition to stressful life events and genetic factors, the following are also commonly mentioned: abuse in early childhood, premature loss of parents, quality of social support, and others [43,44].

In some of the studied clusters, patients have experienced significantly more stressogenic life events compared to their controls. Regarding the type of stressors preceding the episode of depression our results are mostly in harmony with the literature data [45], with some differences however in their frequency. In our sample, the stressors related to poor socioeconomic situation, unemployment, and worse working conditions were the ones more often encountered, which was expected in view of the ongoing social transition in our country. The frequency of individual stressful life events associated with disturbed partner relationships matched the data from the literature.

It has been suggested that stress and depression produce similar hormonal and immunological effects [45], and that stress that precedes the onset of depression is characterized by some still unexplained pathophysiological mechanisms.

The prevalence of depression after the diagnosis of breast cancer is even up to 33%.

Depression may have some very serious consequences related to the quality of life and increase mortality of the disease.

In a large national study in Denmark involving breast cancer patients, the researchers examined their psychiatric treatment (women in both early and more advances disease stages); it was shown that those with depression have a moderate, but significantly higher risk of death compared to those without depression, dependent both on the disease course and timing of depression.

Breast cancer mortality is also significantly higher in women with other psychiatric diagnoses as well [46].

Studies show that depression reaches its peak soon after the diagnosis, and then gradually decreases in the period of one to two years. Nevertheless, long-term patient follow-up is essential because of the possible development of depression and anxiety disorders even years after the diagnosis. In the diagnosis and treatment of psychiatric disorders an individualized approach is crucial. Although psychiatrists are not oncologists, they too have to be well acquainted with the treatment options and their possible side effects. It is important that the psychiatrist inform other team members about the psychic status of the patient, and to gain insight into all other medical findings available. Breast cancer brings about other important countertransference and transference issues during the treatment. Lots of doctors have family members and friends with the diagnosis, and we have to be fully aware of our feelings while we are helping our patients. Furthermore, patients often idealize or underestimate some of the team members, especially if the diagnosis was delayed or inappropriate. Patients have to change their behaviors, take good care of their nutrition, they should not smoke, they have to reduce their alcohol intake and stress exposure, and to adapt to complicated and demanding treatments, persisting pain (especially with bone metastases), changes at the workplace or at home, and changes in their sexual and social relationships and in their familial interactions.

In our study, we used both assessment tools to evaluate depression in breast canceer-patients-the HAMD and MADRS.

We found that there was no correlation between the scales at the beginning of the study; however, the correlation was negative in both groups of examinees after 6 months. This was to be expected, since in general there was a statistically significant correlation in the diagnosis of depression between the scales.

Our results agree with the results of other studies using similar methodology [46].

Regarding the degree of depression, there was a statistically significant difference in the depression degree assessment with HAMD scale at the time of diagnosis and after 6

months.

A comparison of individual 21 components of the HAMD score measured at the beginning of the study between the examined groups is presented in **Table 6**. There were statistically significant differences between the groups in 16 components: depressive mood, suicide, early insomnia, difficulty staying asleep, late insomnia, work and activity, agitation, anxiety, somatic gastrointestinal symptoms, general somatic symptoms, genital symptoms, hypochondria, weight loss, insight into the disease, and daily mood variations.

In addition to stressful events, other psychological factors influence the onset of a malignant disease as well, such as social relations and support of the environment; personality traits and suppression of emotions. Cancer naturally changes the patient's lifestyle, and family can thus offer signifact support in that regard. The severity of depression in a woman with cancer can be influenced by her perception of support by her partner/spouse. Some studies have shown that increased social support, a broader network of contacts, and the perception of support by the partner is associated with a lower level of depression in women with breast cancer. There are diverse results concerning the impact of psychic status of women with cancer on their partners and vice versa. The studies suggest that any avoidance of open discussion about the cancer by the partner is associated with increased patient distress. The level of distress is similar in the patient and her partner.

In addition to the family, professionals can also extend a kind of support (psychologists, psychiatrists, psychotherapists). The fact alone that the patient is able to speak openly about the disease and that there are people interested in her case is a relief of a kind. Professional support can take the form of group or individual activity, and this type of help is essential for those without their families. Psychological therapy can certainly help in the treatment of cancer, especially during chemotherapy or radiation therapy [48].

After chemotherapy, together with depression and cognitive disturbances, other mental disorders are also possible (adjustment disorders, insomnia, anxiety). In 2004 alone, 215.990 women were diagnosed with breast cancer, and more than 80% of them were treated with chemotherapy. Possible side effects of chemotherapy should be known to both the patients and anyone involved in their physical and psychological management.

Although depression after chemotherapy is well known and described, the data about other mental disorders are limited, and cognitive disorders after chemotherapy have been of late increasingly examined. Cognitive dysfunctions may involve consciousness (attention, vigilance), cognition (memory, learning), executive functioning (planning, organization), aphasia, apraxia, agnosia. Studies of the cognitive changes after chemotherapy support the hypothesis that the changes occur after the initiation of chemotherapy, especially in the areas of verbal memory and psychomotor functioning. Studies have shown that younger women with breast

cancer treated with chemotherapy after surgical intervention are at an increased risk of adjustment disorder, and sleep disturbances, fatigue and other problems are also common quality of life issues. Patients should be informed about the potential psychic side effects in their informed consent forms, and mental help should be made available to all women with breast cancer [49].

In women with breast cancer numerous problems related to their body image after the surgery can also occur. Altered body image is also a constant reminder of their disease – the change is abrupt and reconstructive surgery is a commonly used approach in the resolution of this problem. Patients sometimes refuse or delay their treatment because of the altered cosmesis. The disturbances related to the altered body image last about two years after the surgery. Women also commonly have various sensory changes and even difficulties in their adaptation to the breast prosthesis. Chemotherapy, with hair loss, weight gain and similar issues, cause problems in their body image, and radiotherapy can cause dermatological problems as well.

The basic objectives of psychotherapy in the treatment of oncologic patients are as follows: to reduce the patient's resistance to her treatment; to remove possible conflict situations the patient is exposed to (that can markedly influence the processes of diagnosis and treatment); to remove the tendency towards a deeper regression, passivization, and infantilization; to position the patient in an active stance towards the actual (present) and future problems and to motivate her for her treatment and to teach her to live with her disease.

In spite of a high prevalence of psychiatric disorders in oncology patients, there have been few studies elaborating in more detail the use of medicaments in the prevention and treatment of these disorders. However, numerous clinicians' observations suggest the need and justify the use of psychopharmacotherapy (anxiolytics, antidepressants, and antipsychotics, increasing noradrenergic, serotonergic and dopaminergic neurotransmission) in oncology in the treatment of anxiety and depression disorders and for the conditions occurring after using the drugs that may induce or mimic anxiety or depression [50].

In the introduction of psychopharmaceuticals in the treatment of oncologic patients it is necessary to assess disorder severity, comorbid conditions, premorbid personality traits, and take into account organ-involvement with the malignant process, pain intensity, type of oncologic treatment and its adverse effects, and possible development of a metabolic disorder.

Antidepressants in cancer patients can help to alleviate the adverse effects of chemotherapy (e.g., insomnia, loss of appetite), in pain control (due to their analgesic action and effect in increasing the action of narcotics), and in the treatment of depressive disorder (they improve sleeping, appetite and energy) [51]. However, antidepressants may exert their action via prostaglandin as well, known to regulate every component of the cellular microanatomy and physiology [52]. An ideal anticancer drug should inhibit the creation of prostaglandin in a way that

prevents cancer pathogenesis. Some recent studies have indicated that antidepressants have the properties such as these. Their immunostimulating and antimicrobial action can help in the resolution of infections occurring after chemotherapy or irradiation [53,54].

A larger number of examinees would improve the validity and reliability of conslusions in such studies. Furthermore, attention should be focused on genetic/molecular factors of risk as well.

A psychological response to breast cancer and its treatment varies in accordance with the age of the affected woman, personality traits, familial and social relations, and a varying impact of the fertility, body image and treatment side effects on the marital, parental, professional and social roles a woman may have. Psychiatric consultations and supportive psychotherapy are useful in women with breast cancer, offering them a chance to work on their existential, somatic, emotional, social, psychosexual and marital problems. Psychiatric consultations and psychotherapy also present the chance for the patient to express emotions openly, to obtain support and alleviate one's anxiety, fear, and depression.

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Chapter 4

Breast Cancer in Saudi Arabia and its Possible Risk Factors and Control

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Abstract

Breast cancer is the commonest females' cancer and the leading cause of cancer death worldwide. There have been several factors implicated to the etiology of breast cancer, which differ for different geographical locations. The aim of this review was to discuss the most important etiological factors available in the literature with its possible association in the Kingdom of Saudi Araba (KSA). A number of etiological factors have been involved in the etiology of breast cancer. These factors act independently or together to cause breast cancer. The etiological factors discussed in this review include: age factors, age at first birth, early menarche, gender, dietary factors, tobacco smoking, alcohol consumption, low-dose irradiation, obesity, physical activity, lactation, hormonal factors, hormone replacement therapy, steroid hormone receptors, mammographic density, benign breast disease, and genetic factors. This in addition to the role of Her-2/Neu Antigen and triple negative breast cancer (Tnbc). These factors were discussed in view of the existing literature in general and in KSA in particular, in association with the future scope of the prospective research orientations. Such review can provide necessary information to identify individuals at high risk for prevention, early detection, identifying gaps for future research, and appropriate subsequent management, as well as, caregivers' coordination. The ultimate benefit will be a future dramatic decline in the morbidity and mortality of the breast cancer in KSA.

Keywords: Etiology; Breast Cancer; Early Detection; KSA

1. Introduction

Breast cancer is the second most prevalent cancer worldwide and, by far, the utmost common females' cancer with about 1.67 million new cancer cases (25% of all cancers) diagnosed in 2012 [1]. Incidence rates of breast cancer vary almost four-fold across the world's different regions [2]. Prevention of new cancer cases from beginning, researchers investigate the risk factors and protective factors. Some risk factors for breast cancer can be escaped, but many are un-avoidable.

Several studies have well-established the relationship between breast cancer and etiological factors including; age factors [3], age at first birth [4], early menarche [5], gender [6], dietary factors [7], tobacco smoking [8], alcohol consumption [9], low-dose irradiation [10], obesity [11], physical activity, lactation [4], hormonal factors, hormone replacement therapy [12], steroid hormone receptors [13], mammographic density [14], benign breast disease [15], and genetic factors [16]. This in addition to the role of Her-2/Neu Antigen [17] and triple negative breast cancer (Tnbc) [18].

However, it is still uncertain, which of the risk factors has the most effective role over others, in the carcinogenesis of the breast cancer. Here, we reviewed linkage studies that have established evidenced based breast cancer risk factors and what was already published in that regard from KSA. Our stress will be on the strengths of a particular risk factors and its potential to yield substantially contribution to the etiology of breast cancer in KSA.

2. Epidemiology

Females' breast cancer incidence rates are tremendously increasing in Arab countries in recent years, and a sensible number of cases are still being diagnosed at advanced stages of the disease [19]. An epidemiological records associated with breast cancer cases diagnosed from 2001 to 2008 among Saudi women indicated that, 6,922 females with breast cancer were registered in the Saudi Cancer Registry. The highest prevalence was found in eastern region of KSA, representing 0.266 per 1000 women followed by Riyadh, and Makkah, constituting 0.205, 0.194, respectively [20].

In 2009 breast cancer accounting for 25.1% of all newly diagnosed female cancers and the median age at diagnosis was 48 years. The age-standardized incidence rate (ASR) for females was 19.2/100,000 and the median age at diagnosis was 47 years. Breast cancer constituted 27.4% of all newly diagnosed female cancers in the year 2010. The average age at the diagnosis was 48; weighted average was 49.8, and range 43-52. The ASR was 24.9/100,000 in 2010 representing an increase in 2010 compared to 2009 [21].

There view described the cases ofbreast cancer among Saudi females during the period from January 1990 to December 2014, indicated that the number of cases is ascendingly increasing. In 2008 there were 1152 cases of females breast cancer compared to 1308, and 1473 in 2009 and 2010 in this order. The proportion distributions of breast cancer was 40.2, 38.7 and 41.2 in 2008, 2009 and 2010, respectively with minorchanges of 33.5, 33.9, and 36.1 at the age group 45-59 [21].

3. Gender

Globally, female breast cancer incidence have been largely described compared to slightly male breast cancer incidence rates. The highest male's incidence rate was reported from Israel at 1.24 per 100,000 man-years, and the highest female's incidence rate was reported from the United States at 90.7 per 100,000 woman-years [22]. In study from KSA that reviewed 1005 breast cancer cases, 2.3% were found to be males [23]. Another study investigated 87 specimens obtained from males, (90.6%) were benign and 9 (9.4%) were malignant [24]. So the prevalence of male's breast cancer in KSA is relatively lower compared to international figures. Furthermore, no study from KSA has related the cause of male's breast cancer to specific etiologic factor.

4. Age Factors

It was well established that the chance of cancer occurrence increase with the increase with age in general [25], and breast cancer in particular [26]. The prevalence of multiple cancer risk factors is high at midlife and incidence rates start to increase for various cancer types [25]. The alarm in this perspective is the increasing incidence of cancer among younger age groups. In KSA, there is a significant increase in the incidence of breast cancer, which happens at an earlier age than in western countries [27]. The average age at the diagnosis of breast cancer was 48 years; weighted average was 49.8, and range 43-52 [21]. Several factors may be hypothesized for the elevated breast cancer among younger Saudi women including; prolonged light exposure at night time, obesity, reduced physical activities, increased awareness programs with availability of early detection. Habitual night sleep shortage, which associated with the lack of melatonin are prevalent in KSA [28]. Melatonin is a small, highly conserved indole with diverse utilities comprise; circadian rhythm regulation, sleep, and cancer inhibition. Melatonin has the ability to detoxification of the free radicals, is a major function for protecting critical molecules (such as DNA) from the destructive effects of oxidative stress, there by preventing cancer. A recent study found that melatonin is effective against breast cancer stem cells inhibiting the cell viability through octamer-binding transcription factor 4 (OCT 4) also known as POU5F1 gene. Consequently, melatonin has a high potential to be used as a treatment for breast cancer [29].

5. Age at First Birth

This entry provides the mean (average) age of mothers at the birth of their first child. It was strongly proven that women having their first birth before the age of 18 years old have only about one-third the breast cancer risk of those whose first birth after the age of 35 years. These facts explains the earlier detected reverse link between overall parity and breast cancer risk, since women having their first birth earlier tend to become eventually of high parity. The influence of age at first birth in reducing breast cancer risk involves testing of varieties of etiological hypotheses [30].

There is a lack of literature from KSA regarding the relationship between age at first birth and risk of breast cancer. In study included 786 Saudi women, the mean age at the birth of the first child was 21.13±3.68 [31]. Another documented indicated that in KSA men get married at age 27, while women get married at age 25 (age at first marriage only) [32]. These facts may indicate a relatively delayed age at first birth with might increase the risk of breast cancer. Therefore, this factor is common in KSA.

6. Early Menarche and Late Menopause

Menarche and menopause denote the start and end, correspondingly, of reproduction age, and both affect breast cancer risk. In a meta-analysis included 118 964 women with breast cancer were gathered from 117 epidemiological studies, the risk of breast cancer was found to increase by a factor of 1•050; p<0•0001) for every single year earlier at menarche, and independently by a lesser amount (1•029; p<0•0001), for each year older at menopause [33]. It was anticipated that excess obesity and high meat consumption are the possible causative factors leading to the decreasing age of puberty [34]. These factors are common in KSA.

A recent study from KSA evaluated the present age at menarche of young girls and examined its link to that of mother's age at menarche. The mean menarche age for the mothers (12.97 ± 1.71 years) was significantly higher than the daughters (11.5 ± 1.48 years). Moreover, a significant positive correlation was detected between mother's age at menarche and daughter's age at menarche (r=0.264, P=0.023) [35]. Although, the mechanisms underlying this association are not well understood, but may be due to elevated levels of estrogen both earlier [36] and later [37] in life in girls with earlier menarche.

7. Dietary Factors

The role of certain dietary factors in breast cancer causality is not totally determined. Several studies do not support the concept that fat intake in middle life has a strong association with breast cancer risk. However, weight gain in middle life contributes considerably to breast cancer risk [38]. On the other hand, strong evidence is existing that breast cancer risk can be

reduced by escaping weight gain and limiting alcohol consumption throughout adult life.

There is a strong evidence that breast cancer risk is influenced by dietary factors. Blood lipid and lipoprotein levels are also influenced by environmental factors and are linked to breast cancer risk [39].

Decreasing of animal protein and increasing of vegetable intake before puberty, may lower peak height growth velocity, proposing a mechanism for vegetable intakes to protect against breast cancer. Based on dietary data, a significant reduction in peak height growth velocity for girls who increases vegetable intakes and decreases animal protein intakes at ages 3 to 5 years which, would delay menarche [40,41].

There is a lack of literature from KSA regarding the relationship between dietary intakes. A study examined the association between dietary fat and breast cancer in KSA, found a significant positive association breast cancer risk and eating of fats, protein and calories [42]. In KSA, the intakes of high protein and caloric food is common all over the country. The main meal for the great majority of Saudi is meat with rise. The intake of fast food in tremendously growing in recent years. This in addition to the comprehensive dairy products consumption. Dairy products include a miscellaneous group of food in terms of the factors that might possibly influence risk. Dairy foods, such as whole milk and various types of cheese, have a relatively higher saturated fat contents, which may increase breast cancer risk. Moreover, milk products may enclose contaminants such as pesticides, which have carcinogenic potential, and growth factors such as insulin like growth factor I, which have been found to stimulate breast cancer cell growth [43].

Difference in the levels of consumption dairy product greatly vary among populations and with the absence of standard method, many studies have very sensibly made assessments based on quantities of consumption within their specific population. Average intake differs substantially amongst different populations such that a level of consumption that is ascertained “high” in one population might be ascertained “low” in another population. Such evidences, however, should be considered in the future prospects of factors influencing breast cancer risk in KSA.

7.1. Tobacco smoking

The epidemiological evidence on the role of tobacco smoking in breast cancer risk was reliable, sustainable literature supports the association between smoking and breast cancer [44]. This association is mainly perceived in women who smoke for anelongated duration, or who smoke for a long time preceding their first pregnancy [45]. It is difficult to assess the burden of smoking among Saudi females, since tobacco smoking is considered as social stigma usually practiced in hide among females [46,47]. Therefore, the reported figures from KSA are

very low. Consequently, it is difficult to assess this factor at present among Saudi population.

7.2. Alcohol consumption

The International Agency for Research on Cancer (IARC) categorizes alcoholic beverages as carcinogenic to humans; alcohol consumption causes several cancers including breast cancer [48]. Alcohol is a well-established risk factor for breast cancer, and public health advice to women is to limit alcohol consumption [49,50].

A prevalence of 7.5% of alcoholic beverages consumption was reported from Northern KSA [51]. Alcohol usage among Saudi is also considered as social stigma more than the case in tobacco smoking. This in addition to the fact that it is completely prohibited by law.

7.3. Low-Dose Irradiation

While high-dose ionizing radiation is associated with amplified breast cancer risks, the link with protracted low-dose-rate exposures left overs blurred. The US Radiologic Technologist study offers an opportunity to study the association between breast cancer incidence and-low to moderate dose radiation. Occupational radiation to the breast was positively associated with breast cancer risk. The risk was more notice able for women born before 1930 who started working before 1950 when mean annual doses (37 mGy) were substantially higher than in later years (1.3 mGy). Because of the doubts and possible systematic miscalculations in the occupational dose estimations before 1960, these outcomes should be dealt with caution [52]. However, we did not come across any study in this regard from KSA.

7.4. Obesity

Overweight and obesity, a global problem, affects more than one billion individuals [53]. Obesity has been linked to various health disorders, including breast cancer [54]. In the Arab population, the risk of breast cancer was significantly greater among females who were overweight or obese equallyfor pre and post-menopausal (Odd Ratio (OR) =2.73 and OR =2.22 respectively; $p < 0.0001$). A study from KSA, found 75.8% of the cases of breast cancer had abnormal weight. Obese women show more than 2-fold greater breast cancer risk (OR =2.29) compared to those with normal body mass index [55].

Recent studies from KSA reporting very high obesity's prevalence rates. A study from northern KSA had reported a prevalence rate of 71% for obesity among females [56]. Another study had reported prevalence rates for obesity ranging from 55%-70% from 4 different areas [57]. In our opinion obesity is one of the most affected risk factors that strongly contributes to the etiology of breast cancer in KSA.

7.5. Physical activity

An inverse association between early physical activity and breast cancer risk was proved several studies which, assessed physical activity among those under the age of 20 [58]. The average decrease in breast cancer risk associated with physical activity at all ages groups was 16% for adolescence, 8% for early adulthood, 15% for middle adulthood, and 17% for age 50 and older [59]. Some studies have stated that current physical activity has a stronger outcome than activity far in the past [60].

During the past three decades, the KSA has experienced remarkable lifestyle changes, including physical activity and food intake habits. These quick lifestyle changes have absolutely had a significant negative influence on the health of the community. This lifestyle revolution is believed to be responsible for the epidemic of numerous non-communicable diseases in KSA [61]. A study from KSA has reported a prevalence of 96.1% for physical inactivity level among Saudi adults. There were significantly ($p<0.001$)) more inactive females (98.1%) than males (93.9%) [62]. However, a recent study from KSA has showed a decrease in the levels of physical inactivity. The study reported prevalence rates of physical inactivity of 66.6%, for males and 72.9% for females [63]. The results of these studies signify the role of physical inactivity as a strong factor that may contribute to the etiology of breast cancer in KSA.

7.6. Breastfeeding

A systematic literature search found that breastfeeding >12 months was associated with reduced risk of breast cancer by 26% [64]. Breastfeeding is inversely associated with inclusive risk of breast cancer. This association may vary in breast cancer subtypes categorized by receptor status, as they may denote various mechanisms of carcinogenesis. Results of a meta-analysis showed a protective effect of ever breastfeeding against breast cancers with hormone receptor-negative, which are more frequent in younger females and commonly have a poorer prognosis than other subtypes of breast cancer [65].

The World Health Organization (WHO) has endorsed limited breastfeeding for 6 months after birth [66]. The existing practice of Saudi infants'feeding is extremely far from compliance with even the most conservative WHO endorsements of limited breastfeeding for 4 to 6 months. A study from KSA showed that bottle feeding was introduced by 1 month of age to 51.4% of children and to 90% by 6 months of age [67]. These findings at least show that high proportions of Saudi mothers practice breastfeeding for short period of time, which may increase the risk of breast cancer.

8. Hormonal Factors and Hormone Replacement Therapy

Estrogen hormones have an extensive impact on both normal development and tum-

origenesis of the breast [68]. The IARC first established that there was adequate evidence that estrogen-only menopausal hormone therapy (MHT) was carcinogenic to humans in 1999 [69]. In a subsequent report published in 2012, IARC also reported that there was sufficient evidence that estrogen plus a progestogen (combined MHT) was carcinogenic [70]. In a study reanalyzed around 90% of the global epidemiological evidence on the relationship between breast cancer risk and hormone replacement therapy (HRT) usage, breast cancer risk was found to increase in women using HRT and increases with increasing duration of use. This effect is lowered after ending of use of HRT usage and has mostly, if not completely, disappeared after about 5 years [71].

Progesterone is a sexual steroid hormone that has a vital role in reproductive processes in males and females. Progesterone has been linked to several disorders such as breast disease, and also involved in regulating cell proliferation, apoptosis, and metastasis. Membrane progesterone receptors (mPRs) mediate the majority of the non-classical progesterone activities. The role of the various mPRs subtypes in progesterone actions in reproduction and cancer is an evolving and motivating research area [72].

There is a paucity of data regarding HRT from KSA, the only one study undertaken this topic in 2003 and reported a prevalence rate of HRT of 5% [73]. A recent study from KSA have reported long term use of oral contraceptive pills (OCP) in KSA and its association with increased breast cancer risk [74]. In the absence epidemiological data regarding the OCP usage among Saudi women, it is difficult to incriminate this factor as contributing to the etiology of breast cancer in KSA.

9. Steroid Hormone Receptors (SHR)

SHR such as estrogen receptors (ERα and ERβ), are found in the nucleus, cytosol, and on the plasma membrane of target cells. They initiate signal transduction for steroid hormones, which leads to alterations in gene expression [75].

Steroid receptor RNA activator (SRA) is a type of long noncoding RNA (lncRNA) which synchronizes the functions of different transcription factors, improves steroid receptor-dependent gene expression, and also works as a discrete scaffold. SRA can activate androgen receptor (AR), ERα, ERβ, progesterone receptor (PR), and glucocorticoid receptor (GR). SRA plays a key role in both biological processes, such as, myogenesis, steroidogenesis, and pathological changes, such as obesity and tumorigenesis [76]. Studies found that steroid hormones and their nuclear receptors play a key role in the growth and progression of breast cancer [77].

Prevalence of Estrogen and Progesterone Receptor expression in breast cancer in the Saudi population is parallel to that described globally [78].

10. Metabolic and Secretory Factors (MSFs)

MSFs have both protection and risk which, due to variances in the levels of metabolicand secretory activity of the breast. Women of the drycerumen genotype (common among Asians and rare among whites) have lesser levels of secretory activity in the epithelial cells that line the breast ducts than women of the wet cerumen genotype. The difference may decrease risk of breast cancer by protecting the breast ductcells of dry cerumen women from exposure to environmental and dietary carcinogens and cancer promoting substances secreted into breast ducts from plasma [79]. To the best of our knowledge no study has investigated this factor from KSA.

11. Mammographic Density (MD)

MD is a strong risk factor for breast cancer [80]. Dense breast tissue looks light on a mammogram and is consist of of epithelial and stromal tissue while, non-dense tissue, consist of fat, appears dark. Women with over 75 % dense tissue have 4 to 6 times breast cancer risk compared to those with very little to no dense tissue [81]. However, it is indistinct whether high MD is an intermediate phenotype or whether the risk factors of breast cancer affect breast cancer risk and MD independently [82].

Studies from KSA in this context have dealt with Mammography as screening tool rather than reporting epidemiological figures about the MD. Therefore, this risk factor need more research to provide sufficient data for suitable correlation measurement.

12. Benign Breast Disease (BBD)

BBD is a strong later breast cancer risk factor, which can grow in either breast [83]. It involves a variety of histologic pattern, commonly divided into non-proliferative lesions, proliferative lesions without atypia, and atypical hyperplasia, with an elevated risk of breast cancer associated with proliferative or atypical lesions [84].

In study evaluated the profile of female breast lesions in KSA, BBD contained 55.24% of all lesions (mean age 31.7), the most frequently reported being; fibroadenoma 46.9%, fibrocystic disease 23.25% and fibroadenosis 14.5% [85]. Out of 1005 breast biopsies obtained from Saudi, 603 cases (60%) were found with BBD [86]. Out of 969 records reviewed in KSA, BBD accounted for 60.1%. Multiple BBDs were found in 51.1% and more than two lesions in 21.1% of the cases. Non-proliferative BBD with low risk were identified in 81.4%, intermediate risk lesion without hyperplastic atypia were found in 14.6%, where as high risk lesions with atypia were revealed in 4.0% [87]. Another study from KSA have reported a prevalence of 35% of NND [88].

13. Genetic Factors

About 5% to 21.5% of breast cancers arise from germ-line mutations associated genes such as BRCA1, BRCA2, p53 and PTEN, which render an individual at risk for developing hereditary breast cancer [89, 90]. The BRCA1 and BRCA2 genes are located on the long arm of chromosomes 17 and 13, respectively. Patients with positive gene expression have approximately 80% risk of subsequent development of breast cancer mainly around pre-menopausal age [91].

Most of the data from KSA searched for irregularly hereditary mutations rather than to screen for these hereditary mutations to provide epidemiologic data for Saudi population. One study in this context, however, has determined whether any correlation present between single nucleotide polymorphisms in breast cancer associated BRCA1 and breast cancer associated BRCA2 and breast cancer risk. The study showed that neither BRCA1 nor the BRCA2 studied variant illustrate any significant association with the breast cancer among Saudi women [92]. Therefore, this factor needs more research to determine whether it has a significant role in the etiology of breast cancer in KSA.

14. The Role of Her-2/Neu Antigen

Human epidermal growth factor receptor 2 (HER2) is a member of the human epidermal growth factor receptor family. Amplification of this oncogene has been revealed to play an essential role in the development of definite aggressive types of breast cancer [93]. Neu is so named because it was derived from a rodent glioblastoma cell line [94]. HER-2/neu proto-oncogene was found to be over-expressed in 20–30% of invasive breast cancers, and it is associated with worse outcome and short survival [95].

A study from KSA, had shown that, Her-2/neu gene amplification by fluorescent in situ hybridization was noticed in 84.6% of breast cancer that were 3+ and in 18.75 % cases that were 2+ by immunohistochemistry. There is a negative association between hormonereceptors expression and Her-2/neu amplification. Nevertheless not all of the high-grade breast cancers revealed Her-2/neu positive status [78]. Another study from KSA, reported that HER-2/neu oncogene was amplified in approximately 18% of invasive ductal carcinoma of the breast and is associated with poor prognosis [96]. The findings of these study may signify the role of HER2/neu in the breast cancer among Saudi women.

15. Triple Negative Breast Cancer (Tnbc)

TNBC denotes breast cancer patients with negative ER, PR and HER-2/neu receptors [97]. Compared to other breastcancer subtypes, TNBC is more aggressive, and more commonly affects younger patients [98]. TNBC represent about 15%-25% of all breast cancer

cases [99]. Epidemiologic studies strongly support that TNBCs may be distinct entities as compared with ER+ breast cancer, suggesting that the etiologic factors, clinical characteristics, and therapeutic options may differ by molecular subtypes. Many studies propose that reproductive factors and exogenous hormone usage differently or even quite inversely influence the risk of TNBCs and ER+ breast cancers. Arguments regarding the exact role of even the same risk factor in TNBC growth explain that the biological process behind the initiation of both TNBCs and non-TNBCs are entirelyunclear [100].

A recent study from KSA has reported a prevalence of 14.8% for TNBC [101]. However, should be considered in the future research to verify its role in the etiology of breast cancer in KSA.

16. Control and preventive measures

The Collaborative Group on Hormonal Factors in Breast Cancer (2002) estimated that the cumulative incidence of breast cancer in developed countries would be reduced by more than half, from 6.3 to 2.7 per 100 women, by age 70 if women had on average more children and breastfed for longer periods as seen in some developing countries [102]. Given global increases in population growth and the strong evidence that a woman's ability to control her fertility may improve her social, economic, and overall health, it is not considered desirable to increase the birth rate per woman or to encourage pregnancies at a very young age. However, breastfeeding can and should be encouraged for many reasons, including possibly for the reduction of breast cancer risk. Many of the risks of reproductive factors are related to the effects of estrogen as demonstrated by the reduction in breast cancer incidence after an early oophorectomy, by inhibition of the estrogen receptor (ER) by using selective estrogen receptor modulators (SERMs) such as a tamoxifen or raloxifene [103], or by blocking estrogen synthesis by using aromatase inhibitors (AIs) such as exemestane [104] and anastrozole [105,106].

On the other hand the application of measures that are already available, such as chemoprevention and lifestyle prevention, would result in appreciable reductions in breast cancer risk. Another factor is that the pace of advance of our understanding of the biology of breast cancer risk and development is highly likely to give rise to new avenues for prevention over the next 10 years. A major problem is applying what we already know concerning the efficacy of prevention to appropriate populations of women. To apply chemoprevention, we need to have measures in place to assess risk and to explain the pros and cons of treatment and for prescription of appropriate therapies. Lifestyle change is a population problem which involves publicity concerning its risks and benefits of change and providing mechanisms to support women in their choices throughout society [107].

In summary, this review highlighted very important factors that contribute to the etiology of breast cancer in KSA in light of the available evidences and highlighted the possible

gaps that can be addressed in the future. Knowledge of breast cancer risk factors can strongly contribute to the breast cancer prevention efforts, which will have the greatest outcome particularly if initiated at an early age and sustained over a lifetime. Gaps in knowledge are rendered predictable and deserve valuable attention to clarify prevention.

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Recent Studies & Advances in Breast Cancer

Chapter 5

Potential use of Antihistamines on Cancer treatment

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Abstract

Cancer is one of the most frightening diseases worldwide. Several medical options are available for breast cancer treatment, such as surgery, radiotherapy, chemotherapy, hormone therapy and immunotherapy. However, the possible ineffectiveness, the distinct response of cancer to the therapies and the devastating effects of some of these therapies for patients are the major concerns in cancer treatment. So, it is crucial to search for new or at least adjuvant therapies that may improve the lifespan and quality of life of oncologic patients. Antihistamines are one of the most frequently prescribed drugs worldwide. Since the histamine receptors are present in neoplastic cells of several tumors, and the antihistamines have low toxicity and are cheaper when compared with drugs used in chemotherapy, the antihistaminic drugs may be potentially used in the clinical management of oncologic patients.

Keywords: Antihistaminic drugs; Cancer; Histamine; Mast cells

1. Introduction

Cancer is one of the most frightening diseases worldwide, constituting a major public health concern [1]. Approximately 14 million of new cases and 8.2 million cancer deaths were recorded in 2012. Disappointing projections are being pointed for the next years, with an increase in the number of new cancer cases *per* year to 22 million over the next two decades [2].

Cancer may affect any part of the body, until now more than 100 types of cancer were described. Lung, prostate, colorectal, stomach and liver are the most common sites of cancer development in men, while breast, colorectal, lung, cervix and stomach are the organs mainly affected by cancer in women [1].

Cancer is a complex and multistage disease, progressing over several years. It may be divided into four different, but related stages: **initiation, promotion, progression** and **metastization** [3-5]. Cancer initiates with an irreversible deoxyribonucleic acid (DNA) - damage in a cell leading to the conversion of a normal cell into an initiated one. This DNA damage may occur spontaneously or may be induced by physical agents (gamma radiation, X-rays), chemical compounds (arsenic, asbestos, 7,12-Dimethylbenz (a) anthracene (DMBA), Diethylnitrosamine, N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), N-methyl-N-nitrosourea (MNU), tobacco) or biological agents (papillomavirus, hepatitis virus, *Schistosoma haematobium, Helicobacter pylori, Clonorchis sinensis*). Once initiated, the cell grows and divide in an uncontrolled way as a consequence of cumulated abnormalities, originating a population of preneoplastic cells (promotion). The promotion is a relatively long process during which tumor growth may be modulated through different therapeutic strategies, like chemotherapy, immunotherapy, surgery or radiotherapy. A fast increase in tumor size and the conversion of preneoplastic cells into neoplastic ones occurs during progression, as a consequence of additional genetic changes. In the last step of carcinogenesis (metastization) is observed a migration of cancer cells from primary tumor to distant organs through blood or lymphatic system. The capacity to metastasize is exclusive of malignant tumors, despite this not all malignant tumors metastasize [6,7].

Since the success of cancer treatment and prognosis is intimately related to its early detection, screening programs are running in several countries worldwide in order to reduce cancer mortality [8,9]. Although several therapeutic approaches are available for cancer treatment, like surgery, radiotherapy, chemotherapy and immunotherapy, due to the distinct and unpredictable response of cancer to the therapies, the devastating effects of some of these therapies for patients and their ineffectiveness, arises the need to search for new or at least adjuvant therapeutic approaches that may improve the quality of life and lifespan of oncologic

patients [10].

2. Tumor Microenvironment

Cancers are complex organs composed not only of neoplastic cells, but also of other cells that are recruited to tumor microenvironment and may be changed by the transformed cells [11]. Among the non-malignant cells of tumor microenvironment are lymphatic and vascular endothelial cells, pericytes, adipocytes, mesenchymal stem cells, smooth muscle cells, fibroblasts, myofibroblasts, myeloid cells and inflammatory cells (B and T lymphocytes, neutrophils, dendritic cells, eosinophils, basophils, natural killer cells, macrophages and mast cells) [5,12]. These cells may be easily identified in the tumor microenvironment by their specific surface molecules [13]. They synthesize cytokines, reactive oxygen species (ROS), serine and cysteine proteases, metalloproteinases, growth and pro-angiogenic factors, inflammatory and matrix remodeling enzymes, chemokines, and adhesion molecules, acting as a tumor-promoting at all stages of carcinogenesis [14-17]. Taking this into account, targeting the non-malignant cells of tumor microenvironment or their mediators of communication may be used in cancer treatment.

3. Mast Cells

Mast cells are bone marrow derived leukocytes that were first described by Paul Ehrlich more than 130 years ago in his PhD thesis [18]. Since they were identified in all members of vertebrate family [19] and they may be found near common portals of infection, such as skin, gastrointestinal tract, urinary tract and respiratory tract, some authors consider them primitive cells, maybe the surviving remnant of an ancient model of the immune system [20]. Mast cells are not found in avascular tissues like cartilage, mineralized bone and cornea [21].

Inversely to other cells, leaving the bone marrow as fully matured cells, mast cells stem from non-granulated cells (immature precursors) that leave the bone marrow to circulate in the blood [22-24]. Then these precursors migrate into different tissues where they proliferate and differentiate into granulated cells (fully mature cells) through the linkage of microenvironment growth factors, like stem cell factor (SCF), to the c-kit receptor [25-27]. Previous studies observed a low number of mast cells in mice with a defective surface expression or catalytic activity of c-kit when compared with normal animals [28].

Mast cells have the ability to synthesize, store and release several molecules like histamine, serotonin, heparin, chondroitin sulphate peptidoglycans, tryptase, chymase, carboxypeptidase, tumor-necrosis factor (TNF), vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF2), leukotriene (LT) C4, LTB4, prostaglandin D2, prostaglandin E2, platelet-activating factor, interleukins (IL-1α, IL-1β, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-15, IL-16, IL-18), interferon (IFN-α, IFN-β, IFN-γ), chemokins, nitric oxide, oxide

radicals and antimicrobial peptides [29,30].

Mast cells are activated via cross-linking of the cell surface receptor FcεRI and consequent activation of phosphorylation cascades, calcium influx and nuclear importation of transcriptase factors [31,32]. Upon mast cells activation, the granules content may be released from the cell to the exterior through two distinct processes: exocytosis also called anaphylactic degranulation (quick and massive release of granules' content occurring during type I allergic reactions) or piecemeal degranulation (selective release of granules' content occurring in chronic inflammatory processes like cancer) [20,33].

Depending on their location, rodent mast cells may be divided into two groups: connective mast cells (CTMCs) and mucosal mast cells (MMCs). CTMCs are mainly placed in connective tissue and only require SCF for their survival, while MMCs may be mainly found in mucosal tissues and require T-cell derived cytokines additionally to the SCF for their activation [28,34].

Mast cells are involved in several processes, like tissue remodeling, wound healing, fibrosis and angiogenesis. They have also an important role in the central nervous system where the histamine acts as a neurotransmitter [35,36]. Microscopically, they are seen as round to elongated cells with non-segmented monolobed nucleus with condensed chromatin in the periphery [20]. Mast cells may be easily identified in toluidine blue staining by their dark purple granules that fill the cytoplasm. Fully granulated mast cells may contain up to 1000 secretory granules occupying almost half of their cytoplasm [37].

3.1. Mast cells and cancer

Mast cells have been historically associated with response to parasites and bacteria, atopic diseases, allergic reactions and anaphylaxis [38,39]. Indeed, they are important sentinels of the immune system, interacting with invading pathogenic agents [38,40]. The knowledge of mast cells was increasing over the years and currently they are considered versatile tissue elements that play an important role in several biological processes, namely in angiogenesis, immune modulation, tissue repair and remodeling, and cancer [41,42].

Although the mast cells infiltration in carcinomatous tumors was first described by Westphal in 1891, the role of these cells on cancer remains unclear [43,44]. Our research team also identified a mixed inflammatory infiltrate composed of numerous mast cells and less abundant lymphocytes in mammary tumors and urinary bladder tumors chemically-induced by the administration of the carcinogenic agents MNU and BBN in a rodent model, respectively [45,46]. The increase of the number of mast cells in the cancer microenvironment occurs not only due to the migration of mast cell precursors from the blood, but also due to the in situ division of the few mature mast cells [47]. Mast cells degranulation in tumor microenvironment

is activated by stimuli like alarmins, hypoxia, chemokins and cytokines [48].

Previous studies suggest that mast cells may exert a bivalent role on cancer, exerting both pro-tumor (extracellular matrix degradation, angiogenesis and immune suppression) and anti-tumor effects (immune cell recruitment and activation, and cytotoxic activity) [48]. In this way, the overall impact of mast cells infiltration in tumor microenvironment is unknown. The mast cells infiltration is linked with cancer mainly due to the release of potent angiogenic compounds, namely tryptase, chymase, FGF and VEGF [49]. According to Groot *et al.* [50], the mast cells density is an indicator of poor prognosis in different types of cancer, namely in melanoma, Hodgkin's lymphoma, esophageal, lung, prostate, cervical, endometrial, gastric and colorectal carcinomas. The mast cells into action on cancer may be controlled by the regulation of the number of mast cells or by the inhibition of their action (inhibition of mast cells' content release through the administration of a mast cell stabilizer drug or inhibition of the linkage of releasing substances, like histamine, to their receptors.)

4. Histamine

Histamine was discovered and classified as a biogenic amine in 1910 by Henry Dale and Patrick Laidlaw [51]. It is an endogenous physiological substance also called 2-(4-imidazolyl)ethylamine or 5b-amino-ethylimidazole. Histamine is synthetizedby the enzyme L-histidine decarboxylase from the amino acid histidine [52]. Although the mast cells are the main source of histamine, after synthetization it is stored within cytoplasmic granules or vesicles of other cells, namely platelets, enterochromaffin cells, lymphocytes and histaminergic neurons [53,54]. After release into the extracellular microenvironment, histamine has a half-life of approximately one minute, being then degraded by the enzymes *N*-methyltransferase and diamine oxidase [19]. The histamine may be found in all mammary tissues, ranging from less than 1 μg/g to higher than 100 μg/g. High histamine concentrations may be found in lung, gastrointestinal tract, connective tissue and skin [55]. The serum and tissue levels of histamine may be assessed by radio-enzymatic and fluorometric techniques [55].

Histamine is since early associated with allergic reactions. Indeed, high quantities of histamine are released by mast cells during the acute phase of allergic diseases, promoting vasodilation, endothelial permeability, stimulating sensory nerves and promoting smooth muscle contraction [57-60,63]. The histamine is responsible for the clinical manifestations of allergic disease, like edema, rhinitis, sneezing, itching, rhinorrhea, nose obstruction, skin erythema, pruritus and urticaria [57-60,64]. Histamine is also responsible for the activation of inflammatory cells, namely eosinophils and basophils, and release of proinflammatory mediators in chronic allergic inflammation [57]. Furthermore, histamine is involved in several physiological and pathophysiological processes, namely in conjunctivitis, atopic disorders, bronchoconstriction, urticaria, anaphylaxis, asthma, gastric acid secretion, mucus secretion, increase of

vasopermeabilization, stimulation of cardiac contraction, contraction of smooth muscle from the gut and respiratory tract [51,56-60]. It also induces shock-like syndrome when injected in animals and was recognized as a stimulator of the acid secretion in the stomach,and a mediator of anaphylactic and allergic reactions [51,52,56,61,62].

High levels of histamine and high activity of the enzyme L-histidine decarboxylase were observed in several tumors, namely in breast, endometrial, colon and small cell lung cancer, and melanoma [65-72]. Higher blood levels of histamine were found in human patients with breast, prostate and lung malignant tumor, when compared with healthy people. It was also observed a decrease in the histamine levels close to normal levels within three months after surgery [73].

According to some authors, the effects of histamine on carcinogenesis depends on its concentration. Studies on mammary carcinogenesis chemically-induced by the carcinogen MNU in female rats observed that histamine levels up to 50 nM promoted tumor cells proliferation, while higher concentrations inhibited tumor growth [74]. Similarly, the stimulation of human pancreatic carcinoma PANC-1 cells with low levels of histamine (0.01μM) increased tumor cells proliferation, while the stimulation with higher concentrations (10 μM) decreased cells proliferation [75].

4.1. Histamine receptors

Histamine acts by binding to and activating four specific receptors, known as histamine receptors: H1, H2, H3 and H4, which are expressed in several cells and tissues (**Table 1**) [57,64]. Biochemically, these receptors belong to the family of hepatahelical G protein-coupled receptors family [76,77]. These histamine receptors were also identified by genomics-based approaches in human tumors, namely in lymphoma, leukemia, melanoma, breast, cervical, ovarian, vaginal, uterine, vulvar and colorectal cancer [77].

The activation of different histamine receptors is responsible for different physiological reactions. The activation of H1 receptors lead to the activation of pathways that trigger several symptoms of allergy, like pruritus, bronchoconstriction, edema, rhinorrhea and smooth muscle contraction [55,78]. H2 receptors activation promotes gastric acid secretion, and in low grade vasodilation [77,79]. The activation of H3 receptors is responsible for the regulation of pruritus, inhibition of excessive bronchoconstriction and the control of release and synthesis of histamine and other neurotransmitters, such as dopamine, serotonin, noradrenaline, γ-aminobutyric acid and acetylcholine [55,80-83]. H4 receptors induce chemotaxis, regulate the differentiation of promyelocytes and myeloblasts, and have an important role in chronic inflammatory diseases of the skin [55,84-86].

4.2. Antihistaminic drugs

Antihistamines are inverse agonists that inhibit the linkage of histamine to its receptors [57,87]. They have a molecular structure similar to the histamine with which they compete [88]. The first antihistamine was synthetized by Staub and Bovet in 1937 [89]. Phenbenzamine was the first antihistamine applied in humans in 1942 for the treatment of allergies and skin conditions, like pruritus and irritation [55]. Since then, the antihistamines have been routinely used for the treatment of several clinical conditions, namely motion sickness, insomnia, vertigo, and allergic diseases (contact dermatitis, atopic dermatitis, dermatoses, rhinitis, allergic conjunctivitis, mild transfusion reactions, urticarial and hypersensitivity reactions to drugs) [53,57,90,91]. The effects of antihistamines vary among patients [57]. Despite their safety and appropriate use are not fully clarified, the antihistamines are frequently used in children and adults [57,92,93]. The knowledge of antihistamines pharmacokinetics and pharmacodynamics is essential to their correct use [57,92,93]. Their dose should be adjusted in patients with renal or hepatic diseases [92].

Antihistamines may be administered orally or topically applied [90,94]. Antihistamines have a good absorption and reach the plasma concentration within three hours after oral administration [85]. They are mainly biotransformed in the liver by the cytochrome enzyme system (CYP) [95-99]. The simultaneous administration of antihistamines and grapefruit juice change their plasmatic concentration due to the blockage of cytochrome P450 (CYP3A4) [85,95]. The metabolic products of antihistamines are excreted by the kidneys and eliminated in the urine [57,92]. Some antihistamines are eliminated in the feces after biliary excretion, without metabolic alterations [92].

Concerning to their pharmacodynamics, antihistamines may inhibit the action of histamine through the inhibition of the activity of enzyme L-histidine descarboxylase or by the blockage of histamine receptors (acting as inverse agonists) [100]. Some antihistamines, such as desloratadine and ketotiken, have also the ability to stabilize the mast cells' membrane, inhibiting their degranulation [57,101-104]. Antihistamines also inhibit the accumulation of inflammatory cells and their activation, exerting an anti-inflammatory activity [105].

4.3. Antihistamines classes

Considering their chemical structure, H1-antihistamines may be categorized into six groups: alkylamines, ethanolamines, ethylenediamines, phenotiazines, piperazines and piperidines [53,64,106–108] (**Table 2**). According to their toxic properties and side effects, H1-antihistamines may be subdivided into first-generation or second-generation antihistamines [57,92]. The first generation antihistamines affect the cognitive performance of people and prejudice daytime activities that require high concentration, because they cause sedation, incoordination, vertigo, agitation, excitability and lack of concentration. These effects occur due to

their low molecular weight, high liposolubility, their ability to easily cross the blood-brain barrier and high affinity to the H1-receptors of the brain [57,107]. Additionally to the inhibition of H1 receptors, H1-antihistamines also inhibit muscarinic and adrenergic receptors, causing urinary retention, blurred vision, hypotension, tachycardia, and drying of mouth and nasal secretions [57,85]. The first-generation antihistamines have a short half-life, being necessary the administration of multiple daily doses [107,108]. When compared with the second-generation antihistamines, the first-generation ones are less expensive [93].

The second-generation antihistamines, frequently named newer antihistamines, were developed in early 1980's to overlap the side effects of the first-generation antihistamines [107]. Conversely to the first-generation antihistamines, the second-generation antihistamines have a high specificity to H1-receptos and low affinity to non-histamine receptors, such as adrenergic and muscarinic receptors [57,95,107,109]. The second-generation antihistamines have a high molecular weight, they are low liposoluble and have a low affinity to the cerebral H1 receptors, being unprovided of effects on central nervous system when administered at therapeutic doses [57].When administered in high doses, these second-generation antihistamines may have sedative effects [57]. These antihistamines have a longer half-life when compared with first-generation antihistamines, allowing the administration of less doses (one or two doses daily) [95,107,109]. The third generation antihistamines were created as an attempt to improve clinical efficacy and minimize adverse effects of the first and second-generation antihistamines [106]. However, since they are active metabolites of first-generation antihistamines, their definition as a class is not consensual among the scientific community [57].

H2-antihistamines are frequently used in the prophylaxis of conditions where there is high gastric acidity and in the treatment of gastroesophageal reflux disease and duodenal ulcers [110]. Due to the interaction with H2-receptors, the H2-antihistamines may have a modulatory effect on immune system [110] (**Table 2**).

5. Potential use of Mast Cells and Antihistamines on Cancer Treatment

Mast cells, which are the main source of histamine, migrate into the tumor during the carcinogenesis, constituting one of the major non-neoplastic cell population of tumor microenvironment [111]. Despite this, the role of mast cells on cancer is not fully understood. If in one hand, some researchers suggest that the substances released by mast cells promote carcinogenesis by promoting tumor growth, angiogenesis, invasion and host immunosuppression, other researchers suggest that these substances have beneficial effects for the host by inhibiting tumor growth, inducing apoptosis, inhibiting tumor metastization and stimulating immune system [20]. The mast cells degranulation may be inhibited by antihistamine drugs, namely by the use of ketotifen that not only acts as antihistamine drug by inhibiting the linkage of histamine to its receptors, but also stabilizes mast cells' membranes inhibiting their degranulation. The

use of this antihistaminic drug may be a promisor therapeutic approach on cancer treatment.

The relation between the use of antihistaminic drugs and cancer risk development has intrigued the researchers. Indeed, it was observed in several studies that the aminoethyl ether group of antihistamines is structurally similar to N,N-diethyl-2-(4-(phenylmethyl) phenoxy) ethanamine HCl (DPPE) that is a tamoxifen derivative known to inhibit the *in vitro* growth of MCF-7 breast cancer cells [90,112,113].

In this way, investigators have studied this association with distinct results [114]. Nadalin and coworkers [90] enquired 3,133 women with breast cancer and 3,062 healthy women ranging from 25 to 74 years-old about the regular use of antihistamines, and they found no association between the antihistamines use and the risk of breast cancer development. Kelly and coworkers [115] also studied the association between antihistamines and breast cancer risk in 5,814 women with invasive breast cancer and in 5,095 healthy women between 18 and 69 years of age, finding no association between antihistamines use and cancer development [115].

The association of antihistamines exposition for a long period of time with the development of glioma is also contradictory. If according to some studies the antihistamines use promotes glioma development [116-120], Scheurer and colleagues [121] verified that the risk of glioma development is reduced with the exposition of antihistamines. Is was observed by several researchers that the use of antihistamines inhibit the growth of colorectal cancer [122], human melanoma [123], and leukemic [124] cell lines.

It was observed that C-3 fibrosarcoma and B16F10 melanoma cell lines injected in a syngeneic mice model grown quickly after the administration of human equivalent doses of the H1-antihistamines loratadine and astemizole (for both tumors) and hydroxyzine (for melanoma only), they also verified that doxylamine and cetirizine did not change the growth of any cell lines [113]. It was observed that hydroxyzine was cytotoxic against the MCF-7 and EVSA-T human breast cancer cell lines [112]. The H1 antihistamines terfenadine and loratidine inhibited the *in vitro* growth of HMC-1 human leukemia cell line, C2 and NI-1 canine mastocytoma cell lines [125].

The H2 antihistamine cimetidine is one of the most frequently prescribed medicine worldwide [126], being proposed as an anti-cancer drug in 1979 [127]. Indeed, it may inhibit tumor growth and metastasis by different ways: inhibits cell adhesion of tumor cells, exerts antiangiogenenic effects by the inhibition of VEGF that has been recognized as an important angiogenic factor, induces apoptosis, activates macrophages, activates the immune system through the increase of interleukin levels, increases infiltration of tumors by immune cells and inhibits immunosuppression [110]. Positive effects of cimetidine administration were observed in patients with malignant melanoma [128], renal cell carcinoma [129] and glioblastoma [130].

Jiang and collaborators [131] studied the effects of cimetidine administration on the growth of different cancer cell lines (SGC-7901 human gastric carcinoma metastatic lymph node cell line, MGC-803 human gastric mucinous adenocarcinoma cell line and GES-1 normal human gastric epithelial cell line) and they observed that cimetidine induced apoptosis in neoplastic cells and had almost no effect in the normal gastric cells. Jiang and collaborators [131] also observed that cimetidine injection two times a week, during four consecutive weeks in a xenograft model of BALB/c nude female mice subcutaneously injected with SGC-7901 cell line decreased tumor volume and weight in a dose-dependent manner.

Brandes and collaborators [113] did not observe any effect of the daily intraperitoneal injection of cimetidine for 18 days in B16F10 melanoma and C-3 fibrosarcoma cell lines subcutaneously injected in C57BL and C3H female mice.

Takahashi and colleagues [132] observed a reduction in volume and weight of colon adenocarcinoma in a sygeneic model of CT-26 mouse colon adenocarcinoma cell line intradermally injected in the lumbar region of BALB/c female mice.

Several researchers performed *in vitro* and *in vivo studies* with human cell lines of different types of cancer, namely leukemia, lymphoma, melanoma, breast, ovarian, vaginal, cervical, uterine, vulvar and colorectal cancer, demonstrating the positive involvement of histamine in cancer cell proliferation migration and invasion [77] (**Table 3**).

In a study performed by our research team, where the role of mast cells was evaluated in the initiation and progression of mammary tumors chemically-induced by the carcinogen agent MNU in Sprague-Dawley female rats, through the inhibition of mast cell degranulation by the administration of ketotifen, we observed that animals from ketotifen-treated groups developed less number of mammary tumors (palpable masses) but higher number of mammary lesions when compared with non-treated animals. A lower proliferation (Ki-67 immunoexpression) and apoptotic index (caspase-3 and -9 immunoexpression) was observed in mammary tumors from ketotifen-exposed animals. The main positive effect of mast cell inhibition seemed to be the reduction of tumor proliferation when the mast cell degranulation was inhibited before tumor development [133].

Table 1. Distribution of histamine receptors in cells and tissues [96,103,110,123,124,126–136].

Histamine receptors	Cells and tissues
H1	Adrenal medulla, Cardiovascular system, Chondrocytes, Dendritic cells, Endothelial cells, Eosinophils, Epithelial cells, Gastrointestinal tract, Genitourinary system, Hepatocytes, Monocytes, Nerve cells, Neutrophils, Smooth muscle, T and B cells
H2	Chondrocytes, Dendritic cells, Endothelial cells, Eosinophils, Epithelial cells, Gastric parietal cells, Heart, Hepatocytes, Monocytes, Nerve cells, Neutrophils, Smooth muscle, T and B cells
H3	Eosinophils, Histaminergic neurons, Monocytes
H4	Basophils, Bone marrow, Colon , Dendritic cells, Eosinophils, Heart, Hematopoietic cells, Hepatocytes, Lung, Mast cells, Monocytes, Nerve cells, Neutrophils, Small intestine, Spleen, Stomach, T cells, Thymus

Table 2. H1, H2, H3 and H4 antihistamines more frequently used [53,57,64,79,85,92,106–108,134].

<table>
<tr><th colspan="2">H1 antihistamines</th><th>H2 antihistamines</th><th>H3 antihistamines</th><th>H4 antihistamines</th></tr>
<tr><th>First generation</th><th>Second generation</th><td></td><td></td><td></td></tr>
<tr><td>Alkylamines: Brompheniramine; Chlorpheniramine; Dexbrompheniramine; Dexchlorpheniramine; Dimethindene; Pheniramine; Triprolidine</td><td>Alkylamines: Acrivastine</td><td rowspan="6">Burimamide; Cimetidine; Dimaprid; Famotidine; Lafutidine; Nizatidine; Pibutidine; Ranitidine; Zolantidine</td><td rowspan="6">Ciproxifan; Imoproxifan; Impromidine</td><td rowspan="6">Alobenpropit; Clobenpropit; Thioperamide</td></tr>
<tr><td>Ethanolamines: Bromazine; Carbinoxamine; Clemastine; Dimenhydrinate; Diphenhydramine; Doxylamine; Ophenadrine; Phenyltoloxamine</td><td>Piperazines: Cetirizine; Levocetirizine</td></tr>
<tr><td>Ethylenediamines: Antazoline; Mepyramine; Pyrilamine; Tripelennamine</td><td rowspan="4">Piperidines: Astemizole; Bilastine; Desloratadine; Ebastine; Fexofenadine; Ketotifen; Levocabastine; Loratadine; Mizolastine; Olopatadine; Terfenadine; Rupatadine</td></tr>
<tr><td>Phenothiazins: Methdilazine; Promethazine; Trimeprazine</td></tr>
<tr><td>Piperazines: Buclizine; Chlorcyclizine; Cyclizine; Hydroxyzine; Mebhydrolin; Meclizine; Oxatomide</td></tr>
<tr><td>Piperidines: Azatadine; Cyproheptadine; Diphenylpyraline</td></tr>
</table>

Table 3. *In vivo* and *in vitro* studies performed in order to evaluate the potential role of antihistamines on cancer treatment.

Model	Drug	Specie		Dose	Effects	Reference
In vivo studies	Chlorpheniramine	Mice	Syngeneic; Ehrlich carcinoma cells were inoculated	i.p.; 0.2mL/day of 6.4mM chlorpheniramine solution; for 7 or 11 days	Decreased tumor growth	[135]
	Cimetidine	C57BL mice	♀ LL57B004 (mice Lewis lung carcinoma); subcutaneous or intramuscular injection	p.o. in drinking water; 100mg/Kg/day; for 20 days	Decreased cell growth	[66]
		BALB/c nude mice	♀ Xenograft; SGC-7901 (human gastric carcinoma metastatic lymph node cells)	Intratumoral injection; 100mg/Kg for 2 days; 200mg/Kg for 2 days; for 4 weeks	Decreased tumors volume and weight	[131]
			♂ Xenograft; C170 and LIM2412 (human colon adenocarcinoma cell lines)	Subcutaneously implanted; 100mg/Kg/day; for 21 or 28 days	Inhibited tumor growth (lower number and volume of tumors)	[136]
			Xenograft; KK (ovarian carcinoma cell lines)	p.o. in drinking water; 25, 50 or 100mg/Kg/day; for 20 days	Decreased tumor growth	[137]
		♂ nude mice	Xenograft; MKN45G (gastric adenocarcinoma cell line); subcutaneous injection	p.o. in drinking water; 100mg/Kg/day; for 20 days	Inhibited proliferation of tumor cells	[138]
		50 Grey horses	Melanoma	p.o.; 3.5mg/Kg/2 times days or 7.5mg/Kg/day; for 60 days	It was not effective in the treatment of horses melanoma	[139]

		Human	Colorectal cancer	(1) p.o.; 400mg/Kg; for 2 years after surgery. (2) p.o.; 400mg/Kg; for 5 days before surgery. (3) p.o.; 800mg/Kg; for 5 years before surgery.	(1) Increased patients surveillance in 40 months (1) or 14 months (2 and 3)	[140,141]
	Cimetidine; Dipheny-dramine	Sprague-Dawley rats	Colonic tumors chemically-induced by 1,2-dimethylhydrazine	p.o. in drinking water; 100mg/Kg/day; for 26 weeks	Cimetidine did not reduce the incidence of colon tumors; both drugs did not affect the staging and degree of differentiation of tumor	[142]
	Clobenpropit	Immunodeficient nude mice	Xenograft; Mz-ChA-1 (human cholangiocarcinoma cell lines); subcutaneous injection	i.p.; 20mmol/Kg/day; for 39 days	Inhibited tumor progression and decreased tumor volume	[143]
	Cyproheptadine	DBA2 mice	Syngeneic; MDAY-D2 (mouse leukemic cells); subcutaneous injection	i.p.; 10mg/Kg/day; for 5 or 10 days	Abolished formation of malignant ascites; inhibited tumor growth; induced apoptosis of tumor cells	[144]
		Sublethally irradiated NOD/SCID mice	Xenograft; LP-1(human multiple myeloma line); subcutaneous injection		Delayed tumor growth; lower tumor volume; induced apoptosis of tumor cells	[144]
		Sprague-Dawley rats	Colonic tumors chemically-induced by 1,2-dimethylhydrazine	i.p.; 1mg/Kg; single doses	Reduced number of tumors; increased necrosis in neoplastic cells	[145]

		Human	Mastocytosis	0.38mg/Kg/day; for 33 months	Reduced degree of blistering; child grown and developed normally without sign of disease	[146]
	Loratadine, astemizole, cetirizine, hydroxyzine	♀ C57BL mice/ ♀ C3H mice	Syngeneic; B16F10 melanoma cells and C-3 fibrosarcoma cells; subcutaneous injection	i.p. administration; human-equivalent dose; once a day; for 18-21 days	Loratadine and astemizolepromoted the growth of both tumors Hydroxyzine promoted the growth of melanoma. Cetirizine did not have any effects	[113]
	Mepyramine	♂ Syngeneic; McB6-1 (mice fibrosarcoma cell line); subcutaneous injection		i.p.; 0.2mg; 7days/ week; for 35 days	Induced a slight increase in tumor growth; decreases animals' survival	[147]
	Ranitidine	♂ nude mice	Xenograft; C170 and LIM2412 (human colonic adenocarcinoma cell lines)	p.o. in drinking water; 25, 50 or 100mg/ Kg/day (C170); 10, 25 or 50mg/Kg/day (LIM2412); for 28 days	Ranitidine had no effect in C170 cell line. Ranitidine stimulated tumor growth in LIM2412 cell line	[148]
		Human	Colorectal cancer	i.v.; 100mg intraoperatively followed by 150mg/Kg p.o. for 5 years	Increased patients surveillance in 80 months	[149]
	Ranitidine, cimetidine	Immunodeficient SCID mice	Xenograft; HT168 (human melanoma cell line); intradermal injection	p.o. in drinking water; 50mg/Kg/day	Both drugs inhibited tumor growth	[150]
	Ruptadine	Human	Mastocytosis	Concentration of 20mg/day; for 28 days	Controlled symptoms and improved quality of life	[151]

In vitro studies	Astemizole	SUM-229PE and T-47D (human invasive ductal carcinoma cell lines)		Concentration of 0.5-4.5 μM, for 6 days	Inhibited tumor cells proliferation	[152]
	Chlorpheniramine	MDA-MB231 and MCF-7 (human breast cancer cells)		Concentration of 250μM, for 48 hours	Induced a dose-dependent decrease in cell number	[153]
	Cimetidine, Terfenadine	A375 (human melanoma cell lines)		Concentration of 0-10μM; for 2-10 hours	Cimetidine did not show effects on cells; terfenadine induced a dose and time-dependent cytotoxicity	[154]
	Clobenpropit	Mz-ChA-1; SG-231; HuCCT-1; CCLP-1; HuH-28; TFK-1 (human cholangiocarcinoma cell lines)		Concentration from 1-50μM; for 48 hours	Inhibited cells proliferation in a dose-dependent manner	[143]
	Cyproheptadine	HBL-2, Granta-519 and Leko-1 (human lymphoma cell lines)		Concentration of 25μmol/L, 30 μmol/L and 40 μmol/L	Decreased mitochondrial membrane potential at high concentrations; induced apoptosis	[155]
	Loratadine followed by radiation treatment	HT29 (human colon carcinoma); DU145 (human prostate carcinoma); SF295 (human glioblastoma)		Concentration of 75μM	Pre-treatment with loratadine increased radiation induced cititoxicity	[156]
	Meclizine	HT29 and COLO 205 (human colon adenocarcinoma cell lines)		Concentration of 10-100μM, for 24 hours	Induced a dose-dependent decrease in cell number	[157]

	Ranitidine	Tumors N-methyl-N-nitrosourea induced in ♀ Sprague-Dawley rats		Concentration of 10μM	Inhibited tumor cells proliferation	[158]
	Terfenadine	A375, HT144, Hs294T (human melanoma cell lines)		Concentration of 0-20μM; for 24 hours	Induced apoptosis	[159]
	Terfenadine, astemizole, diphenydramine, tripolidine	A375, HT144, HSs294T and MJOI (human melanoma cell lines)		Concentration of 0.1-1mM for diphenydramine and tripolidine; 1-10μM for terfenadine and astemizole, for 6 to 24 hours	All drugs induced apoptotic cell death in all cell lines	[123]
	Terfenadine, loratadine	HMC-1 (human mast cells leukemic cell line)		Concentration of 10μM, for 6, 12, 24, 48 or 72 hours	Both drugs induced apoptosis in neoplastic mast cells	[125]

Abbreviations: i.p: intraperitoneal injection; i.v: intravenous administration; p.o: oral administration

6. Conclusion

The existing therapies for cancer treatment have devastating effects for patients and are frequently insufficient to eradicate the disease. Since the histamine receptors are present in neoplastic cells of several tumors, and the antihistamines have low toxicity and are cheaper when compared with drugs used in chemotherapy, the antihistaminic drugs may be potentially used for the clinical management of oncologic patients.

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Chapter 6

Surgical Anatomy of Mammary Gland

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1. Introduction

1.1. Gross Anatomy

The mammary gland is an important accessory organ of the female reproductive system which lies in the superficial fascia of the pectoral region.

The mammary glands constitute secondary sexual features of the females and are a source of nourishment for the neonates.

Breasts (mammary glands) are modified sweat glands which lie in superficial fascia of pectoral region. The mammary gland is separated from underlying deep fascia by retromammary space which is occupied by loose areolar tissue.

1.2. Extent and Relations

VERTICAL-from second to the sixth ribs

HORIZONTAL- From the lateral sternal margin to the mid-axillary line

ANTERIORLY- skin over breast

POSTERIORLY - retromammary space, deep fascia covering mammary bed structures.

The retro mammary space with loose areolar tissue allows free movement. This mobil-

ity is restricted in case of advanced breast carcinoma.

The mammary bed structures include 3 muscles namely pectoralis major, serratus anterior & external oblique abdominis.

The axillary tail of spence is a prolongation from the superolateral quadrant of the gland extending to the level of the third rib in the axilla. It is in close contact with anterior group of axillary lymph nodes. The axillary tail pierces the deep fascia of axilla (axillary fascia) & lies deep to it. The opening in axillary fascia is referred as Foramen of Langer. It lies upon the deep pectoral fascia which in turn lies over the pectoralis major, serratus anterior and external oblique.

The clinical relevance of the axillary tail is that if enlarged it may be mis- diagnosed as a lipoma or axillary lymphadenopathy.

2. Internal Structure

The breast is a compound tubuloalveolar gland & is made up of glandular tissue & fibrofatty stroma. The glandular tissue consists of 15-20 lobes each drained by a separate lactiferous duct. The lobes are arranged in a radial manner like the spokes of the wheel. The lobes are further divided into lobules. The lobule is the basic structural unit of the breast. There are about 10-100 lobules which drain via ductules into a lactiferous duct. Each lactiferous duct is lined by a spiral arrangement of contractile myo-epithelial cells. The lactiferous duct has a dilated portion deep to the areola named as lactiferous sinus which acts as reservoir of milk . As the neonate sucks, compression of the areola and the lactiferous sinus below it expresses the droplets encouraging the neonate to continue sucking. This is referred to as the Milk Let Down reflex which is hormonally controlled.

Studies of myoepithelial cell lines reveal that these cells exhibit a natural tumor suppressor function. Breast cancer and pre cancer cells are influenced by important paracrine regulation from the breast micro environment, which might be a determinant of breast cancer behavior as the specific oncogenic or tumor suppressive alteration occurring within the malignant breast cells.

The knowledge of central and peripheral ductal anatomy is very important for developing the intraductal approach to the breast cancer.

The gland is firmly attached to the overlying skin & underlying pectoral fascia by fibrous bands named as Suspensory ligaments of Cooper. These ligaments are of paramount importance in supporting the lobes and lobules of the mammary gland.

In case of cancer breast, the malignant cells invade these ligaments and lead to contrac-

tion of the strands resulting in dimpling of the skin referred to as Peau de orange appearance.

Further if cancer cells grow along the ligaments of cooper, it attaches the breast to the pectoral fascia causing the breast fixity to the chest wall.

2.1. Nipple

The nipple is a conical projection below the centre of the breast at the level of fourth intercostal space in most young women. The nipples are devoid of fat, sweat glands and hair. The nipple is an erectile tissue containing concentrically and longitudinally arranged smooth muscles.

Its shape varies from conical to flattened depending on various factors such as nervous, hormonal and developmental. Occasionally the nipple may evert during pre natal development and remains permanently retracted thus causing difficulty in suckling.

2.2. Areola

Areola is a pigmented circular area of skin which encircles the nipple and varies in color according to the race ranging from pink to light brown. It contains involuntary muscles arranged in concentric rings as well as radially in the subcutaneous tissue. The areolar epithelium contains numerous sweat glands and sebaceous glands, the latter enlarge during pregnancy and serve to lubricate the nipple during lactation.

3. Blood Supply of the Mammary Gland

The Blood Vessels of the Mammary Gland have the following salient features:

1) Enter from superomedial &superolateral aspects
2) Penetrate deep surface of breast
3) Exhibit extensive branches & anastomosis

Arterial Supply of the Breast is received from the following sources:

- Lateral (mammary) thoracic artery
- Internal (mammary) thoracic artery
- Intercostal arteries (Posterior intercostal arteries-2^{nd}, 3^{rd}, 4^{th} spaces)
- Superior thoracic artery
- Thoracoacromial artery

Internal (Mammary) Thoracic Artery is the major source of arterial supply to the breast especially to the Nipple Areola Complex (NAC). The internal thoracic artery is a branch of subclavian artery. It descends vertically along the posterior aspect of anterior thoracic wall &

supplies anterior thorax & medial aspect of mammary gland, the latter through medial mammary branches. It runs along with internal thoracic vein which is a tributary of brachiocephalic vein.

The Thoracoacromial Artery is a minor source of blood supply to breast. However, the following points of importance for the breast surgeons are as follows:

- It is a branch of second part of axillary artery (under pectoralis minor)
- It gives off 4 branches namely pectoral, clavicular, acromial & deltoid branches supplying corresponding areas
- Its accompanying vein is a tributary of the axillary vein

The venous drainage of the mammary gland

The veins form circulus venosus- a plexus underneath areola, from which venous blood drains into axillary, internal thoracic & intercostal veins.

- All accompanying veins are tributaries of the axillary vein

The venous drainage of the mammary gland is supplemented by the Cephalic Vein.

- Tributary to axillary vein.
- Only major superficial vein in vicinity of breast
- Primarily drains Upper extremity into deltopectoral triangle
- provides superficial venous drainage to the breast partly

3.1. Innervation of the mammary gland

The nerves are derived from anterior & lateral cutaneous branches of 4^{th} to 6^{th} intercostal nerves which convey both somatic & sympathetic fibres. The sensory fibers supply the skin of the breast and sympathetic fibers are mostly vasomotor. The rich neurovascular supply to the nipple course along regularly located suspensory apparatus. The Surgeons should avoid damage to the anterior and lateral branches of the third, fourth and fifth intercostal nerves with a special attention to the fourth intercostal nerve as it is the consistent nerve to the NAC.

The nerves innervating the nipple and areola are best protected if the incisions are made at the base of the breast and at the lateral areolar border.

The Intercostobrachial Nerve deserves a special mention in this regard. It is the Lateral cutaneous branch of T2 spinal nerve.

- Emerges from 2nd intercostal space
- Supplies skin on medial aspect of the arm
- Associated with referred pain from angina or heart attacks
- Heart sympathetic nerves carry afferents back to upper thoracic cord
- Visceral heart pain referred to somatic thoracic nerve.

3.2. Lymphatics of the mammary gland

The lymphatics of the Breast assumes great relevance in the invasion and treatment of the carcinoma breast. The lymph from the nipple, areola and the lobules passes into the subareolar lymphatic plexus of Sappey. Sappey observed that from sub areolar plexus, lymph drains into the axillary lymph nodes.

The axillary lymph nodes receive 75% of the lymph of the breast. The anatomical classification of axillary nodes comprises of 5 groups :

1. Anterior- along the lateral thoracic vessels
2. Posterior- along subscapular vessels
3. Lateral- along axillary vein
4. Central- embedded in axillary fat
5. Apical- lying above the level of pectoralis minor tendon

The anterior group of axillary lymph nodes drain 75% of lymph from mammary gland. The anterior, posterior & lateral groups drain into central group which in turn drains into apical group. The apical group nodes lymph into the supraclavicular drains which in turn drain into the subclavian lymph nodes.

From the surgical point of view the classification of the axillary nodes is in accordance with their relation to the pectoralis minor muscle.

Level 1- lying below the pectoralis minor

Level 2- lying behind the pectoralis minor

Level 3- lying above the pectoralis minor i.e. between the upper border of pectoral minor and lower border of clavicle.

The staging of cancer breast uses the lymphatic mapping with sentinel lymph node biopsy as on of the vital techniques. Involvement of the sentinel node is regarded as a poor prognostic marker and indicates the need for axillary dissection and clearance.

The sentinel lymph node biopsy makes it possible to comprehend and estimate the metastatic risk in breast cancer.

3.3. Modes of spread of breast cancer

1. LOCAL spread- the tumor may involve the skin, pectoral muscles and even chest wall.

2. LYMPHATIC METASTASIS- it occurs primarily to the axillary lymph nodes and even to the internal mammary chain. The lymph node involvement is a marker for the metasatic potential of the tumor.

3. HEMATOGENOUS route- may lead to skeletal involvement and organs such as liver and lungs.

4. Clinical Aspects

4.1. Nipple discharge

The incidence of nipple discharge is only 3-5 %. Additionally all nipple discharges may not have a pathological etiology and galactorrhea needs to be differentiated from physiological and pathological causative factors. Galactorrhea is not a symptom of breast cancer or primary breast pathology. It is usually bilateral, milky and from multiple ducts. Lesions of the hypothalamus, chest, kidney or non pituitary prolactin producing tumor may be the usual causes of galactorrhea.

A pathological discharge may be the warning sign of an underlying intraductal papilloma or duct ectasia or even cancer.

Non lactational, unilateral, spontaneous and bloody discharge is more likely to be of pathological etiology and therefore histopathological diagnosis becomes mandatory.

Usually 5-10% patients with malignancy present with nipple discharge and this fact increases with age.

4.2. Breast Pain

4.2.1. Skin changes

Lesions of the breast include benign conditions like herpes, psoriasis and malignant lesions like Paget's disease, basal and squamous cell carcinoma.

Paget's disease may present with symptoms of nipple itching or discomfort and may be a sign of underlying in situ ductal carcinoma. Diagnosis may be made on the basis of biopsy which shows large cells with clear cytoplasm.

4.2.2. Mastitis

It is cellulitis of the breast and may be puerperal or nonpuerperal in etiology. Puerperal mastitis may be endemic or epidemic. The usual organism responsible for epidemic mastitis is staphylococcal aureus which may involve the lactiferous ducts and glands.

The endemic type of puerperal mastitis is often polymicrobial and periductal in origin. The major difference between the epidemic and endemic is that the latter occurs immediately after delivery.

4.3. Cancer breast

Adenocarcinomas arising from the epithelial cells of the lactiferous ducts are the commonest form of Ca Breast. Metastatic cancer cells usually traverse lymphatic vessel that enters into 2-3 groups of lymph nodes before spreading onto the venous system.

SIGNS

1. Lymphedema- due to interference of the lymphatic supply by cancer cells leading to excessive fluid accumulation in the subcutaneous tissue.

2. Peau ‘d’ orange- Dimpling of the skin along with puffy skin- due to glandular invasion and ensuing fibrosis caused by shortening of the suspensory ligaments.

3. Retraction of the nipple- due to cancer cells infiltrating the lactiferous ducts

4.4. Lymphatic spread

Typically, cancer cells spread via the lymph nodes, primarily the axillary lymph nodes. Communications between the axillary, cervical and para-sternal nodes may result in spread into suparclavicular nodes, contralateral nodes and the abdomen. Early detection of the axillary lymphadenopathy may alter the disease progression of the cancer. Although lack of enlarged axillary lymph nodes may not necessarily mean absence of the cancer as it may have metastasized into other nodes.

4.5. Venous spread

The posterior intercostal veins drain into the Azygous/hemiazygous veins adjacent to the vertebral bodies, communicating with the internal vertebral venous plexus surrounding the spinal cord. Cancer cells metastasize into cranium and brain by these venous routes.

4.6. Invasion into Retro-mammary gland

This invasion causes attachment to the pectoral fascia overlying pectoralis major mus-

cle. Hence elevation of the breast is caused whenever the muscle contracts. This is a clinical sign of advanced cancer.

5. Four Boundaries for Mastectomy

- Clavicle – superior boundary
- Inframammary fold (above rectus sheath) – inferior boundary
- Sternum (midline) – medial boundary
- Latissimus dorsi (ant. border) – lateral boundary

5.1. Simple mastectomy

Breast is removed down to the retro-mammary space.

5.2. Radical mastectomy

Extensive removal of the breast including the pectoral muscles, fat, fascia and axillary lymph nodes. The lymph nodes of the pectoral region are also removed.

Alternatively, a breast conserving approach followed by radiotherapy may be employed by the operating breast surgeon, depending on the stage and grade of the cancer.

6. Investigations

1. Mammography

2. Ultrasound

3. Fine Needle Aspiration Cytology

4. MRI Scan

5. Positron Emission Tomography (PET) - radionuclide imaging method where a tracer labelled with a positron emitter is detected. 18F Fluorodeoxyglucose accumulates in the tumor cells as they have increased glycolytic activity. This method is a very useful tool for detection and staging if the cancer.

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