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Breastfeeding Effect on The Genetic Variations of Primary Teeth Emergence in Relation to Salivary Transforming Growth Factor-B 1 Among Iraqi Infants

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ABSTRACT

Backgrounds: Mother`s milk has many necessary nutrients and cytokines, for instance transforming growth factor b1 (TGF-b1), which all are essential for teeth eruption and development. Additionally, human milk is considered as the best source of nucleotides, hence it may have effect on genetic variations of infants.

Objectives: This study aimed to assess the effects of breastfeeding on timing of teething in relation to bone morphological protein type 4 (BMP4) gene polymorphisms, and TGF-b1.

Patients and Methods: This study is cross sectional comparative study concerning salivary biochemical analysis with prospective view concerning timing of eruption. Sample was composed of 100 breastfed infants compared to 100 infants depending on formula milk. For the biochemical analysis including, saliva was collected only form subsample infants' groups (40 from each group). While clinical examinations and follow up for teeth emergence was done for the whole sample.

Results: Findings showed that there were highly significant differences between breastfeeding and early eruption time. While there were no significant differences between time of teething and both of salivary TGF-b1, and BMP4 gene by feeding pattern. However, there was an antagonistic effect between BMP4 and TGF-b1, especially with AA genotype by feeding pattern.

Conclusions: The current study confirmed the actual and pure effect of human milk on early primary teeth eruption in comparison with formula-milk fed infants. However, there were no effects of other factors (salivary TGF-b1, and BMP4 gene) on the timing of teething. In addition, an antagonistic effect between BMP4 and TGF-b1 was confirmed in this study.

Keywords: Breastfeeding, BMP4 gene, Eruption time, Salivary TGF-b1.

INTRODUCTION

Infant feeding habits include the sorts of food the child consumes beginning at birth. The milk is basic material of an infant's diet, and it can be either breast or bottle milk [(1)]. The WHO recommends starting weaning not earlier than 4 months old and exclusively breastfeeding until age of 6 months [(2)].

In addition to its nutritional benefits, breastfeeding has many other documented positive effects on baby's health. Human breast milk components shield breastfed newborns from infections and inflammation. Breast milk has distinct nutritional advantages over formula because its nutrient profile is tailored specifically for infants and it adapts over time to a child's changing demands [(3, 4)].

Human milk is the best source of nucleotides for infants. Nucleotides are essential for encoding genetic information, controlling energy metabolism, signal transduction, and enabling fast growth in early infancy. They are the monomeric units of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Whereas, infants' ability to produce the endogenous nucleotides required to satisfy their increased metabolic demands for growth and development is limited [(5, 6)].

Numerous studies have demonstrated that early in development or during key times, the environment and diet may have an impact on the expression of genes that have both short- and long-term consequences on the organism [(6, 7)]. Some elements of human breast milk may directly influence epigenetic alterations, while the underlying mechanisms are still unknown. Numerous research shed light on the actual impact of human breast milk on genetic expression, particularly when it comes to the risk of non-communicable diseases, with the potential to improve the health and long-term development of the infant [(8-10)]. On another hand, several animals and humans studies support the idea that genetic factors may be linked with early or delay teeth eruptions [(11-13)].

Bone morphological proteins (BMPs) belong to the secreted protein family known as the TGF-b superfamily [(14)]. One of the important routes controlling craniofacial development is BMP signaling. It controls the growth of its mineralized structures, including the cranium, mandible, maxilla, palate, and teeth [(15, 16)]. Genome-wide association studies disclosed a linkage of BMP4 in the SNP rs17101923 is likely to contain novel height-associated variants with earlier primary tooth eruption and craniofacial growth [(11)].

Numerous non-syndromic and syndromic human craniofacial abnormalities have been linked to gene polymorphisms and mutations in the BMP pathway, as in cleft palate, tooth agenesis and hypodontia [(17, 18)].

Breast milk contains necessary nutrients for healthy teeth development such as phosphate, calcium, and vitamins A, C, and D. For hydroxyapatite crystals to form properly, calcium and phosphorus are necessary. So, deficiency in the previous nutrients can have an effect on tooth development [(4, 19)].

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Breastfeeding status is a possible source of variation in the timing of emergence of primary teeth [20, 21]. Tooth eruption is a continuous biological activity by which evolving teeth emerge across jaws and the overlying mucosa into the oral cavity [22]. Tooth eruption also reflects the body's growth in general. Thus, when tooth eruption is delayed, the body's growth in general can be said to be constrained. It is a complex and tightly regulated process. Hence, many factors can influence teeth eruption, for instance: genetics, ethnical, racial, geographical differences, hormones, cytokines, gender and nutritional differences [23, 24].

Human milk contains ideal components more than nutrients for instance, hormones, growth factors, and cytokines, including tumor necrosis factor α, transforming growth factor-b1 (TGF-b1), and interleukins (IL): IL-6, IL-8, IL-10, and IL-1. These cytokines are all immunomodulatory, and the majority of them have anti-inflammatory properties, which may prevent the spread of infections [4,25]. A tightly coordinated chain of cellular and molecular activities that drive tooth movement through the eruptive pathway are involved in tooth eruption. Tumor necrosis factor-, transforming growth factor-b1, interleukin-1 (IL-1), colony-stimulating factor-1 (CSF-1), and receptor activator of nuclear factor kappa B ligand (RANKL) are involved in dental follicle. Hence encourage bone marrow precursor mononuclear cells to migrate and differentiate into the dental follicle (DF) and formation of osteoclasts that are needed for bone resorption to form an eruption pathway [23, 26, and 27].

TGF-Bs is important regulators during craniofacial development, including tooth formation and eruption. In particular, the cascade of molecular signals that would drive the beginning of tooth eruption might be started by either epidermal growth factor (EGF) or TGF-b1 [23, 25]. Many studies supported that early primary teeth emergence and breast feeding have a positive correlation [21, 28]. These findings were disagreed with other studies revealed that breast feeding had no influence on the timing of teething [29, 30].

The purpose of this study was to show how breastfeeding affects the timing of the eruption of primary teeth, in relation to salivary biomarker (TGF-b1), and genetic polymorphisms of BMP4 gene, SNPs (rs17101923) in comparison with bottle-feeding infants.

MATERIALS AND METHODS

Cross-sectional comparative study concerning salivary biochemical analysis with longitudinal prospective concern for timing of eruption. The selected sample composed of 100 breastfed infants compared to 100 infants depending on formula milk. All infants were examined and followed for timing of teeth emergence. However the salivary biochemical analysis was done only for subsample infant groups. A subsample was selected randomly from the whole sample (40 from the breastfeeding group and 40 from a bottle-feeding group). Both groups were examined when they attended the health centers of Baghdad during primary health care. Only infants aged between 4-9 months were selected in the study where in this age the infants depend mainly on breastfeeding as essential nutrition taking in consideration the matching in complementary food intake. The salivary analysis used in this study throughout was done by two salivary swabs (Saliva Bio Infant's Swab SIS). The swabs were used for both genetic evaluation and salivary transforming growth factor- beta 1 (sTGF-b1) level assessment. Saliva was collected for a maximum of 1 minute. Saliva was collected from natural flow by gently placing the swab in the flour of the mouth for the salivary biomarker assessment, while through gently swab rubbing on the buccal mucosa for genetic analysis. Finally place the saturated swab, which has been sliced to remove the free saturated section before being placed in the centrifuge tube, into the swab storage tube to be recovered by centrifugation [31].

The salivary pro-inflammatory cytokine TGF-b1 was measured in both infants' groups using ELISA kit (enzyme-linked immune sorbent assay (ELISA). While the genetic investigation and DNA extraction were carried out by using Quick-DNA™ MiniPrep kit (Catalog Nos. D3024 & D3025).

The time of primary teeth emergence in infants was examined through the intraoral examination by using the index finger, feel the incisal edge or tooth cusp tip on the alveolar ridge. When the tooth's crown edge was clearly visible in the oral cavity, it was regarded to be erupted [32]. The infant was placed in the mother's lap during the intraoral examination, which was done in the knee to knee position. A mouth mirror was used to inspect the mouth without the use of radiographs. Infants with no teeth emergence were followed at monthly intervals, until the precise eruption time was recorded. Parents were instructed to consider a tooth erupted as soon as any part of the crown emerged through the gingival surface, and to record the date at which this occurred.

Exclusion criteria: Infants with malnutrition or chronic illness.

Ethical considerations: The Ethical Committee of the College of Dentistry, University of Baghdad approved this study. Legal permission was obtained from Ministry of health to perform clinical examinations at health centers. Also, a special informed consent in accordance with the Collage of Dentistry in University of Baghdad prepared and distributed to mothers to obtain permission to participate in this study. The Declaration of Helsinki, the World Medical Association's code of ethics for studies involving humans, guided the conduct of this work.

Statistical analysis

Statistical analysis using Statistical Package for social Science (SPSS version 22, Chicago, Illinois, USA). Mean and standard error (SE). Inferential statistics are: Student T test, one way analysis of variance (ANOVA), and Pearson correlation. Level of significance was at 0.05. Additionally Hardy-Weinberg equilibrium law was used for calculating allele and genotype frequencies.

RESULTS

Genetic analysis in the current study revealed that there was no significant association between BMP4 gene/SNP (rs17563) and feeding patterns, as shown in **table (1).**

Infants' eruption time for primary teeth in relation to feeding pattern is shown in **table (2).** Data revealed that the mean eruption time of primary teeth among breastfeeding group was significantly higher than bottle-fed infants. Also, there were no significant differences between BMP4 gene polymorphisms and mean values of timing teeth emergence according to feeding pattern, as revealed in **table (3).**

However, the level of salivary TGF-b1 was significantly higher among breastfeeding in comparison with formula milk feeding group as seen in **table (4).**

The data revealed no significant correlation between sTGF-b1 and timing of teething by feeding pattern as shown in **table (5).** Moreover, although no significant differences between the mean values of salivary transforming growth factor (TGF-b1) and gene polymorphisms of BMP4, SNP (rs17563) by feeding pattern, however infants with AA genotype, salivary TGF-B were significantly higher among breastfeeding infants as demonstrated in **table (6).**

Table (1): Genotype distribution and allele frequency of bone morphological protein type 4 gene/ SNP (rs17563) by feeding pattern.

Genotype	Breastfeeding No. (%)	Bottle-feeding No. (%)	Chi-Square (χ^2)	P- value
AA	14 (35.00%)	19 (47.50%)	0.757 NS	0.384
AT	14 (35.00%)	10 (25.00%)	0.666 NS	0.414
TT	12 (30.00%)	11 (27.50%)	0.043 NS	0.834
Total	40	40	---	---
Allele	**Frequency**			
A	0.525	0.60	---	---
T	0.475	0.40	---	---

Table (2): Eruption time (mean ± SE) by feeding pattern

Feeding pattern	Mean	SE	T test	P value
Breastfeeding	8.440	0.1863	2.363*	**0.019**
Bottle-feeding	9.095	0.2052		

*Significant P≤0.05

Table (3): Eruption time of infants' teeth (mean, SE) in relation to polymorphisms of bone morphological protein type 4 gene, SNP (rs17563) by feeding pattern.

Genotypes		Feeding pattern				T test	P value
		Breastfeeding		Bottle-feeding			
		Mean	±SE	Mean	±SE		
rs17563	AA	8.214	0.447	8.974	0.440	1.185	0.245
	AT	8.500	0.609	7.900	0.526	0.707	0.487
	TT	8.375	0.603	9.864	0.626	1.712	0.102
F		0.385		3.654			
P value		0.933		0.074			

Table (4): Salivary transforming growth factor-beta 1 (mean ± SE) by feeding pattern.

Feeding pattern	Mean	SE	T test	P value
Breastfeeding	12.457	.167	3.003*	0.004
Bottle-feeding	11.747	.168		

*Significant P≤0.05

Table (5): Correlation between salivary transforming growth factor-beta 1 and primary teeth eruption time by feeding pattern.

Feeding pattern	Salivary transforming growth factor-beta 1 / Eruption Time	
	r	P
Breastfeeding	0.063-	0.700
Bottle-feeding	0.077	0.638

Table (6): Salivary transforming growth factor (mean, SE) in relation to polymorphisms of bone morphological protein type 4 gene, SNP (rs17563) by feeding pattern.

Genotypes/ rs17563	sTGF		Feeding pattern		T test	P value
	Breastfeeding		Bottle-feeding			
	Mean	SE	Mean	SE		
AA	12.519	.310	11.535	.236	2.569*	0.015
AT	12.640	.265	12.292	.308	0.853	0.403
TT	12.172	.296	11.617	.333	1.249	0.226
F	0.662		1.861			
P value	0.522		0.170			
Total	12.457	.167	11.747	.168	3.003*	0.004

*Significant P≤0.05

DISCUSSION

This study was performed to assess how breastfeeding affects timing of teething in relation to genetic variants of BMP4 gene, SNP (rs17563). Moreover, due to cytokines roles in the regulation of teeth eruption, the current study tried to find the relation between selected salivary TGF-b1 as a biomarker of eruption time in comparison between the two feeding patterns. Despite of all diverse proposed benefits of breastfeeding on the factors associated with teeth emergence, there were no previous Iraqi data studied these relationships and concerning the effect of breastfeeding on teeth emergence in relation with these variables. The result of the present study showed that the breastfed infants had earlier eruption time in comparison with those depending on bottle feeding. This finding confirmed many previous studies [21, 28].

This could be attributed to that breast milk contains nutrients in nearly optimum amounts [10, 20]. In addition to other components that aid in the absorption of essential nutrients such as calcium, phosphorus, and vitamins A, C, and D. To create hydroxyapatite crystals successfully, calcium and phosphorus are needed [19, 20]. Also it has been proposed that breastfeeding is the best stimulus for the physiological development of the orofacial complexes skeletal and muscular components, as well as the formation of teeth [33]. However the present results disagree with several studies who did not show effect of breast feeding on the time of eruption and pattern of teeth eruption within the first 12 month of children life [29, 30].

Many studies clarified the precise impact of human breast milk on genomic expression as other nutrients and environmental factors effect on genes [9]. Hence this study tried to find the relation of genetic factor in human milk on timing of teeth emergence among breast-fed infants in comparison with bottle-fed infants where there was no previous studies concerning these variables. Genetic analysis in the current study revealed that there were no significant differences between BMP4 gene polymorphisms/SNP (rs17563) and mean timing of teeth emergence according to feeding pattern. This may be attributed to the pure effect of human milk in the eruption time due to its nutrients benefits. Additionally, human milk can affect epigenetically on the gene phenotype (gene expression and function). A variety of bioactive compounds found in human milk, such as transforming growth factor-b1 (TGF-b1), are considered to be crucial for growth and development [4, 25], which may be also associated with teeth eruption pathway. In particular, TGF-b1 may trigger a series of chemical signals that drive the emergence of teeth [23, 27]. The current study showed that the mean values of sTGF-b1 were significantly higher among breastfeeding. This may be attributed to the presence of TGF-b1 in human milk, which is associated with this high level among infants

depending on breast-feeding. Moreover, there were no significant differences between salivary transforming growth factor TGF-b1 (mean, SE) in relation to genotype variations of BMP4 gene, SNP (rs17563) by feeding pattern, except with AA genotype, which was lowered in frequency among breastfeeding group, where TGF-b1 mean values were increased. This is parallel with many studies confirmed that BMP4 act antagonistically to TGF-b1 [34, 35]. This may be regarded to the role action of BMP4 in activation of osteoblasts for stimulation of bone formation, adversely to TGF-b1 in activation of osteoclast cells for stimulation of bone resorption. Thus, human milk benefits were not restricted just to the nutritional benefits, and growth factors, but the genetic effect of human milk had crucial role in health and development. Therefore many high qualities, prospective, longitudinal research need to support this epigenetic effect of breastfeeding with the underlying mechanisms, and more sample size is recommended.

CONCLUSIONS

The current study confirmed the actual and pure impact of human milk in the teeth eruption and development. Throughout demonstration of the highly significant differences between the breastfeeding and early eruption time of primary dentition. However there were no significant effects for both salivary biomarkers (TGF-b1 and genetic polymorphisms of BMP4 gene) on eruption time by feeding pattern in this study. This study also confirmed the antagonistic relation between BMP4 and TGF-b1.

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