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The Influence of Breastfeeding and the Infant's Social Environment on Neuroplasticity and Brain Development: The First 1000 Days

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Abstract

There is substantial evidence that breastfeeding and an enriched environment provide significant contributions to the infant's brain development. In the past 2 decades, there have been overwhelming data on the benefits of breastfeeding for 1 year and longer and its association with higher intelligence in later life. There is clear and convincing evidence from a number of disciplines, neuroscience, genetics, animal experiments and magnetic imaging techniques that indicate breastfeeding results in optimal brain development and higher IQ in later life. Magnetic imaging studies of infants, children and adolescents have provided significant evidence that the higher IQ in later life in breastfed infants is associated with larger brain size and higher degree of myelination of the white matter. Furthermore, observational studies of infants have provided clear evidence that breastfeeding and mother-baby sensory interaction result in significant cognitive and behavioral development of breastfed as compared to formula fed infants. Large-scale longitudinal studies of infants' development have shown clear and convincing evidence of higher intelligence in children who were breastfed during infancy, and that the higher IQ persists through adulthood. In this communication, we provide evidence that breastfeeding and an enriched environment result in accelerated developmental potentials in the first 1000 days last a life time. The first 1000 days last the rest of our lives.

Keywords: breastfeeding, oxytocin, neuroplasticity, myelination, brain development, enriched environment

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1. Introduction

The newborn's brain grows at an accelerated rate during the first 1000 days and this is associated with myelination of the white matter. Furthermore the brain growth and myelination correlates with the stages of brain development, including motor, speech, vision, acquisition of social skills, attachment, learning math and logic. All aspects of infant's growth and development are contingent on his genetic code "nature". However gene expression is influenced by epigenetic factors "nurture". The positive environmental factors result in neurobiological changes and improved neuroplasticity. The neurobiological effects of environmental enrichment was first reported by landmark work of Volkmar and Greenough which clearly demonstrated the superior problem solving abilities of rats raised as pets compared with rats raised without such human interactions [1]. Anatomically the environmental enrichment resulted in greater cerebral volumes, larger number of synapses and increased complexity of dendritic branches [1]. Extensive animal research demonstrate that maternal grooming and nurturing can lead to changes in DNA methylation, resulting in gene suppression or histone acetylation, up-regulation of the gene expression, which increase grooming in the offspring when it matures and the cycle repeats itself [2].

2. The role of oxytocin on brain development

Neuropeptide hormones and their receptors have properties that identify them as candidates for the induction of early life experiences into both short and long-term behavioral changes [3].

Animal research has provided a vast knowledge on the significance of the role of mother-baby interaction in child development. The elegant experiments on prairie voles, small monogamous mammals, point to the significant role of oxytocin on our behavior. Injection of oxytocin receptor blockers resulted in changes in pair-bonding behavior in these mammals [3]. Additionally oxytocin is implicated in all aspects of romantic and maternal love, including courtship, mating, pregnancy, childbirth and breastfeeding [3].

Anthropological studies demonstrate significant differences between the breast milk of large primates such as humans, monkeys and apes, referred to as "carrying mammals" vs. "nested mammals". Carrying mammals' breast milk contains lower amount of fat and sugar, while the nested mammals' breast milk contains much higher sugar and fat. Therefore carrying mammals require to breastfeed their infants more frequently than nested mammals.

The human infant's central nervous system depends on a micro-environment that is similar to the maternal uterine environment which is full of sensory exchanges involving heat, sound, movements, transportation, feelings, touch, smell and access to nutrients in the mother's breast milk. For species such as primates, the mother is the environment (Sarah Blaffer Hrdy).

3. The first 1000 days last a life time

The first 1000 days is the most significant period of an infant's life. During the first 3 years there is great potential for the infant and toddler to acquire immense cognitive, visual, speech, motor and emotional development.

Newborn infant's brain is quite immature, however it has great potentials to grow and most of the growth occurs in the first 1000 days of his life. Normal brain function requires rapid messaging which is mediated by the myelinated nerve fibers. However myelination depends on a number of factors including the genetic makeup of the infant "nature" and the infant's social environment "nurture". The trajectory of infant's brain growth and myelination is more robust in breastfed than in formula-fed infants. Therefore developmental milestone, speech, social skills, emotional and motor development are achieved at an earlier age in breastfed than in formula-fed infants. Furthermore we postulate that the infants who were fed breast-milk via a bottle, without sensory interactions with the mother may not reach their fullest potentials. Therefore we make a distinction between breastfeeding as the infant being on the mother's breast and not receiving breast-milk via a bottle, a cup or any other gadgets. Many maternal hormones including estrogens are present in the mother's milk and estrogens are transcriptional promoters for oxytocin and its receptor's genes [4, 5]. Oxytocin is released from supra-optic (SON) and peri-ventricular nuclei (PVN), only upon infant's sensory stimulations, auditory, tactile, visual and vestibular. The rise of oxytocin level in the infant's brain is associated with improved neuroplasticity. The magnitude of environmental influence on the infant's brain development may be ascertained by comparing the white matter growth using magnetic imaging of breastfed infants and infants given breast-milk via a bottle. Alternatively salivary cortisol level measurements may be an index of blood oxytocin because of their inverse relationship [3].

4. Breastfeeding in the twenty-first century

Mother's breast milk is an extension of her placenta which provides nutrients, hormones and epigenetic factors that carry messages to the infant to regulate his metabolic pathways.

World Health Organization (WHO) recommends breastfeeding for 2 years. Extended breastfeeding up to 3 and 4 years of age is highly recommended for additional calories, proteins, lipids, vitamins and factors that regulate the child's metabolic processes. The myelination of the nerve fibers continues, although at a slower pace until early adolescence.

The global breastfeeding rate in the past 10 years has improved only marginally from 33% in 1995 to 37% in 2014 [4]. Suboptimal breastfeeding results in higher health care expenditure for pediatrics and maternal care, and global productivity-related economic losses of \$302 billion or 0.49% of world gross income annually [4].

Maternity and pediatric care providers in many parts of the world do not follow the recommendations of WHO on breastfeeding for 2 years or longer. The policies of several governments do not support breastfeeding including the Chinese government which subsidizes domestically manufactured formulas and US government that provides free formulas to low income families on WIC, Women, Infants and Children's program. WIC program was originally only intended for low income mothers who were breastfeeding their infants, however the program was extended to formula feeding mothers 2 years later [5].

International efforts to promote and protect breastfeeding on a global scale began in 1981 by WHO and World Alliance of Breastfeeding, WABA, in Geneva, Switzerland. The conference resulted in a significant document, International Code of Marketing of Breast Milk Substitutes. This was followed in 1990 by Innocenti Declaration and the Baby Friendly Hospital Initiative

which promotes breastfeeding and rooming in for every newborn resulting in an increase in breastfeeding initiations. Many college educated mothers are cognizant of numerous benefits of breastfeeding and choose to breastfeed their infants.

Majority of nations have ratified the International Code of Marketing of Breast Milk Substitutes, however there are serious violations of marketing by formula industry and court challenges to overturn the marketing code. It is not unusual to hear that some unscrupulous providers in 3rd world countries will give samples of formula to the mothers upon delivery to feed their newborn and deprive the newborn from the benefits of colostrum feeding and later breastfeeding.

There are many obstacles to exclusive breastfeeding of a newborn in US hospitals, some of which are due to flawed hospital policies for newborn jaundice and neonatal hypoglycemia. Some physicians wrongly believe that exclusive breastfeeding will place the newborn at risk of developing hypoglycemia as well as severe jaundice which may lead to kernicterus. Other obstacles to exclusive breastfeeding include the use of pacifiers, nipple shields and providing the mothers with a breast pump to give the breast milk via a bottle to feed the newborn infants.

The high rate of cesarean section in some countries is an obstacle to breastfeeding, because of reduced oxytocin and delayed lactation [6]. Additionally epidural block may result in lower oxytocin release due to the inhibitory effect on Ferguson reflex [6]. Pitocin infusion may result in reduced release of oxytocin via a feed-back inhibitory effect [6]. Therefore it is clear that natural child-birth without epidural block and pain medications result in better outcomes immediately after birth with the newborn latching on and suckling at the mother' breast. Infants born vaginally have higher oxytocin level than infants born by cesarean section, 69 pg/ml vs. 33 pg/ml [6].

Newborns placed on the mother's chest latch on to the mother's breast, attracted to the Montgomery glands around the nipples, which secrete a lubricating liquid. Newborns latch on to both nipples, however when one nipple is washed with soap and water the newborn would latch on only to the unwashed nipple. When the mother's amniotic fluid was applied to the washed nipple, the newborn latched to that nipple. It is generally believed that the liquid discharge from the Montgomery glands have the scent of the mother's amniotic fluid [7]. We have also encountered the statement from the breastfeeding mothers that for the first 2–3 weeks their infants refuse to breastfeed immediately after a shower.

There is strong and convincing evidence that attachment and bonding between the mother and infant is associated with the release of oxytocin from hypothalamic nuclei, peri-ventricular and supra-optic and its binding to peri-aqueductal gray (PAG), insula, inferior and posterior temporal nuclei [8].

Oxytocin and many of the maternal hormones are present in the mother's breast milk. However the concentration of breast milk oxytocin is very low and decreases quickly. Oxytocin is probably digested in the infant's stomach and is not absorbed from the intestinal tract into the circulation. However oxytocin activates cholecystokinin which then activates sensory vagal nerves which release oxytocin from the infant's hypothalamic nuclei [6]. Sucking in the newborn is associated with infant's oxytocin release. Furthermore sensory interactions between mother-infant dyad result in the rise in oxytocin in infant's brain. All the sensory stimuli are transmitted via nucleus tractus solitarius (NTS) to release oxytocin from the hypothalamic nuclei, SON and PVN [6]. The act of suckling in calves at the udder result in oxytocin release, however drinking from a bucket is not associated with a rise in oxytocin [6].

5. Attachment parenting

During the early and mid twentieth century there was a gradual decline in breastfeeding rate in industrialized nations. This decline is attributed to the global conflicts when bread-winners went to war and the women went to work. The infants and young children were left with a relative to care for them and the majority were given breast milk substitute (formula) or cow's milk. In England, majority of infants and young children were placed in large nurseries in the countryside away from London during World War II. The mothers were allowed to visit their children occasionally. Many children were noted to develop behavior problems and maladjustment as they grew older. The emotional and behavioral problems were attributed to maternal deprivation. Several prominent psychologists and psychiatrists promoted the concept of maternal separation as the root cause of delinquent and maladaptive behavior. Sir John Bowlby who was a member of an aristocratic family was the most prominent of these researchers who was personally affected by maternal deprivation. It was customary in the aristocratic families in England to employ a nanny to take care of the infants and young children. They believed that close contact between the mother and their infants and young children would spoil them. Sir John Bowlby writes that he was only allowed to see his mother for an hour after her evening tea. Naturally he developed attachment to his nanny and when she suddenly left the household when he was only 4 years old, he was quite devastated like losing a mother. Bowlby states that his early life experiences provided the idea of what he later called the theory of attachment parenting. When he was 10 years old he was sent to a boarding school, which was common in aristocratic families in England. After completion of high school he studied psychology, medicine and psychiatry. John Bowlby wrote his first paper in 1958, titled, the nature of the child's tie to his mother, which was followed by a number of papers and books on attachment parenting. However his writings do not include the benefits of breastfeeding, primarily because of the lack of knowledge regarding young infants' nutrition at the time. Subsequently there have been a great number of articles and books which have been published on the subject of attachment parenting which still lack the prominent role of breastfeeding and its role in mother-baby bonding. A number of researchers including Marshall Klaus and later John Kennel have published articles and books regarding the benefit of breastfeeding in bonding and attachment between mother and her newborns [9, 10]. They recommended that the newborns should stay with their mothers and breastfeed as soon as possible after they are born. They also stated that early breastfeeding enhance the motherinfant bonding, increase the likelihood of breastfeeding, improves child development and prevent child abuse and neglect. Furthermore Klaus stated that early mother-baby bonding is the result of the rise in oxytocin in both mother and infant's brain [10]. Critics had made comments regarding the feeling of guilt by the parents who were not able to bond with their newborns in the hospital and to breastfeed their babies [11].

The theory of attachment parenting is based on psychological principles and lack of solid scientific foundation. The massive data in the past 2 decades have provided strong and convincing evidence that long term breastfeeding and providing an enriched environment result in every child achieving his fullest potential.

6. Breastfeeding and infant's brain development

There is clear and convincing evidence that breastfeeding for 1 year or longer contributes significantly to the higher IQ during adulthood [12]. The relation between length of breastfeeding and adult intelligence may be difficult to substantiate because of the confounding factors including the maternal IQ, which has been substituted with maternal education [13, 14]. Recent studies on the association of breastfeeding and higher IQ demonstrate that higher IQ is associated with larger brain size and higher degree of myelination of the white matter [15, 16]. It is further believed that one or more ingredients of breast milk are implicated in brain growth and optimal brain function. In the past 2 decades comprehensive studies on a number of breast milk ingredients have been carried out to evaluate the causal association of these ingredients with brain growth and IQ of the child. Two major ingredients of breast milk which have generated major interest in scientific circles as well the food industry are docosahexaenoic (DHA) and arachidonic (ARA) acids, which are the components of myelin. A number of published reports have claimed that additional intake of DHA during pregnancy and lactation may improve the IQ of the infants. Furthermore there were claims of supplementation of DHA and ARA of the infant formula result in improved IQ, compared to formulas without supplementation. This resulted in DHA and ARA supplementation of all infant formulas, marketed by the major formula companies, beginning in 2002. More recent studies have demonstrated that these studies are flawed and DHA and ARA supplementation of pregnant and nursing mothers do not increase the IQ of the child [17]. Furthermore supplementation of infant formulas with DHA and ARA did not increase the IQ of the children who were given the supplemented formulas [18].

Breast milk has significantly higher concentration of cholesterol than infant formula and breasted infants at 6 months have higher cholesterol levels than formula-fed infants [19, 20]. Cholesterol is a significant component of myelin and availability of cholesterol is a rate limiting factor in brain maturation in mice [21]. Therefore it could be argued that higher cholesterol in breast milk result in enhanced synaptic connection, higher degree of myelination of the white matter and higher IQ.

Human milk contains significant numbers of maternal hormones and growth factors, oxytocin, thyroxin, estrogens, nerve growth and epidermal growth factors, which could influence myelin production and white matter development. Breast milk estrogens are transcriptional promoters for oxytocin and its receptors in the infant's central nervous system [22]. Oxytocin has a central role in milk ejection and oxytocin knockout (OTKO) mice are unable to nurse their pups [22]. Oxytocin binds to a large number of oxytocin receptors and is implicated in regulating homeostatic functions, social recognition and fear conditioning [22]. Additionally oxytocin reduces

neuroendocrine stress signaling and anxiety and depression symptoms [22]. Activation of beta estrogen receptors increases oxytocin gene transcription and reduces anxiety-related behavior [22]. Endogenous oxytocin can suppress corticotrophin releasing factor, CRF, and therefore reduce the activation of HPA axis and reduce the ACTH and cortisol levels [22].

Oxytocin is a neurotransmitter for oxytocinergic system and therefore involved in homeostatic processes [22]. Adults who were breastfed during infancy have lower total and LDL cholesterol, lower blood pressure and lower risk of cardiovascular disorders [13].

7. First and second messengers

The onset of labor begins with a decrease in maternal progesterone blood level, which results in a rise of maternal estrogens to progesterone ratio. Estrogens are transcriptional promoters of oxytocin gene which initiate uterine contractions. Furthermore estrogens are also transcriptional promoters of oxytocin genes in SON and PVN nuclei in the maternal central nervous system. Oxytocin is transported to the posterior pituitary which then enters the maternal circulation and result in the milk ejection from the breast.

Estrogens in the breast milk enter the newborn's circulation and cross the blood brain barrier. Estrogens act as transcriptional promoter for oxytocin gene in the hypothalamic SON and PVN nuclei. Oxytocin is then transported to a number of oxytocin receptors in the newborn's brain. Imaging studies comparing the activation of oxytocin receptor sites in breastfeeding and formula feeding mothers demonstrate significant enhancement of oxytocin receptors in breastfeeding mothers, which correlates with greater neural response [8] (**Figure 1**) Additionally plasma and salivary concentration of oxytocin are reported to be higher in breastfeeding compared to formula feeding mothers, 36% in plasma and 23% in saliva, respectively [23]. Furthermore the 24 hour urine concentration of oxytocin in breastfeeding mothers is significantly higher compared to formula-feeding mothers [8] (**Figure 2**). Postpartum depression appears to be associated with formula feeding and there is an association between Edinburgh postpartum depression scale and urinary oxytocin concentration [8] (**Figure 3**). Similarly other neuropsychiatric disorders which are highly associated with depression and anxiety may share the low oxytocin levels [3].

There is significant evidence that maternal bonding is mediated by oxytocin and the periaqueductal gray matter (PAG), the limbic system and the lateral orbitofrontal cortex are identified with maternal behavior [8]. There is strong evidence that an enriched environment result in increased release of oxytocin from the hypothalamic nuclei and have permanent effects on the physiology and behavior of mammalian species [9, 24–27]. Furthermore the oxytocinergic system appears to regulate the sensory, emotional, motivational and cognitive pathways which are affected in individuals with neurodevelopmental disorders.

Steroid hormones have significant roles in pregnancy. Progesterone is important in maintenance of gestation and estrogens play a significant role in parturition and lactation. The mother provides the first messenger, estrogens during the labor, via the placenta, to the unborn

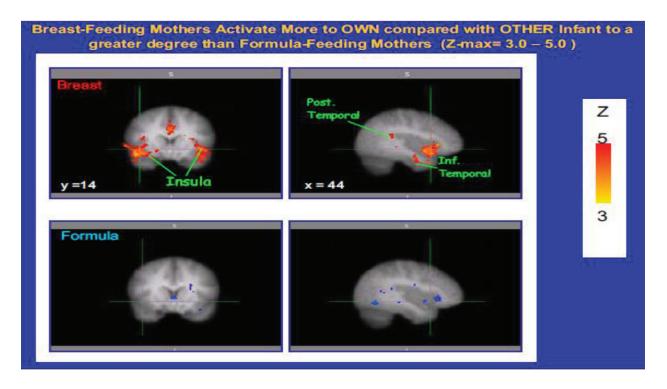


Figure 1. Brain imaging comparing the oxytocin receptors' enhancement in breastfeeding and formula feeding mothers. From Bartel and Zeki [8].

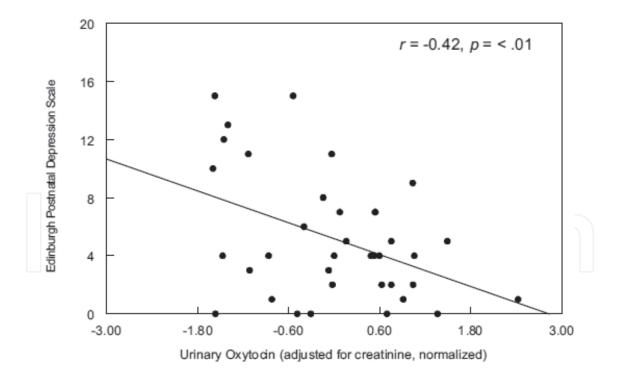


Figure 2. Urinary excretion of oxytocin in post-partum mothers and Edinburgh post-partum depression scale. From Grewen et al. [23].

infant, and after the delivery through her breast milk. Estrogens are transcriptional promoters of oxytocin and its receptors. The second messenger, oxytocin is released by the infant's hypothalamic nuclei, after breastfeeding and mother-baby sensory interactions. A single dose The Influence of Breastfeeding and the Infant's Social Environment on Neuroplasticity and Brain... 35 http://dx.doi.org/10.5772/intechopen.73209

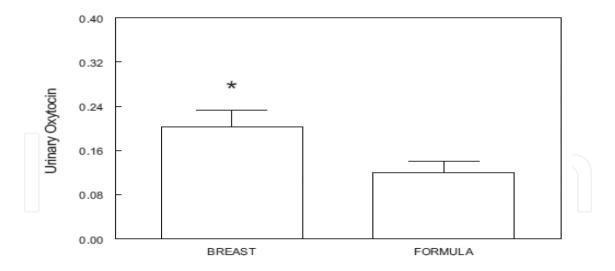


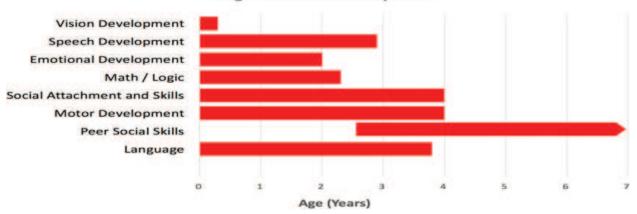
Figure 3. Comparison of urinary excretion of oxytocin in breastfeeding and formula feeding mothers. From Grewen et al. [23].

of estradiol results in an increase in plasma oxytocin level in women [28]. Oxytocin binding with oxytocin receptors result in profound changes in the infant's central nervous system. Other ingredients of breast milk, DHA, ARA and cholesterol significantly augment the role of oxytocin in brain development. However it appears that oxytocin has a central role in brain development and lack of oxytocin or the use of oxytocin blockers significantly alter the behavior and neurodevelopment of mammalian species.

The stages of infant and child development have been well-established and correlate with the stages of neuronal migration and myelination [29, 30] (**Figures 4** and **5**). There is clear evidence that any interruption of neuronal migration and myelination result in impaired brain function. Myelination is an ongoing process into early adolescence, in response to neural activity. Neuropsychiatric and neuro-developmental disorders are strongly considered to be associated with synaptic disconnectivity involving early white matter development.

The incidence of neuro-developmental and neuropsychiatric disorders far exceeds all the other genetic, congenital and sporadic causes of mental deficiencies. Most infants with developmental delays, 30–40 years ago, had chromosomal anomalies (the Downs syndrome), birth defects, cerebral palsy, kernicterus and congenital athyroidism. Autism was quite rare and most physicians had never heard of this disorder. Many physicians are still baffled by it and try to avoid taking care of children with autism. Some physicians do not know what causes autism and believe that the child is born with autism trait. There is also no consensus on which specialist should make the diagnosis and which specialist should be taking care of the child with autism. We believe that autism spectrum disorder is the most common cause of developmental delay in developed countries, because of formula feeding, early weaning and lack of mother-baby sensory interactions [31, 32].

Approximately 50 years ago, Dr. Julius Richmond, who was my first pediatrics professor and later became US Surgeon General, characterized that child development is the basic science of pediatrics [33]. This statement is still true now, because of the ever increasing knowledge accumulated in the past 2 decades on child development.



Stages Of Brain Development

Figure 4. Stages of brain development during infancy and childhood. From Deoni et al. [35].

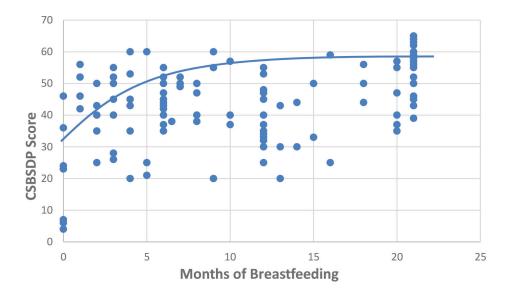


Figure 5. The relationship of CSBSDP scores with the duration of breastfeeding.

8. Infant and toddler developmental screening

We have previously reported on the association of formula feeding and early weaning with autism spectrum disorder using retrospective parent's report [31, 32]. In the present communication we report on the association of breastfeeding and improved developmental milestones using prospective and longitudinal infant and toddler developmental screening.

8.1. Material and methods

All infants had developmental evaluation at the following ages during their well-baby visits, 6, 9, 12, 15, 18 and 24 months. All infants were seen by a pediatrics provider and the mother or the father was asked to answer the questions on the evaluation form. The evaluation form was then scored by the provider and the results were discussed with the parent. Therefore this is

a longitudinal and prospective evaluation of infants and toddlers that identifies the areas of risk and referral for further evaluation and treatment. We have utilized this screening tool to evaluate the association of the score at 24 months of age with the length of breastfeeding. Our hypothesis is that increasing the length of breastfeeding will be associated with a higher score. We are utilizing CSBS DP infant and toddler developmental screen that measures seven language predictors, emotion and eye gaze, communication, gestures, sounds, words, understanding and object use [34]. CSBS DP has been successfully tested for validity and reliability with large samples of children [34]. CSBS DP is not predictor for the child's IQ, however it identifies children at risk of communication disorders and more specific, autism spectrum disorder.

8.2. Results

One hundred and ten children were screened beginning at 6 months of age. The breastfeeding history of the infants were monitored at every well child visit and recorded. All infants including infants with prematurity, chromosomal disorders, birth defects, congenital infections and genetic disorders were also screened for developmental delay; however, they were excluded from this report. The CSBS DP scores were plotted in relation to the duration of breastfeeding as shown in **Figure 5**.

8.3. Discussion

We have previously reported the association of early weaning and formula feeding with autism spectrum disorder [31, 32]. The present study which was based on our direct observation and prospective evaluation of infants and toddlers is further support that breastfeeding results in better developmental outcome. CSBC DP is predictive of the risk of communication disorders and speech problems in toddlers and we have been able to seek services to assist the families with toddlers as young as 18 months of age. Early diagnosis and treatment of at risk children result in better outcome.

In our previous study we reported on the impact of the infant feeding methods on the development of the autism spectrum disorder [32, 33]. With the current study we are able to predict that breastfeeding longer than 1 year is associated with higher CSBS DP score and lower risk of developmental delays.

United States may have the highest rate of autism in the world because of the lack of paid maternity leave. Many mothers who work in low paying jobs return to work 2 weeks after delivery of their infant and a majority do not breastfeed their babies. Modernization has reduced mother-infant sensory interactions. The infants spend more time in the infant seat on the back seat of the family car, in a stroller or a play pan. Mothers do not hold their babies during feeding and the formula bottle is frequently propped up in the infant's crib or the car seat. The combination of formula feeding and absence of mother-baby sensory interaction increases the risk of developmental delay in the child.

Imaging studies of infants and children as young as 10 months to 4 years of age were performed to compare the myelination of the brain on children who were breastfed for at least

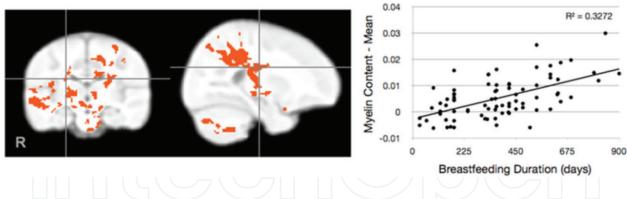


Figure 6. Association of improved myelination with duration of breastfeeding. From Deoni et al. [35].

3 months, those who were only formula-fed and those who were given mixed breast and formula [35]. The results of this study provide further evidence that breastfed infants have higher degree of myelination compared to the other two groups of children who were formula-fed or given mixed feeding. Furthermore longer duration of breastfeeding results in greater brain myelination as shown in **Figure 6**. The myelination occurs primarily in frontal and temporal white matter, peripheral aspects of the internal capsule and corticospinal tracts, superior longitudinal fasciculus and superior occipital-frontal fasciculus. These regions and pathways are associated with higher order cognition, executive functioning, planning, social-emotional functioning and language [35]. Therefore we can connect structure and function, increased level of myelination with increasing breastfeeding duration. Additionally the percentage of breast milk in an infant's diet has been correlated with cortical thickness in the parietal lobe as well as verbal IQ in adolescents [16].

8.4. Conclusion

We have documented the superiority of breastfeeding and an enriched environment resulting in higher cognitive ability and IQ. We have also shown that infants should be at least breastfed for the first 3 years of life to realize the maximal benefits of breastfeeding. We have presented strong and convincing evidence from various disciplines that breastfeeding result in higher IQ and cognitive functions that last into adulthood. Early weaning and formula feeding are associated with higher prevalence of childhood communicable diseases, diabetes, childhood leukemia, sudden infant death and autism. Additionally formula feeding strains the health care and public education systems with increased demands and higher expenditures.

The first 1000 days last a life time the rest of our lives, and every child deserves to reach his fullest potentials.

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Conflict of interest

The opinions expressed in this manuscript are only the opinions of the authors. The authors declare no competing interest.

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