

Recent Advance and Challenges in Diagnosis of Breast Carcinoma in Pregnant Ladies and Lactating Mothers from Human Milk: A Future Prospective

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Abstract: *Breast cancer (BC) is a cancer that occurs when breast cell transforms into malignant cells that grow and transform into tumors. Human milk is the milk that gets secreted from the female's mammary glands due to stimulation of prolactin hormones during a few months of pregnancy and after childbirth. In comparison to other types of breast cancer, breast cancer that develops during pregnancy (PrBC) and after childbirth (PPBC) is typically identified at a later stage, which worsens the prognosis so early detection is mandatory to reduce the mortality rates and its risks. Pregnancy-associated breast cancer (PABC) is commonly defined as a breast cancer occurring during pregnancy, throughout one year postpartum, or during lactation. PABC affects about 1 in 3000 pregnant women, representing about 7% of all BCs in women under 45 years old, 10% in women under 40, and rising to 15.6% in women under 35 aged. The reported incidence of PABC is 15–35 per 100,000 deliveries, and it is higher in the postpartum period, with the great majority of cases detected within six months postpartum. This review paper will help us to gain knowledge on early breast cancer in accepting mothers as well as lactating mothers as early as possible using the use of cell-free tumor DNA (ctDNA) present in breast milk (BM) collected from patients with breast cancer as a new source for liquid biopsy for PPBC & PrBC detection.*

Keywords: Breast milk, ctDNA, breast cancer, liquid biopsy, PPBC detection

1. Introduction

Breast cancer as we know is a leading cause of cancer in women worldwide. We believe always that early detection is mostly important in asymptomatic patients. The risk factors for breast cancer are age and family history, especially if we have a first-degree relative who has the condition (Abraham et al.2023). But when it is of special interest when we are looking for pregnancy-associated cancer and the postpartum period, it's a matter of concern as it is an entity of special aggressiveness due to a delay in diagnosis can lead to chances of double metastatic risk and worst in prognosis in the treatment of the diseases (Saura et al., 2023). Recently, there has been a global growing interest in exploring the human milk as sample for one of the potential biomarkers for any cancer cell detection. Human milk is highly rich in macronutrients & micronutrients along with essential vitamins and enzymes, in research, it has been also highlighted that breast milk contains some unique profiles of genetic and proteomic markers that make be an indicator for the early prediction of the disease. However global detection of breast cancer in breast milk poses significant challenges due to the complex and dynamic nature of breast composition, limited sample volumes, sensitivity, and specific analysis methods (Sanso et al., 2021). Usually the analysis of the samples is a non-invasive method and are collected after 2 hours of the previous feeding at the affected breast versus the health breast for the lactating mother and also from the three other more health woman for a comparison (Aslebagh et al.,2017), all the donated milk samples and cancer diagnosis the cancer cell detection is done from 1 month to 24 months after donation (Aslebagh et al.,2017) and are mainly been focused about the information's on its composition about its proteins, immune cells, and epithelial cells which serves as the indication of the presences of breast cancerous cells and tumors (Whitham et al., 2024).

This review aims to discuss how the challenges in diagnosing breast cancer in breast milk have a potential for early detection and improving the treatment outcomes for those women who are detected with breast cancer during pregnancy and postpartum stages.

2. Epidemiology

Notable results were obtained from the breastfeeding study and its relationship to the risk of breast cancer. The findings showed a strong unfavourable correlation between premenopausal women's nursing duration and breast cancer risk (Abraham et al.2023). Recently published report on 13th March 2024 by WHO (World Health Organization in newsroom, fact sheet, details on breast cancer) as per 2022 reports it has been found that the rate due to breast cancer is approximately 670000, among which out of 185 countries approximately 157 countries are affected where patients have no risk factors and symptoms (WHO et al.2024). Breast cancer cells first proliferate mostly in the milk ducts or breast milk-producing lobules. The first kind, known as "in situ," can be identified early on and is not life-threatening. Cancer cells can invade neighboring breast tissue. Tumors produced by this result in thickening or lumps. Metastasis is the process by which invasive tumors move to neighbouring lymph nodes or other organs. Metastasis can be lethal and perhaps fatal. Pregnancy-associated breast cancer malignancy reported is 1 in 3000 to 1 in10000 pregnancies and is the most common cause of cancer-related death in pregnant and lactating patients (Gemigani et al., 2000 & Hogge et al., 1999).

Global estimates reveal striking inequities in the breast cancer burden according to human development. For instance, in countries with a very high Human Development Index (HDI), 1 in 12 women will be diagnosed with breast cancer in their lifetime and 1 in 71 women die of it. In contrast, in countries with a low HDI; while only 1 in 27

women is diagnosed with breast cancer in their lifetime, 1 in 48 women will die from it. Whereas in males it's only (0.5-1) % (WHO et al.2024).

3. Breast milk parameters and detection of cancer

Alpha-lactalbumin is one of the main elements in human milk that is thought to influence the risk of cancer. Tumor cell death can be induced by HAMLET, a human milk complex consisting of oleic acid and alpha-lactalbumin. Only tumor cells are susceptible to HAMLET's induction of apoptosis;

normally differentiated cells are not affected by it (Abraham et al.2023). Our female body prepares for breast feeding from first trimester where our breast tissues begins to multiply for milk production, by the second trimester milk ducts and glands are formed and gets triggered for colostrum production, as the time of baby's bath triggers by the initiation of hormone prolactin the first is ejected and the body prepares for first breast feeding and the as the breast feeding starts the triggering of oxytocin the milk flow gets initiated (Kim et al., 2020). The human milk in a female undergoes various stages of lactation as the colostrum also known as first milk, transitional milk and matured milk (Ballard et al., 2013). The composition of human milk are as follows:

Stages	Phase 1 (Colostrum)	Phase 2 (Transitional Milk)	Phase 3 (Matured Milk) (fore milk, hind milk)
Time frame	0 to 5 days after birth	5 to 15 days	2 weeks onwards
Color/consistency	Deep Yellow, thick, sticky	Bluish-white, thinner	White, Watery & thinner
Compositions	Higher levels of antibodies and white blood cells	Higher levels of proteins, vitamins, fats and lactose	Optimal amounts of carbohydrates, fats, proteins and 90% of water for hydration
Bioactive components	Immunoglobulins-antibodies- IgA/sIgA, IgG, IgM. Cytokines-IL-6, IL-7, IL-8, IL-10, IFN γ , TGF β , TNF α . Chemokine- G-CSF, MIF. Cytokine Inhibitors- TNFRI &II. Growth factors- EGF, HB-EGF, VEGF, NGF, IGF, Erythropoietin. Cells- Macrophages, Stem cells, EGF, IGF-1 Helps to prevent allergy, intestine mature & intolerance.		
Micronutrients	Vitamins: Vit A, Vit C, Vit D, Vit E, Thiamine, Pantothenic acid, Folic acid Minerals: Iron, calcium, phosphorus, Magnesium, Sodium, Chloride, Potassium, Manganese, Iodine, Selenium, Copper, Zinc. Enzymes & Hormones- Prolactin, thyroid & calcitonin, Somatostatin, adiponectin, Leptin, Ghrelin. Reduces severity of infection and prevents measles, eye disease, and diarrhoea.		
Macronutrients	Carbohydrate – 50- 62 g/L Lactose -20-30 g/L Oligodendrocytes -20-40 g/L Lipids – 15-20 g/L with cholesterol, essential fatty acid (DHA) Proteins -14-16 g/L (casein, α -lactalbumin, Lactoferrin) water	Carbohydrate – 60-70 g/L Lactose-67-70 g/L Oligodendrocytes- 12-14 g/L Lipids – 35-40 g/L with cholesterol, essential fatty acid (DHA) Proteins -8-10 g/L (casein, α -lactalbumin, Lactoferrin)	

Biomarkers Present: Lactoferrin, HMOs, IGA, Lysozymes, EGF

4. The current state of screening and detection:

Currently, to detect breast cancer in lactating mothers we have different types of screening tests that we are practicing to detect the presence of cancerous cells. There are tests using non-invasive methods using samples such as breast milk and blood lactating methods using the most common biomarker among them the most detectable biomarker is the ctDNA, liquid biopsy technique. Using the imaging tools we can have Sonomammography & MR mammography as a gold standard method for routine follow-up and screening studies along with self-breast examination techniques for the early detection of the disease.

4.1 Self-breast examination

A woman with pregnancy or lactation phase-associated breast cancer always can be presented with noticeable changes she will look at breast tissues in shape and size with focal pain in situ. There will formation of palpable lumps with the area of the breast or axillary areas with skin thickening, focal pain, and nipple discharge (bloody or clear fluid). There are also

cases of “milk rejection” signs (Saber et al., 1996) due to reports of a lump in the breast where it changes the taste, odor, or consistency of the milk and the infants refuse to nurse from the affected breast (Goldsmith et al., 1974). In self-breast examination a patient can also observe for nipple position changes, dimpling, puckering, redness, or scaling changes on the skin. For lactating mothers because of milk ducts and glandular tissue, breasts that are nursing frequently feel lumpier than breasts that are not nursing. By self-examination nursing mothers get to know their breast tissue and spot any strange changes early. Because of milk ducts and glandular tissue, breasts that are nursing frequently feel lumpier than breasts that are not nursing. Self-examinations are a good way for nursing mothers to get to know their breast tissue and spot any strange changes early. The self-breast examination is the best way to early look for any changes in the breast areas and is always advice to perform it at least in once in a month intervals.

4.2 Circulating tumor DNA (ctDNA) analysis

CtDNA is a genetic cancer biomarker also known as liquid biopsy, where studies in early BC¹⁰⁸ Saura et al., found that its presence in higher rates in breast milk samples detected in post-partum breast cancer. Both breast samples were

collected from the individual and were stored as fresh-frozen formalin-fixed, paraffin-embedded (FFPE) material, and blood samples, it was collected in anticoagulant treated tubes and plasma fraction and centrifuged at -80 °C and with the analytical parameters of the VHIO-YWBC panel the references DNAs with 1% allelic frequency and 0.5 %AF (SeraSeq ctDNA Mutation Mix v2 AF0.5% cat. #0710-0141) were detected. To detect patient-specific variations in the ctDNA VHIO-300 has designed a droplet digital PCR (ddPCR) which combines the PCR and droplet microfluidics to perform the amplification more accurately and easily. The samples for the study are collected in a sterile container manually, if lumps are noted it's too collected and the date, time and relevant information are noted (Smith et al.2023), then the sample is stored for analysis by the techniques such as high-performance liquid chromatography (HPLC), mass spectrometry, or spectrophotometry to analyse nutrient content and assays specific to the biomarkers of interest (e.g., for cancer detection, use specific antibody-based tests) along with other tests if required (Smith et al.2023).

4.3 Sonomammography

Sonomammography is the gold standard method of detection of palpable breast mass in pregnant or lactating mothers. The unique advantage is its nonionizing safety aspects and higher sensitivity detection of pregnancy-associated breast cancer using the BI-RADS values. The modality can detect tumors from benign to malignant stages via a series of examinations in evaluating tumor responses to neoadjuvant chemotherapy during pregnancy (Wang et al. 2012). The parameters generally we look for in the sonomammogram are as types of lesion (solids or cystic) by the aquostic shadowing or enhancement if cysts are found there will be enhancements and for mass always there is shadowing in the images which reveals a further evaluation (ACR et al.2018). Differentiate between lesions based on their echogenicity (hypoechoic, isoechoic, or hyperechoic). Malignant tumors often appear hypoechoic compared to surrounding tissue and color Doppler will reveal the status of blood flow through the particular area under suspecting, where increased vascularity can be associated with malignancy (ACR et al.2018). The values are always correlated with the BI-RADS score where score ranging from above 2 is an indication of breast carcinoma and score indicating 5 comes under malignancy with surgical excision for score 6.

4.4 MR Mammography

Contrast-enhanced MRI is not recommended during pregnancy as contrast can affect the foetus as classified as pregnancy category C by the U.S. Food and Drug Administration (Schackmuth et al.1993) but can be administered for lactating mothers as a new diagnostic tool for breast cancer detection where the lady is used to pump out the first milk within 48 hrs after the examination and nurse the infant. Contrast-enhanced MRI is the best screening examination for high-risk patients for breast cancer (Sabate et al.2007). Where shape, margin, T1 & T2 values with enhancements, kinetics are noted for masses. As T1 increased indicates lymph node with fat necrosis, haematoma, T2 fatsat increases indicates cyst in lymph node with fibro adenoma, fat necrosis with colloid carcinoma. Non-linear enhancements can be classified as linear, ductal, segmental, clumped etc. Type I indicates 6%ca, Type II –Type III indicates (29%-77% ca). There is a software- based technique to confirm and correlate with lab reports is the MR spectroscopy where presences of cancerous cells always will elevate the chlorine & chloride peaks, and in cases of necrosis we can see the lipids peak elevating.

4.5 Mammography

Mammography is safe during pregnancy and lactation but due to its ionizing properties, it is not much recommended by the referring physicians regarding radiation safety concerns mainly for the foetus or infant (Wang et al.2012). Despite its safety recommendation, it's only advised if an underlying malignancy is suspected or has been proven for core needle biopsy with doses less than 3mGy and estimated dose to uterus less than 0.3 µGy with all possible risks and benefits and radiation precautions clearly explained to the patient (ACR et al.2008). The radiologist will go through series of protocols to look for the abnormal findings as pre contrast T1 weighted sequence for any tissue abnormalities, T2 weighted sequence for lesion and breast tissue surroundings. Post gadolinium sequences to highlight the lesions, dynamic contrast enhanced MRI multi sequences at multiple timings as 1, 3 & 5 minutes for capturing the different enhancement patterns of the lesions (ACR et al.2018). Delayed sequences after 10-15 minutes of the post contrast to record the status washout of the contrast from the lesion along with fat suppression sequences to improve the lesion visibility and reduce artefacts from the lesion (Kuhl et al.2005).

Table 1.1: Current methods of cancer detection with relevant findings is as follows:

S.no	Current stages of cancer detection	Findings
1.	Self-breast examination	“ Milk Rejection technique” , changes in size , colour,
2.	Laboratory samples examination- (Human milk)	Presences of ctDNA in sample as positive indication.
3.	Sonomammography	BIRADS score (2 to 6) Malignant tumors often appear hypoechoic compared to surrounding tissue and color Doppler will reveal the status of blood flow through the particular area under suspecting, where increased vascularity can be associated with malignancy.
4.	MR-mammography	Contrast images mostly will show enhancement in T1 & T2 fatsat sequences and MR spectroscopy will show elevation the peaks Cl, Cho and presence of lipid peaks .

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