International Journal of
Women's Health and Wellness

ORIGINAL ARTICLE

A-FABP Decreases in the Wean Milk of Nursing Women with a Family History of Breast Cancer

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Abstract

Adipocyte/Macrophage Fatty Acid Binding Protein (A-FABP) is linked to breast cancer. Proteins up regulated in breast cancer tissue are often increased in milk from women with and at increased risk of developing breast cancer. We measured A-FABP in human milk from 76 lactating women based on a) Phase of lactation (within 10 days of delivery: transitional-T, mid: 2 month, and wean-W), b) Family history, c) Body mass index, d) Age, and e) Age at First Full Term Pregnancy (FFTP). A-FABP expression was a) Lower in 2 month than T milk independent of family history ($p < 0.01$), b) Lower in 2 month than W milk only in women without a family history ($p < 0.01$), and c) Lower in W than T milk only in women with a family history ($p = 0.055$). A-FABP was not associated with body mass index, age, or FFTP. Lower A-FABP in W than T milk only in women with a family history of breast cancer suggests breastfeeding has a greater breast cancer protective effect in women with than without a family history.

Keywords

Breast cancer, Fatty acid binding protein, Pregnancy, Lactation

Abbreviations

 A-FABP: Adipocyte Fatty Acid Binding Protein; BCa: Breast Cancer; BMI: Body Mass Index; FFTP: First Full Term Pregnancy; FH: Family History; KLK: Kallikrein; PABC: Pregnancy Associated Breast Cancer; SD: Standard Deviation; T: Transitional; TGF: Transforming Growth Factor; W: Wean

Introduction

The breast is the second most common site of cancer (after skin cancer) diagnosed in women in the U.S. and worldwide. Despite notable advances in the treatment of Breast Cancer (BCa), the number of U.S. women dying of breast cancer, over 40,000, has not changed appreciably in the past 10 years [1]. Advances in early detection are limited to breast imaging technology, which has not eliminated the need for an invasive diagnostic biopsy. Moreover, less than a third of the biopsies based on imaging detect breast cancer [2], suggesting the need for a more specific diagnostic marker than breast imaging. There are no noninvasive clinically useful tools for the early detection of breast cancer, and no biomarker expression laboratory tests to accurately assess risk.

Pregnancy Associated Breast Cancer (PABC) is an aggressive form of the disease which occurs in women during and up to 10 years postpartum [3]. Breastfeeding after childbirth may influence breast cancer risk. The time of weaning the newborn from breastfeeding appears to be crucial to future breast cancer risk [4]. Adipocyte/Macrophage Fatty Acid Binding Protein (A-FABP or FABP4) is highly expressed in adipocytes and, to a lesser degree, in macrophages. A-FABP expression is up regulated in invasive BCa compared to normal breast tissue, and in tumors of advanced disease stage compared to early stage BCa [5]. Our prior studies led us to conclude that increased levels of A-FABP are associated with BCa. Moreover, we observed that protein expression in breast milk can be informative regarding risk of current BCa [6], and may prove useful in future BCa risk

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prediction. Of the 3 phases of lactation, the late phase is especially important, since during weaning and involution the breast microenvironment, enriched in growth factors, is tumor promotional [3].

BCa risk has been linked to obesity, especially in postmenopausal women. Preliminary evidence suggests that the expression of A-FABP in serum is associated with obesity and breast cancer risk. We evaluated the expression of A-FABP in milk samples from nursing women to determine if expression was associated with markers of risk, and if there was a biological explanation for a recent report involving over 60,000 parous women which observed that breastfeeding decreased BCa risk (hazard ratio 0.41, p = 0.03) compared to women that did not, but only in those with a FH [7]. The authors proposed that BCa risk may be higher among women who did not breastfeed because of disordered involution.

Materials and Methods

Specimen collection: Seventy-six healthy women were prospectively recruited prior to or soon after delivery. They were eligible if they delivered a ≥ 37 weeks gestation infant and were planning on breastfeeding. Mean age of subjects at the time of enrollment was 28.3 years; mean age at the time of First Full Term Pregnancy (FFTP) was 25.4 years. It was the FFTP for 35 of the participants. After agreeing to enroll in an Institutional Review Board approved project by signing an informed consent document, three milk samples were requested from each participant: Transitional (T), defined as within 10 days of the initiation of lactation, 2 months after lactation started, and when the woman was Weaning (W). The participant was asked to provide the wean sample once they had decided to stop nursing and had started to decrease the number of daily feedings. Each sample was collected from the same breast.

Mothers were asked not to breast feed their infants for at least two hours before milk collection, which involves draining the breast using manual extraction or a breast pump. Samples were immediately frozen after collection. Collected milk was thawed, centrifuged (1500 xg, 20 min, 4 °C), and the fat and cellular layers separated. The aqueous phase was then centrifuged at 12000 g for 15 min at 4 °C, the second lipid layer removed and stored at -80 °C prior to analysis.

Specimen analysis: Milk was prospectively collected from female adult (>= 18-years-old) participants. Milk was analyzed by immunoassay for the expression of human A-FABP using an immunoassay kit from Cayman Chemical, Ann Arbor, MI, USA, following the manufacturer's instructions.

Statistical analysis: We first summarized the expression of A-FABP at different time points by mean, Standard Deviation (SD), and number of observations (n) as mean ± SD (n) (Table 1), then we examined the expression of A-FABP at different time points according to whether the subject has a FH of BCa (Table 2). We applied linear mixed effect models and Wald tests to examine whether A-FABP expression was associated with time and FH, controlling for Body Mass Index (BMI), age, and age at FFTP.

Results

Samples from 76 women were evaluated for the expression of A-FABP. Samples from three women were excluded because of extreme values (> 3 SD from the mean). Compared to T expression, A-FABP was lower at 2 months but not W time period (p < 0.001, Table 1).

We then evaluated A-FABP expression based on whether the patient had a FH of breast cancer. Compared to T expression, A-FABP was lower at 2 months for both women with (p = 0.001) and without a FH (p < 0.001, Table 2). A-FABP expression was also lower at W compared to T for women with a FH (p = 0.03) but not those without (Table 2). Controlling for BMI, age and age at FFTP, women with a FH had lower W than T milk A-FABP levels (p = 0.055), which was not observed in women lacking a FH (Table 2). A-FABP was not significantly associated with age, FH, or BMI (p > 0.05 for each).

Discussion

PABC is an aggressive form of breast cancer which is difficult to diagnose and, stage for stage, has a worse survival than BCa diagnosed at other times [3]. We report that A-FABP levels in breast milk are lower at 2 months compared to transitional milk for all women, both those with and without a FH. We previously reported that total protein expression is significantly lower at 2 months than either T or W milk [8], which likely explains this difference. On the other hand we observed that T and W total milk protein expression is similar, but observed that A-FABP expression was lower in W than T milk only among women with a FH.

Based on the epidemiologic association between FH and a protective role for breastfeeding, we previously

Table 1: Mean ± SD (n) of A-FABP for different visits.

Transitional	2 month	Wean
12.98 ± 12.40 (73)	6.32 ± 11.22 (71) (p < 0.00)	11.61 ± 12.34 (45) (p = 0.26)

SD: Standard Deviation; A-FABP: Adipocyte/Macrophage Fatty Acid Binding Protein.

Table 2: Mean ± SD (n) A-FABP stratified by visits and FH.

	No FH	FH
Transitional	13.81 ± 12.79 (49)	11.29 ± 11.65 (24)
2 month	7.04 ± 11.79 (49) (p < 0.001)	4.73 ± 9.91 (22) (p = 0.001)
Wean	15.1 ± 13.62 (29) (p = 0.77)	5.28 ± 5.80 (16) (p = 0.055)

SD: Standard Deviation; A-FABP: Adipocyte/Macrophage Fatty Acid Binding Protein; FH: Family History.

The results are after controlling for body mass index, age and age at first full term pregnancy.

reported that four proteins (KLK5, KLK14, TGFβ-1, and TGFβ-2) were differentially expressed in breast milk based on whether the woman had a FH of BCa [9], most notably comparing 2 month and W protein expression levels. On the other hand, KLK13, which is a favorable prognostic marker in women with BCa [10], decreased in women without a FH in wean compared to T milk, whereas it increased in women with a FH (p = 0.0047), suggesting a protective effect of breastfeeding only for women with a FH. We observed that A-FABP, expressed primarily in adipocytes but also in macrophages, is down regulated at the time of breast weaning and involution in the milk of women with a FH, but not in those lacking a FH. Our current studies suggest that A-FABP is only expressed in certain subsets of macrophages, those which promote BCa cell growth and metastasis. A-FABP + macrophages typically exhibit a MHCII-CD11c-phenotype and have reduced capability of mediating adaptive immune responses and antigen presentation. As such, macrophages present in the tumor promotional microenvironment that is present at the time of breast weaning and involution may be less able to suppress tumor formation and/or progression. The current observation is consistent with our earlier finding that breastfeeding is more protective in women with a FH than in those without a FH. A strength of our paper is this consistency, which supports the earlier report. A weakness of our study is the small sample size.

The reasons for this are worthy of further study. As we try to personalize our care for patients at risk for developing breast cancer, confirmation that breastfeeding is especially protective for women with a FH could be helpful for women at greatest risk of developing the disease, allowing them to redouble their efforts at breastfeeding and to consider more sensitive methods of breast cancer screening such as breast magnetic resonance imaging.

Declarations

Ethics approval

All participants in this study agreed to enroll in an Institutional Review Board approved project by signing an informed consent document.

Competing interests

The authors declare that they have no competing interests.

Funding

Authors contributions

Author contributions: BL helped design the project and supervised specimen analysis; JH analyzed the samples; XY performed the statistical analysis, MK designed the statistical analysis plan and supervised the analysis; ERS helped design the project, consented the patients, collected the samples and the clinical data.

Acknowledgments

NA.

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