MINI-REVIEW

Breastfeeding and its Relationship with Reduction of Breast Cancer: A Review

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Abstract

In this review, we describe the patterns of known immunological components in breast milk and examine the relationship between breastfeeding and reduced risk of breast cancer. The top risk factors for breast cancer are a woman's age and family history, specifically having a first-degree relative with breast cancer. Women that have a history of breastfeeding have been shown to have reduced rates of breast cancer. Although the specific cause has not been elucidated, previous studies have suggested that breastfeeding reduces the risk of breast cancer primarily through two mechanisms: the differentiation of breast tissue and reduction in the lifetime number of ovulatory cycles. In this context, one of the primary components of human milk that is postulated to affect cancer risk is alpha-lactalbumin. Tumour cell death can be induced by HAMLET (a human milk complex of alpha-lactalbumin and oleic acid). HAMLET induces apoptosis only in tumour cells, while normal differentiated cells are resistant to its effects. Therefore, HAMLET may provide safe and effective protection against the development of breast cancer. Mothers should be encouraged to breastfeed their babies because the complex components of human milk secretion make it an ideal food source for babies and clinical evidence has shown that there is a lower risk of breast cancer in women who breastfed their babies.

Keywords: Breast cancer - breastfeeding - breast milk - alpha-lactalbumin

Introduction

Breast cancer is the most common gynaecological tumour in young women, the second most common cancer worldwide and the most frequently diagnosed cancer among women (Cordero et al., 2010). Its profound implications for healthcare and the increasingly earlier age of diagnosis have resulted in the careful analysis of its causes and possible preventive measures; thus, the study of the causes and potential methods of prevention of breast cancer are the primary goal of epidemiological research. Each year, about 22% of new cancer cases diagnosed in women are breast cancer. In Brazil, estimates performed by the Inca (National Cancer Institute) in 2010, which are also valid for 2011, indicate that the annual number of new cases of breast cancer expected in Brazil is 49,240, with an estimated risk of 49 cases per 100,000 women. In southeast Brazil, breast cancer is the most common type of cancer in women, with an estimated number of 65 new cases per 100,000 women. Without the exception of non-melanoma skin cancers, this type of cancer is also more common in women of south (64/100,000), central west (38/100,000) and northeast (30/100,000) Brazil. In the northern region of the country, breast cancer is the second most frequently encountered tumour type (Inca, 2010).

The relationships between breastfeeding and the development of a number of chronic diseases, including obesity, diabetes and breast cancer, have been extensively studied (Taylor et al., 2005).

Breastfeeding is the best way to provide young infants with the nutrients they need for healthy growth and development (França et al., 2010; Morceli et al., 2011). There is evidence that the protective effect caused by breastfeeding persists into the postmenopausal years (Enger et al., 1998).

Support and advice should be routinely available during antenatal care to help mothers to initiate breastfeeding at the time of birth and to ensure that breastfeeding is fully established during the postnatal period. Poor breastfeeding rates and complementary feeding practices are widespread. Worldwide, it is estimated that only 34.8% of infants are exclusively breastfed for the first 6 months of life, while the majority receive other types of food or fluid during their early months (WHO, 2009).

Breastfeeding provides short-term and long-term benefits for the both child and the mother, including protection for children against a variety of acute and chronic disorders. The long-term disadvantages of electing

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to not breastfeed are increasingly being recognised as significant (Geister et al., 1996; WHO, 2007).

This review summarises many of the known immunological components of human breast milk and examines the relationship between breastfeeding and reduced risk of breast cancer.

Breast Cancer

The most important risk factors for breast cancer are a woman's age and family history, specifically having a first-degree relative with breast cancer. Breast cancer is the most prevalent in women between the ages of 40 and 69 years. There are also other established risk factors for cancer development related to the reproductive health of women: early menarche, nulliparity, first pregnancy over 30 years, oral contraceptive use, late menopause and use of hormone replacement therapy (Matos et al., 2010).

For pathological breast cancer, the current model of human breast cancer progression is depicted by a linear multi-step process, which begins as flat epithelial atypia, progresses to atypical ductal hyperplasia, evolves into DCIS and culminates in the potentially lethal stage of invasive ductal carcinoma. A major challenge to human breast cancer research has been the identification of the molecular alterations linked with the different stages of breast cancer progression. Until recently, progress in attaining this goal has been hampered by technical limitations associated with applying advanced molecular technologies to the microscopic preinvasive stages of breast tumorigenesis (Bombonati and Sgroi, 2011).

Breast cancer tumours are infiltrated by a heterogeneous population of immune cells, consisting of different proportions of T cells, B cells, natural killer (NK) cells and macrophages (Georgiannos et al., 2003). Although components of the immune system are present, many breast tumours progressively grow and spread; therefore, the role of tumour-infiltrating leukocytes in the tumour microenvironment is unclear (Marsigliante et al., 1999). The association of tumour-infiltrating CD4+ T lymphocytes with lymph node metastases suggests a role for these cells in the spread of tumour cells to the lymph nodes in patients with early breast cancer (Macchetti et al., 2006).

Considering that cancer cells must acquire permanent genetic mutations, cancer is defined as a disease caused by deficiency of DNA repair. Yet, for cells to uncontrollably replicate their DNA and divide, which is the fundamental phenotype of cancer, multiple DNA repair pathways are required. This produces a paradox for the cancer cell, whereby its origin is also its weakness. The cancer cell often becomes dependent on other DNA repair pathways that are distinct from the pathway that led to its initial mutability. The best example of this paradox is breast or ovarian cancers with mutated BRCA1 or 2, both of which are essential components of the DNA double-strand break repair pathway (Shaheen et al., 2011).

An important factor involved in metastasis is the ability of the cells to migrate. Vimentin is a type III intermediate filament protein that is frequently over-expressed in epithelial carcinomas and correlates with invasiveness

and poor prognosis. A previous study that analysed the ability of a panel of carcinoma cell lines to migrate and adhere to a collagenous matrix found that ablation of vimentin expression inhibits migration and invasion of breast cancer cell lines (Mcinroy and Määttä, 2007). Besides vimentin, Gli1 is also involved in metastasis. Gli1 is an established oncogene and its expression in oestrogen receptor (ER) α-negative and triple-negative breast cancers indicates poor prognosis; however, the biological functions regulated by Gli1 in breast cancer have not been extensively evaluated. Reduced expression of Gli1 in human breast cancer cell lines resulted in a decrease in migration and invasion. Gli1 over-expression has been shown to increase the migration and invasiveness of these cell lines. In summary, Gli1 promotes the growth, survival, migration, invasion and metastasis of ERα-negative breast cancer (Kwon et al., 2011).

Recent advances in the treatment of breast cancer and early detection caused increases in survival rates. Today, women diagnosed with breast cancer are almost twice as likely to survive for 10 years or longer as women 40 years ago. However, breast cancer remains a major contributor to cancer morbidity and mortality the majority of patients present with potentially curative disease and surgery is the typical treatment. Many patients receive adjuvant therapy, which reduces the risk of loco-regional and distant disease recurrence (Barrett, 2010).

The therapy of breast cancer include radiotherapy, chemotherapy, endocrine therapy and biological agents and treatments are increasingly being tailored to the individual tumour and patient to provide the maximum survival benefit with little toxicity. Many patients participate in clinical trials examining radiotherapy, novel agents, drug combinations or novel dosing regimens. If there is metastatic disease metastatic disease are offered treatment, but improved quality of life and prolonged survival may be achieved with palliative treatment, including hormonal therapy, chemotherapy, radiotherapy and treatment trastuzumab and bisphosphonates (Barrett, 2010).

Protein kinase Cε (PKCε) is capable of modulating various cellular functions, including proliferation, differentiation and survival. PKCε also promotes tumour metastatic capacity and resistance to anti-cancer therapy. Overexpression of PKCε has been detected in numerous cancer types, including colon, breast, stomach, prostate, thyroid and lung and it is considered to be an important marker of negative disease outcome. Suppression of the gene encoding PKCε using antisense cDNA, inhibition of PKCε using RNAi or inhibition of PKCε using translocation-inhibitory peptides may be further developed into novel cancer treatment strategies (Toton et al., 2011).

Due to the political, social and financial incentives for cancer research, advances have been made in the treatment of many types of cancer. The hypothesis that cancer arises from a small population of cells, termed cancer stem cells (CSCs), is gaining popularity amongst researchers. However, there are still numerous sceptics who question the validity of this theory. Many sceptics do not believe that there is a specific subset of cells that originate with these characteristics; instead, the cells develop certain

features over time, making them more resistant to conventional therapy. It is theorised that many relapses that occur after remission are caused by an inability to destroy the self-renewing CSCs. The central idea that CSCs are biologically different from all other cancer cells has directed research towards the development of therapies that directly target CSCs. One major concern of targeting therapies is the inability to target CSCs as opposed to normal stem cells, thereby inducing the development of myeloproliferative disorders, malignancies of the central nervous system or other malignancies in general. Nevertheless, with the recent advances in the identification of unique molecular signatures for CSCs, along with on-going clinical trials targeting CSCs, it is possible that targeted nanotechnology-based strategies will be used in the near future for the management of different types of cancers (Clayton and Mousa, 2011).

Breast Milk

Several studies using improved epidemiological methods and modern laboratory techniques have documented the diverse and compelling advantages of breastfeeding for infants, mothers, families and society (Kramer et al., 2001).

There is a variety of immunologically relevant components in human milk (Honorio-França et al., 1997; 2001; França et al., 2010; França et al., 2011; Morceli et al., 2011). Secreted IgA neutralises pathogens and simultaneously limits the damaging effects of tissue inflammation caused by other antibody types (Jackson and Nazar, 2006).

There is evidence that human milk may confer long-term benefits, such as reduced risk of certain autoimmune diseases, inflammatory bowel disease and certain malignancies. Human milk has been demonstrated to possibly affect components of metabolic syndromes. Recent studies have also indicated the long-term health benefits of lactation for mothers. A reduced incidence of breast cancer is the most well-documented long-term effect of breastfeeding on mothers. An increasing number of studies have indicated that breastfeeding offers protection against ovarian cancer, rheumatoid arthritis and type II diabetes (Løland et al., 2007).

In addition to these immunological components, breast milk contains important nonspecific factors that have antimicrobial effects. These factors include the enzyme lysozyme, which inhibits the growth of many bacterial species by disrupting the proteoglycan layer of the bacterial cell wall. Lactoferrin, one of the most abundant proteins in human milk, also limits bacterial growth by removing essential iron molecules. Nucleotides in human milk have been shown to enhance immune function in infants. Complex sugars are found only in trace amounts in cow's milk but make up a substantial portion of the sugars in human milk, in which they may prevent adherence of various microbial pathogens by acting as decoy receptors (Jackson, 2006).

Of the many factors with immunological, hormonal, enzymatic and trophic activity, cytokines are believed to play a significant role in immunomodulation and immunoprotection. Most of the cytokines that are known to be deficient in neonates and particularly in preterm infants, have been found in elevated amounts in breast milk: IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12, IL-18, IFNγ, TNFα, transforming growth factor-β (TGF-β), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Ellis et al., 1997).

Studies have suggested that breast milk cytokines reach the neonatal intestinal intact because of protection from digestion by protease inhibitors, mainly α1-antichymotrypsin and α1-antitrypsin, which are present in maternal milk. In addition, gastric digestion of proteins is reduced during the first 3 months of life because of the limited secretion of pepsin and H+ ions and the general immaturity of the newborn's digestive abilities. These factors favour the survival and the intestinal absorption of intact polypeptides, thereby allowing them to exert their biological activities (Maheshwari et al., 2002).

Lactoferrin (LF) exhibits antibacterial, antifungal, antiviral, antiparasitic and antitumoural functions. It protects the intestinal epithelium, promotes bone growth and accelerates the recovery of immune system function in immunocompromised animals. LF has been utilised for the treatment of hepatitis C infection and the intestinal form of graft-versus-host disease (GvHD). Proline-rich polypeptide (PRP) has been shown to demonstrate a variety of immunotropic functions, including the promotion of T cell maturation and inhibition of autoimmune disorders. PRP, in the form of chewable tablets (Colostrinin), was recently found to improve or stabilise the health status of Alzheimer's disease patients. Previous studies have shown that casein and casein-derived peptides have protective effects on enamel demineralisation and act as caries-preventing agents. Protein hydrolysates were also protective in diabetic animals and were able to reduce tumour growth, had antihypertensive activity and diminished colicky symptoms in infants. In addition, glycomacropeptide (GMP), a peptide derived from kappa-casein, exhibited various antibacterial and antithrombotic activities (Zimecki and Artym, 2005).

Another very important component of breast milk is alpha-lactalbumin. Tumour and bacterial cell death have been shown to be induced by HAMLET (a human milk complex of alpha-lactalbumin and oleic acid). HAMLET induces apoptosis in tumour cells, while normal differentiated cells are resistant to its effects. The activity of HAMLET was discovered by serendipity while using human milk fractions to investigate bacteria adherence to lung carcinoma cell lines. In addition to blocking adherence, one milk fraction actually killed the cells by inducing apoptosis. Cell death was accompanied by changes in morphology, nuclear condensation and cytoplasmic blebbing and the formation of apoptotic bodies, all of which are similar phenotypes to those seen in cells that undergo classical apoptosis (Kerr et al., 1972; Gustafson et al., 2005).

HAMLET has unique biological properties because it selectively purges malignant cells using an apoptosis-like mechanism but leaves normal cells unharmed. This suggests that HAMLET bypasses the various blocks of

apoptosis employed by many tumour cells and that it activates other cell death pathways that remain functional in tumour cells. HAMLET illustrates the value of human milk as a rich source of molecules with beneficial effects on a variety of human-disease conditions. The conditions required to form HAMLET are present in the stomachs of breastfed children, where the low pH potentially unfolds the protein by the release of calcium and the acid-sensitive lipases hydrolyse milk triglycerides to release oleic acid. There is evidence that HAMLET kills virus-transformed or premalignant cells in the gastrointestinal tract of breastfed children. This effect might include lymphoid cells in the gut-associated lymphoid tissue because breastfed children have dramatically reduced frequencies of lymphomas compared with age-matched bottle-fed children (Gustafson et al., 2005).

HAMLET has a broad antitumour activity in vitro, and its therapeutic effects have been confirmed in vivo in a human glioblastoma rat xenograft model, in patients with skin papillomas and in patients with bladder cancer (Hallgren et al., 2008).

In a previous study, the authors examined the effects of HAMLET on mammary cells. Plastic pellets with HAMLET were implanted into the fourth inguinal mammary gland of lactating mice for three days. Exposure of mammary tissue to HAMLET resulted in morphological changes typical of apoptosis and the stimulation of caspase-3 activity in alveolar epithelial cells near the HAMLET pellets but not in the areas more distant to the pellet or in contralateral glands. The effect was specific for HAMLET and no effects were observed when mammary glands were exposed to native α-lactalbumin or fatty acids alone. HAMLET also induced cell death in vitro in a mouse mammary epithelial cell line. These results suggest that HAMLET can mediate apoptotic cell death in mammary gland tissue (Baltzer et al., 2004).

Several mouse breast cancer models have been developed to define a prototypic strategy for prophylactic cancer vaccination. We selected alpha-lactalbumin as our target vaccine autoantigen because it is a breast-specific differentiation protein that is expressed at high levels in the majority of human breast carcinomas and in mammary epithelial cells only during lactation. Immunoreactivity against alpha-lactalbumin provides substantial protection against the growth of autochthonous tumours in transgenic mouse models of breast cancer and against 4T1 transplantable breast tumours in BALB/c mice. Because alpha-lactalbumin is conditionally expressed only during lactation, vaccination-induced prophylaxis occurs without any detectable inflammation in normal nonlactating breast tissue. Thus, alpha-lactalbumin vaccination may provide safe and effective protection against the development of breast cancer for women in their post-childbearing, premenopausal years, during which lactation is readily avoidable and the risk for developing breast cancer is high (Jaini et al., 2010).

Breastfeeding: benefits for women and the relationship with breast cancer

In previous studies, non-breastfeeding mothers have been shown to have a higher risk of reproductive cancers. Breast, ovarian and uterine cancers have been found to be more common in women who did not breastfeed (Dermer, 2001).

Although there are few studies comparing the practice of breastfeeding to ovarian cancer, the risk of cancer appears to be lower in women who breastfeed (Labbok, 2001).

In a study by Brun et al, more than 60,000 Norwegian women were followed for 29 years. The authors analysed various aspects of their reproductive lives and their causes of death; in addition, 355 women who suffered from rheumatoid arthritis were examined. A longer duration of lactation was associated with lower mortality in the rheumatoid arthritis group (Brun et al., 1995).

During lactation, women produce between 600 and 1,000 ml of milk per day, with an average daily loss of 200 mg of calcium. This loss of calcium can potentially lead to bone fractures, especially if breastfeeding is maintained exclusively for 6 months (as recommended). It is plausible that breastfeeding increases the risk of fractures because the loss of calcium and hormonal changes that occur during pregnancy and lactation might be responsible for changes that facilitate bone fractures. However, it is known that calcium loss is recovered during the weaning period and after the return of menstruation. In fact, women who breastfed for more than 8 months were demonstrated to have a higher bone mineral density, according to a study conducted in the state of Minnesota in the United States (Melton et al., 1993).

Although the mechanisms are not entirely elucidated, breastfeeding has been hypothesised to reduce the risk of breast cancer primarily through two mechanisms: the differentiation of breast tissue and reduction of the lifetime number of ovulatory cycles; however, previous reviews examining the association between breastfeeding and breast cancer have not consistently found that breastfeeding reduces risk of breast cancer (Yang and Jacobsen, 2008).

In a recent meta-analysis, the authors evaluated a total of 50,302 parous women with incident invasive breast

Table 1. Main Benefits of Breastfeeding for Women

Benefit	Sources
Reduction in brest cancer	Bernier et al. (2000); Dermer (2001); Ip et al. (2007); Silva et al. (2010)
Reduction in ovarian cancer	Labbok, (2001); Dermer (2001)
Lower mortality in rheumatoid arthritis	Brun et al. (1995)
Bone mass was shown with higher mineral density	Melton et al. (1993)
Physiologic Effects (prevent postpartum hemorrhage and promote uterine)	Lawrence and Lawrence (1999); Dermer (2001)
Lactational amenorrhea method	Kennedy and Visness (1992)
High HDL cholesterol	Oyer and Stone (1989)

cancer and 96,973 controls. Included in the meta-analysis were primary studies that analysed at least 100 cases of incident invasive breast cancers per study regardless of menopausal status, with additional information regarding reproductive factors and use of hormonal preparations. Both cohort and case-control studies from developed and developing countries were included. Individual subject data from the primary studies were analysed for homogeneity across study definitions. The majority of the primary studies did not differentiate between exclusive. The average age at breast cancer diagnosis in the studies was 50 years. There were a higher proportion of women with either nulliparity or low parity in the breast cancer group compared with the control group. In addition, fewer parous women in the breast cancer group had breastfed their infants compared with the control group. There was a statistically significant reduction in the risk of breast cancer by 4.3% (95%CI: 2.9-5.8) for each year of breastfeeding. Decreases in the relative risk of breast cancer associated with each year of breastfeeding remained homogeneous across the studies in regards to developed versus developing countries, age at diagnosis, menopausal status, family history of breast cancer and study design. In addition, the decrease in the relative breast cancer risk in parous women, according to breastfeeding history (some breastfeeding versus never breastfed) and number of births, was more pronounced after four or more births (Ip et al., 2007).

Another study evaluated the relationship between breastfeeding and histologically diagnosed breast cancer in parous women. The meta-analysis combined 25,871 cases and 44,910 controls from 23 primary case-control studies. The meta-analysis used both fixed and random effects models. There was a small but statistically significant decrease in the risk of breast cancer in women who had breastfed their infants compared with women who had not breastfed. The decreased risk was further explored by examining the menopausal status at the time of diagnosis of breast cancer and the duration of breastfeeding. Breastfeeding duration was divided into categories of 1-6 months, 7-12 months and more than 12 months. When each of these categories were compared with non-breastfeeding women, only those whose lifetime duration of breastfeeding was longer than 12 months (compared to never) had a small but statistically significant reduction in the risk of breast cancer using the subgroup analysis (Bernier et al., 2000).

A case-control study in women aged 30-64 years in selected healthcare facilities in the western province of Brazil analysed a total of 100 recent cases of breast cancer (histologically confirmed) and 203 controls (age- and parity-matched). Detailed information regarding breastfeeding, menstruation, reproductive factors, passive smoking and other confounders was collected using a structured questionnaire. The multivariate analysis found that women who breastfed for at least 24 months during their lifetimes had a significantly lower risk of developing breast cancer than those who breastfed for less than 24 months. Compared with women who breastfed a total of 0-11 months over their lifetimes, there was a 66.3% reduction in breast cancer risk in women who breastfed

for 12-23 months, an 87.4% reduction in women who breastfed 24-35 months and a 94% reduction in women who breastfed 36-47 months. The mean duration of breastfeeding per child for at least 12 months was also associated with reduced risk of breast cancer (Silva et al., 2010).

Concluding Remarks

Women should be encouraged to breastfeed their babies because the complex components of human milk make it the ideal food source for babies; in addition, breastfeeding is beneficial for maternal health. Breastfeeding improves the quality of life for mothers. Non-breastfeeding mothers have been shown in previous studies to have a higher risk of reproductive cancers. Breastfeeding for longer periods results in statistically significant reductions in the risk of developing breast cancer, the most common gynaecological tumour in young women, the second most common cancer and the most frequently diagnosed cancer among women worldwide. There is strong evidence that breastfeeding reduces the risk of breast cancer; however, further studies must be conducted to further elucidate the mechanisms involved in the protective effects of breastfeeding.

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