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Breast Milk: A Free Radical Scavenger and Role of Mother's Diet

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Abstract

Breast milk is a vital source of antioxidants that play a crucial role in protecting infants against oxidative stress and supporting optimal growth and development. This review highlights the presence and significance of key antioxidant enzymes in breast milk, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and lactoferrin (LF), which neutralise free radicals through enzymatic and non-enzymatic mechanisms. Malondialdehyde (MDA), a marker of lipid peroxidation, is shown to be the lowest in colostrum due to higher concentrations of these antioxidants, underscoring the importance of early breastfeeding. The antioxidant profile of breast milk is influenced by maternal diet, environmental factors, and lactation stage. Trace elements like zinc, copper, and iron, which serve as cofactors for antioxidant enzymes, also regulate oxidative balance through their interaction with binding proteins such as ceruloplasmin and ferritin. Additionally, maternal intake of polyphenols, carotenoids (lutein, zeaxanthin), and essential fatty acids affects breast milk composition, with evidence showing enhanced antioxidant capacity in women consuming plant-rich or fish-based diets. However, certain nutrients like taurine and vitamin B12 may be lower in vegan or vegetarian mothers, potentially influencing infant neurodevelopment. Overall, breast milk offers superior antioxidant protection compared to infant formulas and reflects maternal nutritional and lifestyle factors, emphasising the need for targeted dietary support during lactation.

Keywords: *B12; CAT; GPx; LF; MDA; SOD*

Introduction

Breast milk is very important for a newborn because it is easy to digest, readily available, and a low-cost natural food. It not only gives immunity, growth and development but also has other roles in the psychosocial growth of the mother and baby.

Besides giving high nutrition and immunity, it has its antioxidative properties. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the breast milk destroy free radicals created by oxidative stress (Matos, Ribeiro & Guerra, 2015).

Cellular metabolism creates free radicals, oxidants and oxygen radicals, which cause harmful effects inside living organisms. Breast milk is more powerful at reducing free radical damage and structural changes of DNA in newborns than infant formulas. The enzymatic antioxidants like Superoxide dismutase (SOD) and Catalase (CAT) prevent the free radical formation of H_2O_2 and other peroxides (Yuksel *et al.,* 2015).

Compared with formula milk, breast milk has an increased number of PUFAs (c20-c22) [long-chain polyunsaturated fatty acids], which are highly susceptible to lipid peroxidation and associated with increased oxidative injury. Antioxidants in human breast milk may protect long-chain polyunsaturated fattyacids in human milk against oxidation (Hanson *et al*., 2016).

Overall growth and development of an infant is dependent on breast milk composition, which contains nutritive elements, antioxidants and micronutrients. Breast milk contains antioxidants, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase and glutathione S-transferase. The relation between GPx, SOD and GST and the number of pregnancies was found. GST activity and BMI show an inverse relationship. Cu and catalase activity show a negative correlation with GPx and GST activity (Li *et al*., 2009).

Breast milk is healthy and nutritious food for premature babies. When a mother is not capable (in a hospitalised condition), she cannot provide breast milk to her child, then donor milk is an ideal alternative to mother's milk. Premature babies do not have 1) an ample number of antioxidants at the time of birth and 2) the ability to synthesise antioxidants, so they become susceptible to different diseases like chronic lung disease, disease of the retina (Retinopathy), necrotising bowel disease and periventricularleukimalacia (PVL). So, it is crucial to make certain that premature babies get an ample amount of antioxidants through diet (Hanson *et al*., 2016).

A hypothesis was made that preterm mothers' milk would have much better antioxidative protection than milk from the full-term mothers. In this regard, three experiments were designed to assess the power to resist the oxidative stress of breast milk and powdered formulas for both full-term and preterm infants and differences in resisting oxidative stress between milk from full-term and preterm infants. The experiments also examined factors that are responsible for increased resistance to oxidative stress in the mother's breast milk (Castillo-Castaneda *et al*., 1980).

In this review, research will be made about the ability of breast milk to eliminate the free radicals from the bodies of the infants and the role of the mother's diet in enhancing different useful components in breast milk.

Different Antioxidants in Human Milk Which Act as Free Radical Scavenger

Vit-A: liposoluble compound, acts against ROS. Carotenoids, which are structurally similar to vitamin A, show antioxidative activity by quenching reactive oxygen, neutralising thiol radicals, and stabilising peroxyl radicals to protect cells from peroxidative damage. The activity of enzymes involved in lipid peroxidation is suppressed by vitamin A and prevents oxidative disorders in cell membranes. Vitamin A is not transmitted through the placenta, so newborn babies are dependent on breast milk consumption (Matos, Ribeiro & Guerra, 2015).

Vit-E: Plasma membranes, fatty deposits and circulating lipoproteins contain vitamin E, which reacts with reactive oxygen species and molecular oxygen to protect PUFAs and lipoproteins from peroxidation. Vitamin E is not transferred through the placenta, so breast milk is the main source of nutrition for infants.

Vit-C: Reacts with free radicals and has a low reduction potential. It plays a vital role in breast milk antioxidative properties and is associated with a reduced risk of atopy in high-risk infants.

Different Proteins and Enzymes in Breast Milk

B casein and the primary whey protein (α-lactalbumin), iron-binding glycoprotein (lactoferrin), IgA, serum albumin protein and protein hormones are moved to breast milk from plasma. These proteins and peptides show free radical scavenging activity and inhibit lipid peroxidation.

a) Lactoferrin: It is an anti-inflammatory compound as it takes up iron from the inflammatory compound. Its main mode of action is antimicrobial.

b) Glutathione peroxidase: Contains a selenium component in the form of selenocysteine. It provides protection against oxidative damage and protects lipids from oxidative stress.

c) Enzymatic antioxidants: Mn and copper/zinc superoxide dismutase (SOD) have been recognised in breast milk, which converts a superoxide anion to hydrogen peroxide.

d) Ceruloplasmin: Copper-containing multifunctional protein inhibits the formation of hydroxyl radicals through a chemical process (Fenton reaction) and lipid peroxidation. It prevents damage to DNA, proteins, and lipids.

e) Coenzyme Q10 or ubiquinone: Liposoluble vitamin, potent antioxidant and free radical scavenger.

f) Melatonin: A derivative of serotonin secreted by the pineal gland, a highly effective hormone (antioxidant) and free radical scavenger (Matos, Ribeiro & Guerra, 2015).

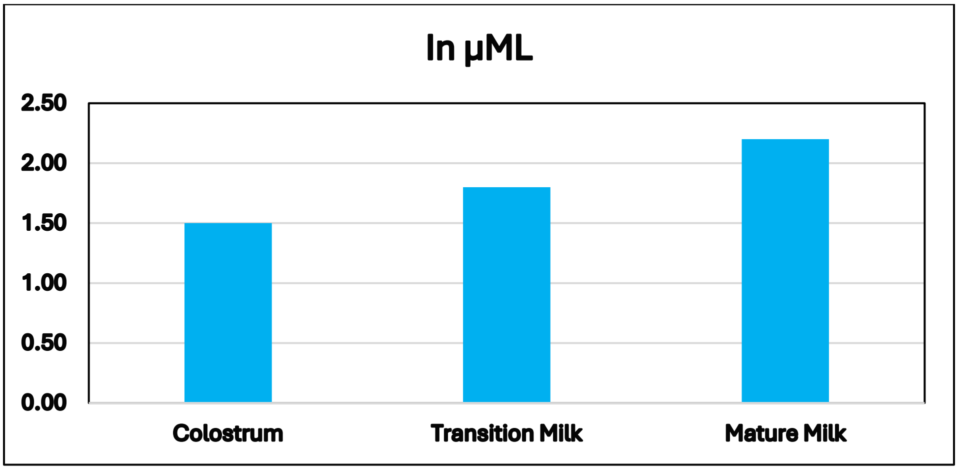

Figure 1: Malondialdehyde, (secondary products of lipid peroxidation). (MDA) levels in Colostrum, Transition Milk and Mature Milk [In µML]

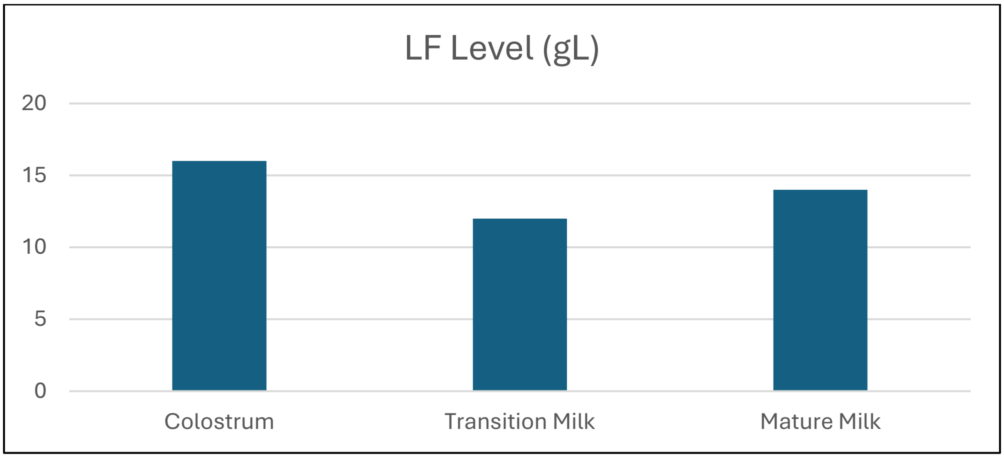

Figure 2: Lactoferrin (LF) levels in Colostrum, Transition milk, and Mature Milk [LF Level in gL]

Phenolic compounds play a major role in anti-tumour activity. Phenolic compounds in breast milk reduce inflammation by inhibiting the transcription factor nuclear factor-B. When mothers consume a diet rich in phenolic compounds, their breast milk protects the newborn babies from diseases (Hanson *et al*., 2016).

Infant health is dependent on nutritional status, antioxidant level and quality of mother's milk. Breast milk contains enzymatic antioxidants like superoxide dismutase (SOD), several vitamins like tocopherols, ascorbic acids, soy-based phytoestrogens and coenzyme-Q10, selenium, thirodoxin, etc. (Hanson *et al*., 2016).

Relationship between Different Antioxidant Enzymes and Different Micronutrients Like Fe, Cu, and Zn in Breast Milk

1) Role of Fe in BM: Iron-containing glycoprotein (Lactoferrin) present in human milk helps in the cognitive development of babies.

2) Role of Cu in BM: Copper is essential in the formation of bone, myelin, collagen, neuropeptides, and haemoglobin and contributes to the electron transport chain.

3) Role of Zn in BM: Insufficient consumption of Zn by the infants may cause nervous system and growth defects.

In this study they have shown there is a correlation between antioxidant enzyme activity and number of pregnancies. Mothers who have more than 3 pregnancies show the highest Glutathione peroxidase activity. They also showed a correlation between enzymatic antioxidant activities and concentrations of copper, zinc, and iron by BMI value. Women who have a body mass index (BMI) < 25 show the highest GST activity. (Li *et al*., 2009). The Glutathione peroxide (GPx) activity (0.08umg-1) was found in women with 3 or more pregnancies. Women whose body mass index is >25 and < 30 show significant differences. Zn, Cu and Fe concentrations did not differ with respect to the number of pregnancies and body mass index value (Li *et al*., 2009).

Preterm babies are prone to oxidative stress as they lack adequate antioxidants at the time of birth, which leads to diseases like chronic lung disease (BPD), disease of the retina (ROP), necrotising bowel disease (NEC) and periventricular leukomalacia (PVL). So, dietary antioxidants are very essential in preterm babies. Samples of matured breast milk, donor milk and formula milk were analysed at the Biomarker Research Institute at the Harvard School of Public Health. samples were quantitated by high-performance liquid chromatography (HPLC). The result showed that breast milk contains higher levels of natural carotenoid pigments like β-cryptoxanthine (a precursor of vitamin A), lycopenes (effective antioxidants), Lutein + Zeaxanthene (natural carotenoid pigments), vitamin A, and different isoforms of vitamin E than donor milk. Premature babies who are formula-fed have lower levels of carotenoid pigments in the bloodstream than breastfed premature babies (Hanson *et al*., 2016).

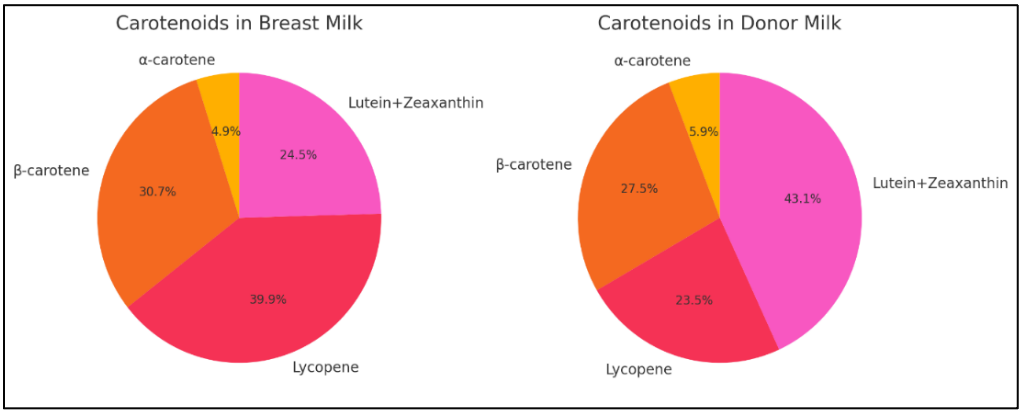

Figure 3: Percentage of α -carotene, β- carotene, Lycopene and Lutein + Zeaxanthin Breast Milk v/s Donor Milk Samples

Lycopene percentage is higher in mother's breast milk than donor milk. Research shows that Lutein + Zeaxanthin plays a major role in the vision, learning, thinking and understanding ability of the babies. Lutein +zeaxanthin concentration in different brain regions of preterm babies was remarkably lower compared to matured babies. infant in most of their brain regions. Because of the increased proportion of bioavailability of lutein concentration in breast milk, breastfed infants have more lutein concentration than formula-fed infants. Infant formula needs four times more Lutein than human milk to achieve similar serum lutein among breastfed and formula-fed infants (Hanson *et al*., 2016).

Breast milk colostrum has the ability to reduce cytochrome - c spontaneously, depletes hydrogen peroxide produced by polymorphonuclear leukocytes, and protects the epithelial cells from polymorphonuclear leukocyte-mediated detachment.

Cow's milk is modified to formula milk, which is very similar to human milk. These powdered formula milks have increased radical trapping antioxidants in contrast with human milk. According to Goldman *et al*. antioxidants are absent or present in a very poor amount in cow's milk or other feedings. The premature babies fed with human milk had greater free radical trapping ability in vitro than formula-fed babies (Castillo-Castaneda *et al*., 1980).

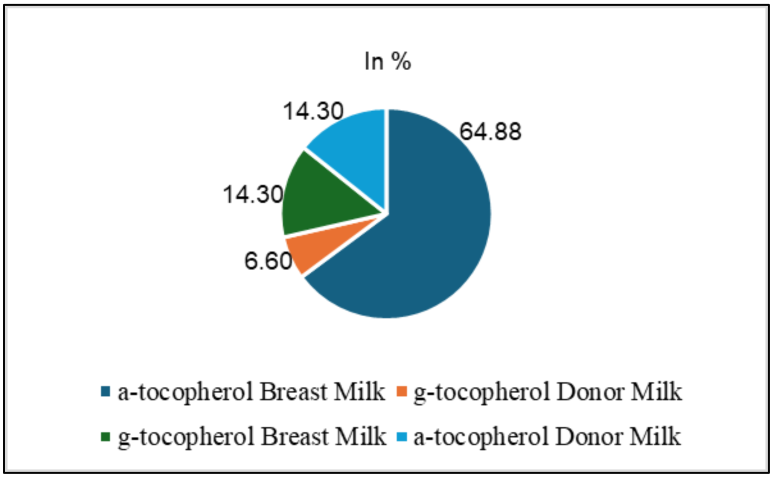

Figure 4: The Concentrations of α-tocopherol and γ-tocopherol in Maternal Breast Milk vs. Donor Milk Samples

Early feeding of human milk by premature babies reduces the occurrence of inflammatory bowel disease and disease of the retina (Retinopathy of prematurity). Due to some properties of human milk, which reduces the incidence of necrotising enterocolitis. Their experiments indicate that oxidative stress has very little impact on human milk compared to very effective formula milk. The report says the presence of GPx and SOD, CAT in human milk, plays a role in providing antioxidants in vitro. When formula milk is added with different enzymatic antioxidants like catalase, Superoxide dismutase, and Glutathione peroxidase it shows greater protection against oxidative stress and lipid damage. This indicates that formulas when added with these enzymatic antioxidants may help in reducing the damaging effects of excessive oxidative stress and inflammation caused by ROS (reactive oxygen species), which results in decreased symptoms, speedy recovery and normal development (Castillo-Castaneda *et al*., 1980).

Breast milk of lactating women contains different polyphenols and their metabolic products like flavonoids, phenolic acids, ellagic acid and urolithin, which are plant-based natural compounds transferred to breast milk through the mother's blood circulation. The metabolic products of polyphenols in mother's milk undergo a new cycle of digestion when they are consumed by the newborn babies (Carregosa *et al*., 2023).

Polyphenols get broken down into small molecular weight phenolics by gut microbiota by undergoing Phase-I and Phase-II metabolic reactions. All these compounds enter the liver and are transferred to circulation, where they may reach the mother's milk. Several studies indicate that breast milk contains phytoestrogens like daidzein and genistein when mothers consume diet isoflavone-rich foods like soya milk and roasted soybeans (Carregosa *et al*., 2023).

Mother's diet has an impact on breast milk composition. When grapefruit juice is consumed by mothers, her breast milk shows flavanones. After consumption of red raspberry, breast milk shows different flavonoids, microbial metabolites and phenolics. According to Lu *et al*., when mothers consume plant-based foods and polyphenols, it remarkably affects the phenolic composition of her breast milk.

Quercetin was the flavonol detected in the highest concentration in human breast milk, ranging from 0.05 to 0.15 µM. Zhang *et al*. (2018) showed that quercetin was more plentiful in breast milk and urine than in plasma of mothers.

Different studies have suggested polyphenols have a protective role against inflammation, oxidative damage and cardiometabolic stress. Vegetable consumption and antioxidative properties of polyphenols in breast milk are positively correlated. (Carregosa *et al*., 2023).

The concentration of Arachidonic acid and docosahexanoic acid was studied by Kelishadi *et al*. (2012). They found that DHA content was low in comparison to other studies. DHA is important in infant brain development. According to WHO recommendation AA/DHA ratio should be 2:1 in colostrum, as DHA is important in brain development. In a well-developed country, this ratio varies from 5:1 to 15:1. In their study, the AA/DHA ratio was 2.6:1 higher than the WHO recommendation. It was shown that in the regions where fish consumption is high, the DHA content of breast milk is higher.

The antioxidant status of infants is closely influenced by the mother's antioxidant levels during pregnancy and breastfeeding. Human milk contains a variety of antioxidant substances—such as vitamins, enzymes, and trace elements – that work together to protect the infant from oxidative stress. The total antioxidant capacity (TAC) of breast milk is affected by the mother's diet, with healthy dietary patterns like the Mediterranean diet enhancing the presence of beneficial compounds like polyphenols. Since the body cannot synthesise certain antioxidants, they must be obtained from foods such as fruits, vegetables, and whole grains. Studies show that breast milk offers superior antioxidant protection compared to infant formulas, underlining the importance of maternal nutrition for infant health (Karbasi *et al*., 2022).

Karcz and Królak-Olejnik (2021) have shown in their review vegan, vegetarian and non-vegetarian mothers' breast milk is comparable in their nutritional value. Some differences are found in fatty acids and some microcomponents, primarily Vitamin B12. Their study suggests that vegetarian and vegan mothers produce milk of the same nutritional value, except some nutritional supplement is added to the mother's diet. An appropriately planned plant-based diet can contribute to the sustainable growth of infants. As vitamin B12 helps in the proper maintenance of the nervous system, lactovegetarian mothers are also advised to take B12 supplements regularly.

Discussion

Antioxidants like catalase, superoxide dismutase, Glutathione peroxidase and Lactoferrin present in the breast milk can eliminate reactive oxygen species by enzymatic and nonenzymatic reactions. MDA, which is a metabolite of lipid peroxides, acts as a measure for LPO. Research indicates that mothers' milk contains crucial protective mechanisms, which are absent in powdered formula or bovine milk. The current study shows that colostrum contains decreased levels of MDA compared to transition milk and mature milk because enzymatic antioxidants' (CAT, SOD, NO, and LF) levels were highest inside colostrum. The high oxidative stress requires high antioxidant levels from the 1st day of lactation (Yuksel *et al.,* 2015).

The major whey proteins lactalbumin and lactoglobulin, the iron-binding protein lactoferrin, serum albumin and immunoglobulins present in milk show antioxidant activity by ferrous ion-chelating abilities as well as by inhibitory effects on LPO.

High molecular weight milk catalase enzyme induces the decomposition of H_2O_2. The current investigation shows that catalase activity markedly decreased as the lactation period began. Superoxide radicals are changed into H_2O and O_2 by superoxide dismutase, having an antitoxic effect against superoxide anion. (Yuksel *et al.,* 2015).

It has been reported that breast-fed infants have significantly lower oxidative stress than formula-fed infants. Granot *et al*. (1999) suggest that breastfed babies showed more oxidative injury compared with formula-fed babies having similar antioxidant capacity because of differences in fatty acids between mother's milk and formula milk.

Long-chain polyunsaturated fatty acids (PUFA) in breast milk may get protection against oxidative injury by the antioxidants present in it. Antioxidant composition varies among different racial groups of the same region. It has been found that Nigerian women's breast milk has a higher antioxidant content than the women of Nepal. Antioxidant levels decrease in smoker mothers and their passive smoker babies when compared with those of nonsmokers. During the freezing process and refrigeration, antioxidant activity notably decreased (Hanson *et al*., 2016).

This has been found in this review that for the first six months of life, breast milk provides the infants with the best nourishment, which reduces the risk of morbidity in ininfant, and provides growth, development and immunity. Proteins, fatty acids, vitamins, antioxidants, and microelements such as zinc, copper, and iron contribute to the proper growth and development of the child. Metabolism, foreign chemicals (Xenobiotics) and the smoking of the mother produce free radicals and create oxidative damage in breast milk.

Free radicals (ROS) cause oxidative damage to DNA, proteins, Lipids and other substrates and also cause structural and functional damages to the nutrients in milk. Breast milk contains enzymatic and non-enzymatic antioxidants that provide infants' antioxidant defences and may protect nutrients in milk from oxidative damage.

The microelements zinc, iron, and vitamin C have an impact on copper absorption. In the current study shows that zinc concentration has a positive correlation with Cu content in breast milk. However, the Cu:Zn ratio can measure the oxidative stress as well as the immunological and nutritional condition.

Copper takes part in the Fenton reaction instead of iron. In this reaction Cu^{2+} changes to Cu^{+} and reduces H_2O_2 to. OH, a more reactive free radical. Breast milk contains ceruloplasmin, which transports copper. Copper, when bound with these proteins, which decreases its accessibility to take part in the Fenton reaction, consequently, stops oxidative damage (Li *et al*., 2009).

Level of free iron is decreased as it remains in a soluble, non-toxic form as ferritin, preventing oxidative reactions. The activity of enzymatic antioxidants like superoxide dismutase and Catalase is influenced by the concentration of Cu, Zn and Fe, which are cofactors of these enzymes. These microelements also influence (GPx, GST), by reducing their substrate (H_2O_2) availability or by intervening with their functional groups (Li *et al*., 2009).

Research shows that Lutein + Zeaxanthin plays a major role in optical, learning, thinking and memory development. Lutein and zeaxanthin concentration was remarkably lower in premature babies in major brain areas compared to full-term babies. Because of the higher amount of lutein concentration in breast milk, breast infants have more lutein concentration than formula-fed infants (Hanson *et al*., 2016).

The mother's diet plays an important role in enhancing the quality of breast milk. Metabolic products of polyphenols, mainly flavonoids, phenolic acids and ellagic acid, are found in the breast milk of lactating mothers. Studies indicate that isoflavone is present in breast milk when mothers take isoflavone-rich food like soyamilk and roasted soybeans. Flavonone is also detected in breast milk after grapefruit juice consumption (Castillo-Castaneda *et al*., 1980).

Multiple studies indicate that polyphenols protect babies from inflammation and cardiometabolic stress. Antioxidative properties of breast milk are associated with a higher vegetable consumption by mothers (Castillo-Castaneda *et al*., 1980).

During pregnancy DHA plays a very important role in infant brain development. It requires the mother's dietary intervention. DHA contents of breast milk become higher where fish consumption is high. Women having reproductive potential are recommended to consume food rich in fatty acids (Kelishadi *et al*., 2012).

Baby’s antioxidant level depends on the mother’s antioxidant level throughout the entire gestation period. Infant synthesizes antioxidants through its endogenous component at the expense of exogenous component transmitted to him or her through breast milk. Vitamins A, E and C and different enzymes like (GPx,SOD) different chemicals like copper, Zinc, and Selenium act as antioxidants and neutralise free radicals. Among the most essential antioxidants are phenolic compounds and flavonoids, which are derived from plants and are powerful natural dietary antioxidants (Karbasi *et al*., 2022).

The ratio of LA/ALA (Lenoleic Acid and Alpha Lenoleic Acid) was higher, whereas omega -6 and omega-3 ratios are higher in the case of vegan mothers. The average percentage of alpha-linolenic acid in milk samples was 2.09% for vegan mothers, 1.55 % for vegetarian mothers and 1.19 % for omnivore mothers.

Bijur *et al*. and Debski *et al*. found no significant difference in particular dietary groups in interms of protein concentrations in human milk. According to them, the mean values in the case of lactovegetarians were 1.122 gm/dl, 1.221 gm/dl for occasional meat eaters and 1.216 for frequent meat eaters. From the above report, it indicates that a vegetarian or nonvegetarian diet does not have an impact on milk protein concentrations (Karcz & Królak-Olejnik, 2021).

Rana *et al*. in their experiment compared the taurine concentration in two different groups – one group of omnivores and another of vegans – and found that vegan milk samples contained significantly lower taurine concentration compared with omnivore as vegan mothers consumed less protein containing preformed taurine. In the human body, taurine acts as a neurotransmitter in the brain. Adults can synthesise taurine from cysteine and hypotaurine. It is present in high amounts in meat and fish (Karcz & Królak-Olejnik, 2021).

Patel and Lovelady (1998) reported that cobalamin, or Vitamin B12 is also low in vegetarian mothers. Women of India showed lower B12 concentrations than omnivore control groups. Specker *et al.* (1990) said that a vegetarian diet has an inverse correlation with breast milk B12 levels (P1/40.03). Finley *et al*. analysed that in the incase of minerals like Cu, Zn, Ca, Mg, K, and Na concentrations, there was no notable contrast in breast milk of vegetarian and nonvegetarian mothers.

Plant based vegan diet contains huge amounts of linoleic acid, omega-6 polyunsaturated fatty acids and monounsaturated oils. Vegetarian and vegan mothers' diets contain a good amount of omega-6 fatty acids, but lower omega-3 fatty acids compared to nonvegetarians (omnivores) (Karcz & Królak-Olejnik, 2021).

Conclusion

From the above discussion, it is clear that breast milk is the best source of antioxidants in newborn babies, as it contains a lot of antioxidants for reducing the free radicals. There are several microelements which are important. Breastmilk contains an essential defence mechanism to protect the newborn from free radicals, which is not present in formula or bovine milk. A mother's diet plays an important role in breastmilk composition. Vegan and vegetarian mothers' breast milk contains all the components, but omega-3 fatty acids, taurine (an essential amino acid), and vitamin B12 are in lower concentrations than in non-vegetarian or omnivorous mothers. Therefore, during the gestation period, vegan and vegetarian mothers need to regularly take a vitamin B12 (cobalamin) supplement to ensure the quality of their breast milk.

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