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EFFECT OF EARLY AND LATE WEANING ON IMMUNOLOGICAL, HEPATIC AND HEMATOLOGICAL PARAMETERS IN EGYPTIAN INFANTS

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Abstract

Breast milk is the optimal form of nutrition in infancy. Breastfeeding protects infants from a wide array of infectious and non-infectious diseases. Weaning is the process of introducing other foods and reducing the supply of breast milk.

Aim of the work: To study the effects of early and late weaning on certain immunological patterns, blood parameters including serum iron and copper and some hepatic functions in Egyptian infants.

Patients and Methods: The present study was carried out on 80 Egyptian infants who were attendants to clinic and immunization room in the health care centre in Tanta for routine child care visits (during vaccination sitting). The infants in this study were divided into two groups: Group I: Forty breast fed infants with early weaning. Group II: Forty breast fed infants with late weaning. All infants of the study was subjected to complete history taking, thorough clinical examination and laboratory investigations including complete blood picture, serum iron, copper, liver functions and immunological assay of IgA, IgE ,CD4 and CD8.

Results: There were significantly higher mean weight and weight for age percentile in infants with early than infants with late weaning but there were no significant differences in length, head circumference and incidence of infections between early and late weaning groups. There were significantly higher hemoglobin, serum iron, copper levels, CD4% and CD4/ CD8 ratio in infants with early than infants with late weaning with significant positive correlation between CD4% and Hb % in early weaning group and significant positive significant correlation between CD4% and incidence of infections in late weaning group. There were no significant differences in leucocytes count and liver functions between infant weaned early or late except for serum albumin which is significantly higher in early weaning group.

Conclusion: There were significantly higher Hb%, MCV, MCH, lymphocytes %, serum iron and cupper, serum albumin levels, CD4 % and count and CD4/CD8 ratio in early weaning group compared with late weaning group while there were no significant differences in number of exposure to infections, total leucocytes, eosinophils, platelets and reticulocytes counts, total bilirubin, total serum protein, ALT and AST, IgA and IgE levels and CD8 % and count between early and late weaning groups.

Recommendation: Based upon the above mentioned conclusion criteria; early weaning may be recommended in Egyptian infants.

INTRODUCTION

Breast feeding is the feeding of an infant or young child with breast milk directly from human female breasts (i.e., via lactation) rather than from a baby bottle or other container [(1)]. World Health Organization and American Academy of Pediatrics emphasize the value of breast feeding for mothers as well as children. Both recommended exclusive breast feeding for the first six months of life and then supplemented breast feeding for up to two years or more [(2,3)].

The longstanding debate over the optimal duration of exclusive breast feeding has centered on the so called "weanling's dilemma" in developing countries: "the choice between the known protective effect of exclusive breast feeding against infectious morbidity and the (theoretical) insufficiency of breast milk alone to satisfy the infant's energy and micronutrient requirements beyond 4 months of age. The debate over whether to recommend exclusive breast feeding for 4-6 months versus about 6 months becomes more intense [(4)].

Reilly and Wells 2005 hypothesize that the mean metabolizable energy intake in exclusively breast fed infant at 6 months is 525-574 Kcal/day, and the mean energy requirement is approximately 632-649 Kcal/day, leading to a gap between the energy requirements and the energy provided by breast milk by 6 months for many babies. Infant actually require an excess of energy intake over energy expenditure in order to grow adequately [(5)]. At some point, exclusive breast feeding no longer meets a growing infant's energy and nutrient needs and complementary foods must be added. These additional foods are not intended to replace or interfere with breast feeding. The timing and type of complementary foods is variable, reflecting the numerous cultural foods and practices of society [(6)].

Weaning is the process of introducing other foods and reducing the supply of breast milk. The infant is fully weaned when it no longer receives any breast milk [(7)].Weaning is a learning process and infants will only learn to accept and enjoy new tastes and textures if they are given the opportunity to try them [(8)]. The term weaning comes from the Anglo-Saxon word "wenian" meaning "to become accustomed to something different". Weaning is a natural, inevitable stage in a child's development. It is a complex process involving nutritional, immunological, biochemical and psychological adjustments. Weaning may mean complete cessation of breast feeding ('abrupt' or final wean) or the beginning of a gradual process of the introduction of complementary foods to the infant's diet [(9)].

AIM OF THE WORK

The aim of this study was to study the impact of weaning age on certain hematological, immunological patterns and some hepatic functions in Egyptian infants to choose the best time of beginning weaning and encourage it among Egyptian families.

SUBJECT AND METHOD

This study was done after approval from Ethical Committee of research center of Tanta University and written consent from parents of all infants included in this study and was carried out on 80 Egyptian infants who were attendants to clinic and immunization room in the health care center in Tanta for routine care visits (during vaccination sitting) from April 2013 to April 2014. The infants in this study were divided according to their feeding pattern into two groups: **Group** I: Forty breast fed infants with early weaning (i.e. additional or mixed foods before 6 months)**.** **Group** II**:** Forty breast fed infants with late weaning (i.e. exclusively breast fed from birth up to 6 months)**.**

Inclusion criteria

1- Age of infants (6-8) months.

2-Healthy full term breast fed infants with average birth weight with early and late weaning.

Exclusion criteria

1-Preterm infants with low birth weight.

2-Infants with congenital malformations or congenital diseases as congenital heart, hepatic, renal, metabolic or haematological disorders.

3-Infants with congenital or current infections.

4-Infants with atopic diseases.

Method: All infants of the study were subjected to the followings:

1-Proper history taking: Including dietetic history, duration of exclusive breastfeeding, timing of introduction of solid foods, type and frequency of complementary foods , the nature of the infant's appetite and history of infection regarding number, type and duration.

2-Clinical examination including: Weight, length, head circumference, mid arm circumference and complete systemic examination.

3-Laboratory Investigations including:

Complete blood picture.

Serum iron.

Serum cupper.

Liver functions.

Immunological assay of IgA and IgE.

Assay of T cell populations (CD4 and CD8).

Sampling

Five ml of venous blood were collected using sterile needles through gentle venipuncture after sterilization of puncture site by alcohol, and collected samples were divided into; 2 ml in clean tube with 20 uL EDTA solution for complete blood count using ERMA PCE-210 N cell –counter [10] , immunological assay of IgA and IgE by particle-enhanced immunonephelometry using the BN systems. (BN is a trademark of Siemens Healthcare Diagnosis) [11, 12] and assay of T cell populations (CD4 and CD8) using Flowcytometry [13, 14] and 3 ml in a plain glass tube without EDTA for estimation of serum iron [15] , serum cupper [16] and liver functions tests including: bilirubin, serum proteins, albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [17, 18].

Statistical analysis

Data were analyzed using SPSS version 20. Data were expressed as means (±SD). Comparisons were made using the Mann–Whitney test (Kruskal Wallis test for two groups), and p-value less than 0.05 was considered statistically significant.

RESULTS

There were statistically significant differences between early and late weaning groups regarding weight and mean weight for age percentiles with higher weight and mean weight for age percentiles in early than late weaning groups but no significant differences between early and late weaning groups regarding length and head circumference. Table 1.

There were statistically significant differences between early and late weaning groups regarding serum albumin levels with higher serum albumin levels in early than late weaning groups but no significant differences between early and late weaning groups regarding total bilirubin, total serum protein, ALT and AST levels. Table 2.

There were significant differences between early and late weaning regarding Hb%, MCV, MCH, lymphocytes %, neutrophils %, serum iron and cupper levels, CD4 % and count and CD4/CD8 ratio with higher levels in early weaning group in all previous parameters except for neutrophils % which is significantly higher in late weaning group while there were no significant differences in total leucocytes, eosinophils, platelets and reticulocytes counts, IgA and IgE levels and CD8 % and count between early and late weaning groups. Table 3.

There were no significant differences as regard number of gastroenteritis and respiratory tract infections between early and late weaning groups. Table 4.

There were significant positive correlation between CD4% and Hb% in early weaning group and significant positive correlation between CD4% and incidence of infections in late weaning group. Figure (1, 2).

Table 1: Anthropometric measurements in early and late weaning groups

Parameters	Early weaning group (n=40)	Late weaning group (n=40)	P value
Weight (kg)	7.95± 0.45	7.01± 0.32	0.001*
Length (cm)	64.65±7.35	62.90±2.40	0.318
Head circumference (cm)	43.20±1.38	43.40±1.50	0.633
Weight for age percentile	74.31+17.7 (25^{th} -97^{th})	63.43+25.93 (25^{th} -95^{th})	0.017*

* Significant value with p <0.05.

Table 2: Comparison between early and late weaning groups regarding some hepatic functions.

Parameters	Early weaning group (n=40)	Late weaning group (n=40)	P value
Total bilirubin level (mg/dl)	0.71± 0.11	0.69± 0.09	0.459
Total serum protein (gm/dl)	6.99± 0.72	6.97±0.20	0.906
Serum albumin level (gm/dl)	4.24±0.39	3.92±0.26	0.005*
ALT (U/L)	23.25±6.38	22.70±5.35	0.770
AST (U/L)	29±9.55	32.2±8.56	0.272

* Significant value with p <0.05.

Table 3: Hematological and Immunological parameters in early and late weaning groups.

Parameters	Early weaning group (n=40)	Late weaning group (n=40)	P value
Hb level (g/dl)	10.41± 0.66	8.60± 0.70	0.001*
Mean corpuscular volume (MCV) (fl).	69.83±4.55	5.36± 5.32	0.002*

Mean corpuscular hemoglobin (MCH) (pg)	24.7±2.55	14.52±2.36	0.003*
Leucocytic count (thousand/cubic mm)	10.24±1.01	8.27±1.07	0.096
Neutrophils %	42.4±9.33	50.8±11.4	0.015*
Lymphocytes %	50.1±10.06	41.7±11.8	0.048*
Eosinophils %	2.50±1.10	3.10±1.74	0.201
Platelets count (thousands/cubic mm)	366.8±156.5	314.1 ±44.33	0.156
Reticulocytic count (thousand/cubic cm)	29.63 ± 3.85	33.33± 3.62	0.125
Serum iron level (ug/dl)	34.19± 5.22	30.14± 5.22	0.048*
Serum cupper (part per million) (ppm)	0.68±0.13	0.62± 0.04	0.047*
IgA (mg/dl)	33.15±16.82	37.58±12.66	0.353
IgE IU/ml.	12.00±6.55	9.83±7.32	0.384
CD4 %	39.20± 5.54	34.65±6.79	0.026*
CD4 count/ cubic mm.	1721.8±565.7	1348.5±562.1	0.043*
CD8 %	18.80±2.82	20.00±5.33	0.393
CD8 count/ cubic mm.	803.3±73.60	787.7±80.01	0.844
CD4/CD8 ratio	2.10 ± 0.30	1.81 ± 0.35	0.008*

* Significant value with $p < 0.05$.

Table (4): Comparison between early and late weaning groups regarding number of gastro enteritis and respiratory tract infections.

Disease	Number of episodes	Early weaning group (n=40)		Late weaning group (n=40)		P-value
		Number	%	Number	%	
Gastroenteritis	**No episodes**	18	45	28	70	0.253
	1 episode	8	20	8	20	
	2 episodes	4	10	2	5	
	> 2 episodes	10	25	2	5	
Respiratory tract infections	**No episodes**	6	15	6	15	0.758
	1 episode	20	50	10	25	
	2 episodes	10	25	10	25	

	> 2 episodes	4	10	14	35	

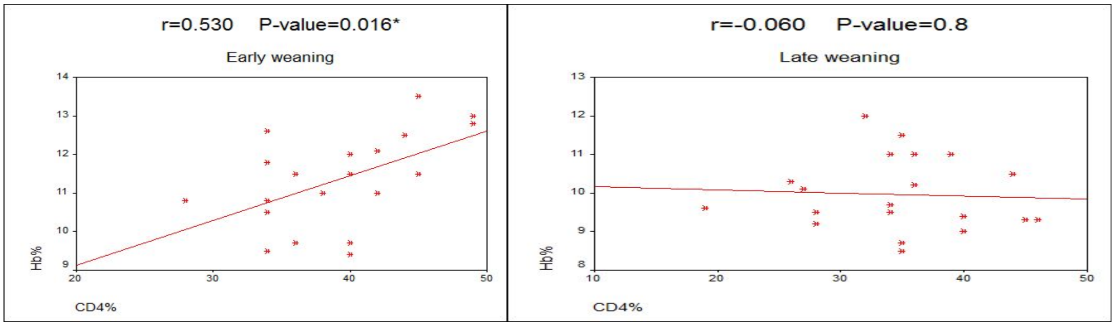

Figure (1): Correlation between CD4% and Hb% in early weaning (Left) and late weaning (Right).

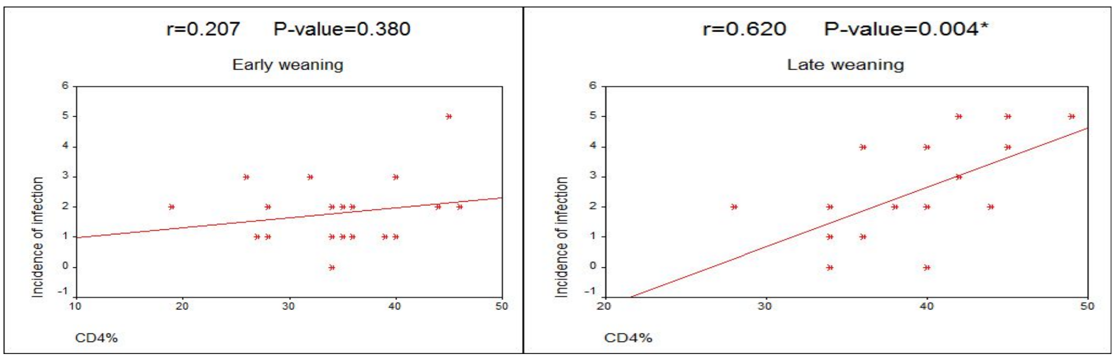

Figure (2): Correlation between CD4% and incidence of infections in early weaning group (Left) and late weaning group (Right).

DISCUSSION

The study included 80 infants who were chosen according to inclusion criteria and divided into two groups**.** Group I: Forty breast fed infants with early weaning. Group II: Forty breast fed infants with late weaning.

In our study we found that early weaning group had significantly increased values regarding weight and mean weight for age percentiles than late weaning groups. These results are in agreement with **Kramer et al 2003 (19)** who found that monthly weight gain with mixed feeding from the age of 3-7 months is higher than those with exclusive breast feeding for 6 months , **Baker et al 2004 (20)** who found that early introduction of complementary feeding (before 16 weeks) was positively associated with infant weight gain, **Ong et al., 2006 (21)** who reported that higher total dietary energy intake at the age 4th month was associated with greater weight gain between birth and ages 1, 2 and 3 years and **El-Shafi et al 2010 (22)** who found that mean weight for age percentiles and mean weight of infants weaned at 4 months was significantly higher than those weaned at 6 months. Better weight gain in infants weaned at 4 months may be explained by lower dietary energy intake for infants 6th month weaned group than that of infants weaned at 4th month **(21)**. **Reilly and Wells 2005** hypothesize that the mean metabolizable energy intake in exclusively breast fed infant at 6 months is 525-574 Kcal/day, and the mean energy requirement is approximately 632-649 Kcal/day, leading to a gap between energy requirements and energy provided by breast milk for many babies. Infant actually require an excess energy intake over energy expenditure in order to grow adequately **(5)**. At some point, exclusive breast feeding no longer meets a growing infant's energy and nutrient needs and complementary foods must be added **(6)**.

The present study showed that there were no significant differences in the length and head circumference between early and late weaning groups, this is in agreement with **Dewey et al., 1999 (23)** who found no significant differences in length and head circumference gain from 4^{th} to 6^{th} month between exclusively breast feeding group and breast-feeding plus solid foods group , **Kramer et al 2003** (19) who found that infants with exclusive breastfeeding up to 6 months were normal as regard anthropometric measurements , **Kramer and Kakuma 2002** who have performed a large Cochrane review of the effects of duration of exclusive breast-feeding which was last updated in 2009. The overall conclusion was that neither controlled clinical trials, nor observational studies from developing or developed countries show deficits in weight or length gain for those who continued to be exclusively breast-fed for 6 months (24, 25) but regarding weight of studied groups it was against our findings.

In this study there were no significant differences as regard number of exposure to gastroenteritis and different types of respiratory tract infections between early and late weaning groups. This is in agreement with **Dewey et al 1999** (23) who found no significant differences between exclusively breast feeding group and breast-feeding plus solid foods group as regard morbidity (fever, respiratory illness and diarrhea) from 4^{th} to 6^{th} months and **El-Shafi et al 2010** (22), who found no significant differences as regard co-morbidity between early and late weaning. In contrast to our results, **Rebhan et al 2009** (26) found that exclusive breast-feeding for at least 6 months decreases the risk of infections. While **Kramer et al 2003** (19) found that the infants that were breast-fed exclusively for 6 months, experienced less morbidity from gastrointestinal infections than infants who received mixed breast-fed after 3-4 months of age but they found no differences in the risk of respiratory infections between the two groups.

This could be due to, babies possess an open gut from birth until 4-6 months of age; this means that the spaces between the cells of the small intestines will readily allow intact macromolecules, including whole proteins and pathogens, to pass directly into the bloodstream. This allows beneficial antibodies in breast milk to pass more directly into baby's bloodstream, but it also means that large proteins from other foods (which may predispose baby to allergies and disease-causing pathogens can pass right through, too. During baby's first 4-6 months, while the gut is still "open," antibodies (sIgA) from breast milk coat baby's digestive tract and provide passive immunity, reducing the likelihood of illness and allergic reactions before gut closure occurs. Baby starts producing these antibodies on his own at around 6 months, and also gut closure occurred by this time (27).

Our results showed that early weaning group had significantly increased hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin levels than late weaning groups. Similar results were reported by **Sultan** and **Zuberi 2003** who reported that late weaning was the most important predictor of iron deficiency anemia at 1-2 years of age (28), **El-Shafi et al 2010** who found significant increase in hemoglobin level in infants weaned at 4 months than those weaned at 6 months (22), **Meinzen et al 2006** who reported that exclusive breastfeeding up to 6 months was associated with increased risk of anemia (29) and **Kramer and Kakuma 2002** who showed that infants with exclusive breastfeeding for 6 months, compared with 3 -4 months was associated with lower mean hemoglobin. (24). In the present there were no significant differences as regard total leucocytes, platelets, reticulocytes counts and eosinophils % between early and late weaning groups but lymphocytes count and percentage was significantly higher in early weaning group while neutrophils % were significantly higher in late weaning group. There is no previous data to compare with.

In our study there were no significant differences in total IgE and IgA levels between early and late weaning groups but mean total IgE was higher in early weaning than late weaning. These results are in agreement with **Sanaa et al 2006** who found higher total IgE level in mixed feeding group than exclusive breast feeding for at least 6 months, these results was distinct but not statistically significant (30).

In present study there were significantly higher CD4% and CD4/ CD8 ratio in early than late weaning group. **Hawkes et al 1999** (31) found that there are some differences in lymphocyte populations following exposure to maternal milk. These differences include a decrease in CD4+:CD8+ cells and an increase in natural killer cells. The functional consequences of a report that breast-fed infants have a thymus twice the size of that of non-breast-fed infants have yet to be explained but support the role of human milk on T cell development (32).

Our study found significant positive correlation between CD4% and Hb% in early weaning but not in late weaning groups and significant positive correlation between CD4% and incidence of infections in late weaning but not in early weaning groups. There is no available previous data to compare with but this may be explained by presence of iron deficiency anemia in most of infants in late weaning group and it is well known that iron deficiency anemia lower proportion of mature T-lymphocytes (CD4 and CD8) (33). **Mullick et al. 2006** also found a trend of lower CD4 cells with increasing iron deficiency anemia (34).

In present work there were significantly higher serum iron in early weaning group than late weaning group, these results were in agreement with **Kramer and Kakuma 2002** [24] who showed that infants with exclusive breastfeeding for 6 months were associated with poorer iron status when compared with those with exclusive breast feeding for 3-4 months and **Domellof et al 2001** [35] who found higher incidence of iron deficiency in susceptible infants who are exclusively breastfed for 6 months.

Breast milk may have evolved to have low iron as a mechanism for protecting infants from infections, the introduction of iron supplements and iron-fortified foods, particularly during the first six months, reduces the efficiency of baby's iron absorption. The specialized proteins in breast milk ensure that baby gets the available iron (instead of "bad" bacteria and such) [36].

Canadian Pediatric Society and American Academic of Pediatrics recommended exclusive breast-feeding for at least 4 months and the introduction of iron-containing complementary foods and foods containing ascorbic acid, which enhance iron absorption, at the age of 4–6 months [37]. As by the age of 6 months iron stores of most babies have been depleted, and from 4 to 12 months after birth the infant's blood volume doubles. Thus, at this age, dietary sources of iron become critical to keep up with this rapid rate of red blood cell synthesis [24], so this iron needs to come from complementary foods, in addition to breast milk or formula. If the mother tries to meet her infant's iron requirement on breast milk alone, the baby would have to consume between 4 and 13 liters of breast milk per day, depending on baby's efficiency of iron absorption from breast milk (estimates range from 15-50% absorption). Most exclusively breastfed babies don't consume much more than 1 liter of milk per day [38].

In our study we found significantly higher serum copper levels in early than late weaning groups but no similar studies is available to compare with. Deficiency of trace elements such as copper can occur in infants for different reasons. It has been reported that copper intake by infants from breast milk is inadequate during the weaning period, especially if weaning foods are introduced at too early stage. Similarly, copper deficiency can occur because of infants' inability to use absorbed copper rather than a dietary insufficiency of the element [39].

In this study we found no significant differences between early and late weaning groups regarding total serum bilirubin, level of hepatic enzymes, and total serum protein level but there were significantly increased serum albumin levels in early weaning group than late weaning group. It has been known that early feeding can affect liver biochemistry because breast-fed infants have a higher risk of hyperbilirubinemia. [40]. **Jorgensen et al 2003** found higher mean serum bilirubin, albumin, and AST levels in breast-fed infants at 2, 6, and 9 months of age than in partially or non breast- fed infants at the same ages [41].

CONCLUSION

There were significantly higher weight and mean weight for age percentiles, Hb%, MCV, MCH, lymphocytes %, serum iron and cupper, serum albumin levels, CD4 % and count and CD4/CD8 ratio and significantly lower neutrophils % in early weaning group compared with late weaning group while there were no significant differences in number of exposure to gastroenteritis and respiratory tract infections, total leucocytes, eosinophils, platelets and reticulocytes counts, total bilirubin, total serum protein, ALT and AST, IgA and IgE levels and CD8 % and count between early and late weaning groups.

RECOMMENDATION

Based upon the above mentioned conclusion criteria; early weaning may be recommended in Egyptian infants.

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