Osteopontin Levels in Human Milk

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Breast milk is a unique source of nutrients that is physiologically tailored to meet the changing needs of the infant during the first six or more months of life. In addition to providing optimal energy and nutrition, breast milk optimally manages the transition of the neonate to extrauterine life through a combination of bioactive proteins, lipids, oligosaccharides, and immunomodulatory components.

Keywords: osteopontin ; breast milk ; human milk ; maternal diet ; infant health ; immune system ; milk protein

1. Introduction

Epidemiological studies have shown that breastfed infants are less likely to develop necrotizing enterocolitis, leukaemia, and lymphomas, infectious diseases and allergies, or immune-mediated diseases such as asthma, celiac disease, or diabetes, than infants unable to be breastfed for a variety of reasons [1][2][3][4]. Furthermore, the intestinal microbiota of breastfed infants has been shown to differ from that of non-breastfed babies. Microbial dysbiosis in early life has been suggested to correlate with an increased incidence of immune-modulated diseases such as asthma and atopic disease, obesity, and neurodevelopmental disorders [2][3][4][5]. Infants fed breast milk have also been shown to have advantages with regard to cognitive development [6].

The superiority of human breast milk over animal milks or infant formulas is thought to be due to its higher concentration of bioactive ingredients [7][8][9]. Antibacterial and opioid agonist effects of peptide components of breast milk such as lactoferrin, lactoperoxidase, lysozyme, IgA, α-lactalbumin and casein, as well as immunostimulatory effects, have been described [10][11][12][13]. However, osteopontin (OPN), a potential bioactive component, has received less attention to date, and its biological functions in breast milk have yet to be fully elucidated [14].

OPN is a glycosylated phosphoprotein that can be synthesized in many different tissues and is also found in body fluids such as urine, blood, and milk [15][16][17]. As OPN undergoes various types of post-translational modification, alternative translation and proteolytic separation specific to different tissues, organs, and body fluids, it can acquire a site-specific function. Breast milk, cord blood, and infant plasma contain exceptionally large amounts of OPN, suggesting that it may play an important role in lactogenesis and/or in immune and nervous system development and the programming of functions vital to long-term health of the neonate [10]. However, the mechanisms of direct and/or indirect involvement of OPN in these functions, are not fully understood [14]. In vitro studies have shown that breast milk OPN is partially resistant to proteolysis in the infant intestinal tract, suggesting that OPN is potentially a bioactive component [18].

Donovan et al. [19] observed that in the first trimester of life, gene expression in infant Rhesus monkeys receiving formula feed with adjuvant OPN was similar to that of breastfed infant monkeys. OPN is thought to be associated with cell cycle programming (e.g., cut homeobox gene 1 (CUX1)), intercellular communication, cell mobility, cell survival (e.g., epidermal growth factor receptor (EGFR)), and digestive system regulation (forkhead box (FOX) genes). The ability of dietary OPN to bind to integrin proteins and its well-defined association with CD44 also reinforce the view that OPN affects many related genes and pathways [19].

OPN is highly expressed throughout the lactation period: its levels were recently shown by Goonatilleke et al. to be at their highest in the second week postpartum, gradually decreasing to approximately 50% of this concentration (similar to colostrum levels) by the 24th week of lactation [20]. While the number of macrophages in breast milk is also known to decrease during lactation, it remains unclear whether it is the macrophages or the epithelial cells of breast tissue that produce the majority of breast milk OPN. However, there is evidence that the presence of OPN-producing epithelial cells in breast milk during active lactation influences immune system development in neonates [14].

The question of whether OPN supplementation in infant formula may be beneficial has recently begun to be addressed. To date, there are limited but important data to support this idea. In one controlled, double-blinded study by Lönnerdal et

al. [21], 240 infants were fed for the first 6 months of life with either a whey-based standard formula or the same compound supplemented with 65 mg/L or 130 mg/L bovine OPN, or with human breast milk. At four months, infants fed standard formula had higher serum TNF-α and interleukin-2 concentrations compared with infants fed OPN-enhanced formula or breast milk. In support of this finding, infants fed OPN-supplemented formula had less frequent occurrence of febrile illness during the first 6 months of life than infants fed the standard formula. Neither the incidence of fever nor the levels of inflammatory markers were found to differ significantly between breastfed babies and those receiving OPN-supplemented formula. High TNF-α levels found in infants fed standard formula indicate a proinflammatory response to early formula use [22]. In contrast, babies receiving OPN-supplemented formula showed TNF-α levels similar to those of breastfed infants, further supporting a positive role of OPN in immune system development.

In summary, recent research has demonstrated that OPN has different tissue-specific functions, and that its levels are high in breast milk. Since breast milk OPN is presumed to be associated with immune system development, other possible biological functions of breast milk are increasingly under investigation [14][23]. However, studies that have examined the structure and function of OPN in breast milk are scarce, and few comprehensive studies have determined OPN concentrations in breast milk [10]. To determine whether OPN should be added to infant formulas, however, it is important to determine natural OPN levels in human breast milk samples.

While the composition of breast milk is largely determined by maternal factors including age and duration of lactation, it is additionally influenced by maternal nutrition. This study aimed to determine OPN levels in mature breast milk, to investigate maternal factors which may influence OPN levels, and to identify possible relationships between breast milk OPN levels and neonatal health. The secondary objectives were to investigate possible relationships of breast milk OPN concentrations with maternal dietary patterns and the incidence of infection in neonates.

2. Development and Findings

Although the bioactive components of breast milk have been a subject of discussion for many years, the functions of OPN and its relation to maternal and infant health have yet to be completely elucidated. OPN in breast milk was first described by Senger et al. in 1989 [24] and Sorensen et al. showed the presence of OPN in cow's milk in 1993 [25]. Shortly afterwards, in 2004, Nagatomo et al. detected human breast milk OPN concentrations of 1493.4 mg/L at 3–7 days postpartum and 896.3 mg/L after one month [10], results that would indicate that OPN comprises approximately 10% of human milk protein. In 2009, however, Schack et al. questioned these findings and performed a new study showing an OPN concentration of 138 mg/L in breast milk and reporting that it made up 1–3% of breast milk protein (ca. 100–300 mg/L) [10]. In 2018, Bruun et al. [26] found that the median concentration of OPN contained in 829 milk samples taken from 629 mothers from different countries was 157.0 mg/L. In the present study, the mean concentration of OPN was determined to be 137.1 ± 56.8 mg/L. This value is consistent with previous studies of human milk. and particularly close to the levels reported by Schack et al.

To date, only a few studies have focused on the functions of human breast milk OPN, and to our knowledge, this is the first to investigate the relationship between OPN levels in breast milk and maternal factors. In our study sample, breast milk OPN levels were not associated with maternal age, age at first pregnancy, total number of pregnancies, or the number of living children. On the other hand, OPN levels were found to be significantly higher in the breast milk of mothers who gave birth via the natural cervical vaginal route (160.6 ± 48.8 mg/L) compared with mothers who delivered by Cesarean section (99.9 ± 48.5 mg/L), independent of age. There are no previous data correlating concentrations of breast milk OPN with the birth route of infants. Interestingly, however, Ge et al. [27] found that in vitro oxytocin exposure promoted gene expression of OPN. Since oxytocin is highly expressed during vaginal birth, this might explain the higher concentrations of OPN associated with vaginal birth compared with Cesarian, where oxytocin is lacking. Nissen et al. [28] found that after vaginal delivery, mothers showed a more pulsatile oxytocin re-lease pattern during breastfeeding two days after delivery (as well as a marked increase in prolactin levels) compared with women who delivered by Cesarian. Reports suggest that delivery method may also affect other aspects of breast milk composition. Dizdar et al. [29] determined a higher protein content in the colostrum of mothers who had a cervical vaginal delivery compared with mothers who underwent Cesarean section. Hahn et al. [30] reported a significant increase in fat content from 0–3 months in the breast milk of mothers who had undergone Cesarean section. In contrast, a significant increase in breast milk carbohydrate content was found in mothers who gave birth by the cervical vaginal route. A study by Affolter et al. [30] suggested that breast milk immune factors may be affected by Cesarean delivery. Taken together, these studies indicate that delivery route may influence breast milk composition, albeit to a limited extent. Our results are in line with this, suggesting that breast milk OPN levels are related to the birth route. However, since our samples were collected only at a single timepoint, during the 3rd month of lactation, it is possible that they may represent a coincidental finding. Further studies

are needed with regular sample collection from a broader and more homogeneous study population, starting with samples of colostrum.

Our data also indicated that during pre-pregnancy, pregnancy and lactation, breast milk OPN levels were significantly lower in the milk of mothers who smoked ($p < 0.05$). However, when interpreting these data, it must be kept in mind that the smokers in all three periods were the same women, some of whom refrained from smoking during pregnancy and lactation. Thus, while these data show an association of smoking with breast milk OPN levels, conclusions cannot be drawn specifically for any one time period. Studies have shown that in lactating mothers who smoke, vitamin C levels are decreased [31], the lipid composition of breast milk is altered, and total fat content is reduced [32]. Mitnerowicz et al. [33] found smoking to have no effect on albumin or lactoferrin levels, but to reduce the volume of breast milk produced. In another study by Bachour et al. [34], smoking did not affect concentrations of albumin, IgA, lactoferrin or casein in breast milk. Uniquely, however, Bachour et al. showed that total breast milk protein levels decreased significantly due to smoking [34]. Our study suggests that the relationship between smoking and breast milk OPN levels is worthy of further investigation.

In the present study cohort, no relationship was determined between pre-pregnancy BMI and levels of breast milk OPN. There are no studies in the literature that directly correlate breast milk OPN with pre-pregnancy BMI. However, Saha et al. [35] reported lactoferrin and lysozyme levels to be similar in breast milk samples taken from women with a BMI below and above 20 kg/m^2 prior to pregnancy. In another study, pre-pregnancy BMI did not affect breast milk IgA levels [34]. Thus, the lack of correlation between OPN and pre-pregnancy BMI is not an unexpected finding.

Weight gain during pregnancy and BMI during lactation were both found to be associated with breast milk OPN concentrations: These were significantly lower in the milk of mothers who gained excessive weight during pregnancy compared with mothers whose weight gain was in line with or below recommended levels. On the other hand, breast milk OPN levels of mothers with inadequate weight gain and those with healthy weight gain were found to be similar. Similarly, whereas breast milk OPN levels did not differ between the “normal” vs. “overweight” BMI groups during lactation, obesity was significantly associated with reduced OPN concentrations compared with the other two groups. In the literature, we found no study directly investigating breast milk OPN levels and maternal BMI and weight gain during pregnancy. In a study by Yangz et al. [36] focusing on factors influencing lactoferrin levels in breast milk, no relation between maternal BMI and breast milk lactoferrin concentrations was found. While Nayak et al. [37] found no correlation between maternal BMI and breast milk composition, Kugananthan et al. [38] linked increased maternal fat tissue volume to increased concentrations of both protein and hormones in breast milk. Moreover, Whitaker et al. [39] reported enhanced levels of inflammatory markers in the breast milk of women with excessive weight gain during pregnancy. Existing data are thus inconclusive concerning possible links of maternal BMI and weight gain during pregnancy to macro- or micronutrients in the breast milk. Therefore, our findings linking these factors with reduced breast milk OPN levels might simply reflect the fact that the interrelationship between OPN, obesity and inflammation is complex and worthy of further investigation.

Low or moderate diversity of the maternal diet has not, in general, been found to cause major variations in breast milk composition or volume [40][41][42]. Nevertheless, studies have shown that maternal energy intake affects breast milk volume, that maternal fat intake does not influence the composition of fat in breast milk, and that deficiencies of some vitamins and minerals influence vitamin and mineral levels in breast milk [7][37]. Therefore, major differences in maternal nutrition may affect the composition of breast milk. In this study, we sought to examine possible effects of maternal intake of energy and macro- and micronutrients on breast milk OPN levels for the first time. A few studies have indicated that IgA, IgG and lysozyme levels are significantly decreased in the breast milk of mothers with malnutrition compared with mothers of a healthy nutritional status [43][44]. However, the influence of maternal nutrition on immunological properties of breast milk remains unclear. Our data showed low to moderate associations of breast milk OPN concentrations with maternal intake levels of energy, vegetable/plant proteins, total fat, poly-unsaturated fatty acids, carbohydrate, and fiber. Additional studies are required to clarify these relationships further.

Breast milk OPN has been linked to a healthy pattern of growth and development in infants. In rhesus monkeys, Donovan et al. [19] found the growth pattern and bone miner-al density of baby monkeys fed OPN-supplemented formula to be similar to those of exclusively breastfed monkeys. Later, Lönnerdal et al. [21] reported similar findings in a human cohort of 240 neonates. To our knowledge, ours is the first study since to focus on growth patterns of neonates in relation to breast milk OPN levels, albeit without a control group of infants receiving formula feed. Our results are consistent with the existing literature. Generally, infants of mothers with higher breast milk OPN levels had a higher mean BW and length at both one and three months. We found no significant correlation between breast milk OPN levels and the BW and length measurements of infants at birth. However, significant positive correlations between breast milk OPN levels and BW and length were found after one and three months, suggesting that OPN levels in milk may be related to the early growth

pattern of neonates. Recent studies also concluded that OPN in human milk could play an important role in brain development and behavior in infancy [45][46], possibly by promoting myelination [46].

The most important function of breast milk OPN in infant health is thought to be its role in regulating immune system development [14]. OPN-producing epithelial cells and macrophages have been found in the actively lactating mammary gland, implicating that high expression of OPN in human milk cells may play an essential role in the immuno-logical development of breastfed infants [23][47]. Both circulating T cells and levels of pro-inflammatory cytokines were found to be reduced in infants fed OPN-supplemented formula compared with infants fed standard formula feed [21][48]. Thus, OPN appears to affect both innate and adaptive immunity. Donovan et al. [19]. reported that pathways of cell proliferation and cell–cell adhesion were upregulated in infants who were breastfed or fed OPN-supplemented formula in comparison to those fed standard formula, thus promoting the production and maturation of immune cells. Plasma OPN is known to play an important role in the immune system response to microbial infections [49]. Mice with OPN deficiency were found to be more susceptible to pathogenic microorganisms such as *Listeria monocytegenes*, *Plasmodium chabuad*, and *Mycobacterium bous* [50][51][52], while in another OPN-deficient murine model, the spontaneous development of colitis was reported [53]. Lönnerdal et al. observed that infants fed OPN-enriched formula had a lower incidence of fever compared with infants fed standard formula [21]. In line with this, we found a significantly reduced incidence of hospital admission due to febrile illness during the first three months of life in the infants of mothers with higher breast milk OPN levels. These data are consistent with the literature and not surprising, given the known immune system-related functions of OPN.

3. Conclusions

In conclusion, we found that OPN concentrations in breast milk seem to be affected by maternal factors including dietary intake (especially energy and fiber intake), BMI and smoking. Our data also indicated an influence of the birth route, with higher breast milk OPN levels found in mothers who delivered via cervical vaginal birth compared with Cesarean section. In addition, we found correlations between OPN levels and measures of infant growth in the first three months of life. Overall, our results are in line with previous findings suggesting that OPN might have important effects on infant growth and health. Our data showing reduced postnatal fever-related hospital admissions in babies fed breast milk with higher levels of OPN substantiate a possible role of OPN-associated mechanisms in immune system development in the neonate. In order to gain more clarity on functions of OPN as a bioactive ingredient of human breast milk, additional studies are needed in a broader and more homogeneous study population with sample collection on a regular basis and over a longer time period. Depending on future findings, the concept of supplementing infant formulas with OPN should be further pursued. In the light of our findings, we believe this may indeed prove a viable option and an important contribution to the health and immune system development of infants who, for whatever reason, cannot receive breast milk.

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