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Cognitive Functions in Protein-Energy Malnutrition: In Relation to Long Chain-polyunsaturated Fatty Acids

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Abstract: Aiming to find out a correlation between plasma LC-PUFA levels and neurodevelopmental status of malnourished infants, the present study was conducted on 42 infants suffering from PEM, with a mean age of 11.28 ± 4.59 months. They were divided clinically into edematous and non-edematous groups. Fifteen age and sex matched well nourished apparently healthy infants were chosen to serve as controls. All patients were subjected to a 3-phase workup, while controls were subjected only to phase 1. Phase I: includes clinical assessment, laboratory investigations including plasma LC-PUFA levels and Neurodevelopmental assessment. In Phase II: An interventional program of 8 weeks duration; where all patients were receiving an initial supportive treatment followed by nutritional rehabilitation according to WHO guidelines, 1999 as well as developmental stimulation. According to the formula supplied to patients, they were randomly divided into either PUFA supplemented (+ve group) or nonBsupplemented (-ve group). In Phase III: All patients were re-assessed clinically and neurodevelopmentaly as well as re-evaluation for plasma LC-PUFA levels. The study revealed that, the mean plasma AA and DHA levels as well as the mean MDI and PDI scores of BSID-II were significantly lower in PEM patients compared to those levels after nutritional rehabilitation and to controls. Moreover, the mean MDI score was significantly lower in edematous subgroup compared to non-edematous one. Meanwhile, the mean rate of change in plasma DHA level was significantly higher in edematous subgroup compared to non-edematous one. However, there was no significant difference in the mean of rate of change in AA level or MDI and PDI scores between the 2 subgroups. Further, the mean rate of change in plasma AA and DHA levels as well as MDI score were significantly higher in PUFA +ve patients compared to PUFA Bve ones after nutritional rehabilitation. Finally, the study showed significant positive correlations between plasma AA and DHA levels and both MDI and PDI scores. From the course of this study we concluded that malnourished infants had impaired neurodevelopmental functions that could be related to the poor status of plasma LC-PUF. Thus, we recommend early intervention including nutritional rehabilitation and LC-PUFA supplementation as well as stimulation program, so as to have a better effect on future cognitive abilities of these infants.

Key words: LC-PUFA, malnutrition, cognition

INTRODUCTION

Protein energy malnutrition (PEM) is a pathological state resulting from insufficient intake of energy and other nutrients (Ge and Chang, 2001). Although the prevalence of malnutrition in developing countries is decreasing, it is still one of the most common causes of morbidity and mortality among infants and children under five years (Hoeree *et al.*, 2002).

Nutrition plays a critical role in prenatal and early postnatal development of brain at different levels including structural, chemical and functional levels (Singh, 2003). The earlier the onset of malnutrition, the higher the incidence of permanent impairment of brain development (WHO, 1999).

The central nervous system is highly enriched with long chain polyunsaturated fatty acids (LC-PUFA) especially arachidonic acid (AA) and docosahexanoic acid (DHA). The experimental studies have shown that dietary supplementation or deprivation of the precursors of LC-PUFA during the early stages of growth affect both LC-PUFA composition in nervous tissue and sensory-motor development (Gibson and Robert, 2004).

The presence of LC-PUFA in human milk but not formula coupled with the higher scores of breast fed versus formula-fed infants on standardized tests of neurodevelopment had focused attention on the importance of LC-PUFA for the optimal development of the vision and nervous system. Thus, many studies had compared fatty acids patterns as well as visual and

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cognitive outcomes of infants fed formula supplemented with LC-PUFA versus those fed unsupplemented formula (Winston *et al.*, 2003).

The plasma LC-PUFA status of children with PEM has been investigated in several studies and it was generally accepted that PEM is associated with decreased levels of PUFA in plasma lipids that may be due to low dietary intake, impaired desaturation and elongation of PUFA and/or increased PUFA peroxidation (Haussaini *et al.*, 2001). Restoration of normal energy, protein and essential fatty acid intake does not appear to readily correct abnormalities of plasma and erythrocyte membrane PUFA levels. Enhanced dietary supply of PUFA and/or improved supply of antioxidant vitamins may represent novel therapeutic modalities in severe PEM (Decsi and Koletzko, 2000).

The aim of this study was to assess the plasma LC-PUFA in infants suffering from PEM and its correlation with their neurodevelopment status as well as the effect of LC-PUFA supplementation with enriched formula on their plasma LC-PUFA levels and their cognitive outcome.

MATERIALS AND METHODS

The present study was conducted in Cairo in the period between January 2006 and May 2006 on 42 infants suffering from PEM, their ages ranged between 6-25 months with a mean age of 11.28 ± 4.59 months. They were 19 males and 23 females. They were recruited from the outpatient clinic, children's hospital, Ain Shams University and were diagnosed clinically as PEM on the basis of presence or absence of edema and Z-score of weight for length according to Gernaat and Voorhoeve (2000) classification,
They were divided into two groups:

- **Non-edematous group:** Included 14 patients having a Z-score < -2SD without edema (marasmic patients).
- **Edematous group:** Included 28 edematous patients; 14 of them had Z-score > -2SD (kwashiorkor {KWO} patients) and the other 14 patients had Z-score < -2SD (marasmic kwashiorkor patients).

Exclusion criteria included infants born prematurely and those with history of perinatal insult and/or abnormal neurological examination.

The study also included 15 age and sex matched, apparently healthy well nourished infants serving as controls. They were 8 males and 7 females with a mean age of 10.40 ± 4.89 months.

All patients were subjected to the following 3 phases while controls were subjected to phase 1 only.

Phase I:

- Full history taking laying stress on perinatal and dietetic history, current illnesses, appetite and history of vomiting, diarrhea, weight loss and edema.
- Socioeconomic status (Park and Park, 1979).
- Thorough clinical examination with special emphasis on vital measures, signs of dehydration or vitamin deficiency, presence of edema or hair and skin changes of KWO as well as chest and abdominal examinations.
- Anthropometric measurements including weight, length, skull circumference, midarm circumference and skin fold thickness centiles as well as weight for length (Z-score).

Laboratory investigations

- CBC by coulter 1660.
- C-Reactive Protein (CRP) by AVITEX-CPR latex test.
- Total plasma proteins, serum albumin, serum electrolytes by autoanalyser, synchrom Cx4/Cx5.
- Plasma LC-PUFA by high performance liquid chromatography (HPLC) supplied by Lepage and Roy (1988).

Principles of the test: 150 (μL) of serum were mixed with 5 mL [Methand (BDH-UK/ acetyl chloride (Aldrich, Germany)] 50:1 volume: volume and incubated for 45 min at room temperature in glass tube.

One milliliter hexane (Romil UK) was added to the tube and the contents were mixed. The hexane layer (upper layer) was then aspirated and evaporated to dryness under nitrogen gas stream.

The residue was dissolved in 25 (μL) of acetonitrite, then injected in HPLC equipment (Beckman -USA) using software system (Gold), detector unit module 166 for monitoring programmable solvent (module 126 AA) and reversed plasma column C18 (hypersil BDS 250×4.6 mm 5 μL) for separation of methyl ester FA (MEFA).

20 (μL) of dissolved FA was then injected in HLPC for separation and quantification using mobile phase methanol = H_2O (90: 10 volume: volume) with a flow rate 1 mL/min.

The MEFAs were detected as peaks by absorbance at 210 nm then quantitatively determined by measuring the peak area in relation to the concentration of the injected standards in 1/4 μg mL^{-1} unit.

Neurodevelopmental assessment: by using the Bayley scales of infant development-second edition (BSID-II) (Bayley, 1993).

Bayley scales of infant development (BSID-II) are individually administered examinations that assess the current developmental functions of infants and children from 1 month to 42 months of age. These consist of three scales: the mental scale, motor scale, and Behavior Rating Scale (BRS).

Mental scale: includes items that assess memory, habituation, problem solving, early number concepts, generalization, classification, vocalizations, language and social skills.

Motor scale: assesses control of the gross and fine muscle groups. This includes movements associated with rolling, crawling, creeping, sitting, standing, walking, running and jumping. It also tests fine motor manipulations involved in comprehension, adaptive use of writing implements and imitation of hand movements.

Behavior rating scale: assesses qualitative aspects of the child's attention/arousal (under 6 months of age), orientation/engagement towards the tasks, examiner and care giver, emotional regulation and quality of movement.

Principles of BSID-II:
Motor and mental scales:
Item sets

- To choose the appropriate item set (based on the child's chronological age), round the child's calculated age to the nearest whole month.
- These item sets were constructed by examining the performance of the children from the standardization sample.
- When testing a child of very low or very high ability, it may be necessary to test outside the item set appropriate for the child's age according to the basal and ceiling rules as shown in the table.

	Mental scale	Motor scale
Basal rule	5 or more no credit items	4 or more no credit items
Ceiling rule	3 or more no credit items	2 or more no credit items

Computing raw scores: The child's raw scores of the mental and motor scales of BSID-II are computed by adding the total number of items for which the child receives credit on each scale and all items below the basal item.

Obtaining Mental Development Index (MDI) and Psychomotor Development Index (PDI) were done according to special tables.

Each raw score has an equivalent index score for each scale. The index scores for each scale range form 50 to 150 with a mean value of 100 and standard deviation of 15.

Behavior rating scale (BRS):
- In the BRS, children were classified into 3 age groups: 1-5 months, 6-12 months, and 13-42 months.
- After completing all of the rating on BRS, obtain the total raw score fro each factor by the sum of scores of all factors.
- Finally, obtain the percentile rank and classification corresponding to the scores by referring to the appendix B of BSID-II.
- Decisions regarding cut off scores were based on the following criteria:

- 26th percentile and above = within normal limits.
- Between 11th -25th percentile = Questionable.
- At or below 1oth percentile = Non optimal.

Phase II: An interventional program of 8 weeks duration for all patients was carried out. Patients received an initial supportive treatment followed by nutritional rehabilitation according to the protocol of WHO (1999).

Initial treatment (The first week of management): Treatment and/or prevention of hypoglycemia, hypothermia, dehydration and electrolyte imbalance.

- Starting to feed the patient by diet which gives 80-100 Kcal/kg/day and 1-2 g protein/kg/day in addition to vitamins and trace elements.
- Treatment of any infection.
- Treatment of other problems i.e., severe anemia.

Rehabilitation (The next 2-6 Weeks of management):

- Encourage the patient to eat as much as possible by changing the diet in order to supply the patient with 150-220 Kcal/kg/day and 3-4 g protein/kg/day.
- Stimulation of the emotional and physical development as much as possible.
- Preparation of the mother or care-giver to continue to look after the infant and to follow-up after discharge.

Follow-up: (After discharge from hospital): Planned follow up of the child was carried out at regular intervals in the nutrition clinic in children's hospital, Ain Shams University so as to prevent the recurrence.

According to the formula supplied to the patients during the 8 weeks of management, patients were randomly divided into 2 groups:

PUFA -ve group: included 21 patients (7 non-edematous and 14 edematous) who received formula which does not contain LC-PUFA.

PUFA +ve group: included the 21 patients (7 non-edematous and 14 edematous) who received LC-PUFA enriched formula.

The fat content of LC-PUFA enriched formula is 3.6 g/100 mL consisting of a mixture of vegetable fat and animal fat (egg lipid). This fat blend provides beside the essential fatty acids (which present in all standard infant formulas), the LC-PUFA derivatives (AA and DHA) as recommended by ESPGHAN, 2005; ώ-6: ώ-3 ratio is 6:1 (ESPGHAN recommended ratio between 5:1 B 15: 1). AA: DHA ratio was 2:1 (ESPGHAN recommended the same ratio).

The estimated amount of AA = 0.17-0.19 g/100 g powder (= 0.02 g/100 mL), while the amount of DHA = 0.06-0.08 g/100 g powder (= 0.01 g/100 mL).

Phase III: After completion of the 8 weeks, all patients were reassessed clinically and neurodevelopmentally as well as for laboratory investigations.

Data were analyzed with statistica software package v.5 (Stat soft, Tulsa, OK, USA). Normally distributed variables were analyzed using student t test to compare mean values of different variables and paired t test for comparison of mean values before and after addition of LC-PUFA. Pearson R correlation coefficient was used to determine the relationship between different normally distributed variables. Mann-Whitney U-test was used to compare non parametric data. Rate of change was also calculated.

RESULTS

The mean values of plasma AA and DHA levels and MDI and PDI scores of BSID-II were significantly lower in PEM patients before nutritional rehabilitation compared to controls. Although, the significant increase of these values after nutritional rehabilitation, these were still significantly lower than controls. Meanwhile, the mean value of AA/DHA ratio was non-significantly higher in PEM patients compared to controls with non-significant decrease after nutritional rehabilitation (Table 1).

The mean values of plasma AA and DHA levels and MDI score of BSID-II were significantly lower in edematous and non-edematous PEM patients before nutritional rehabilitation compared to controls. Meanwhile, the mean value of MDI score was significantly lower in edematous subgroup compared to non-edematous one. However, the mean values of AA/DHA ratio were higher in edematous and non-edematous patients compared to controls and in edematous compared to non-edematous patients but these were statistically non-significant (Fig. 1 and 2).

After nutritional rehabilitation, the mean rate of change of plasma DHA level was significantly higher in edematous subgroup compared to non-edematous one, while, there was no significant difference in the mean rate of change of plasma AA level, MDI and PDI scores or AA/DHA ratio between edematous and non-edematous subgroups (Fig. 3).

Although there was significant increase in the mean values of plasma AA and DHA levels, MDI and PDI scores after nutritional rehabilitation, they were still significantly lower in both PUFA Bve and PUFA +ve

Table 1: The mean values of plasma AA and DHA levels, MDI and PDI scores and AA/DHA ratio among PEM patients (before and after rehabilitation) and controls

| Parameters | Controls (A) | PEM +ve patients | | p-value | | |
		Before rehabilitation (B)	After rehabilitation (c)	A vs B	A vs C	B vs C
AA (µg mL^{-1})	3.61±1.03	2.49±0.68*	2.96±0.78*	<0.001	<0.05	<0.001
DHA (µg mL^{-1})	1.29±0.41	0.79±0.13*	0.94±0.17*	<0.001	<0.05	<0.001
MDI score	97±10.92	76.3±8.78	88.19±8.93	<0.001	<0.05	<0.001
PDI score	97.13±12.38	72.76±11.27	87.39±8.85	<0.001	<0.05	<0.001
AA/DHA ratio	2.97±1.01	3.15±0.79	3.04±0.76	> 0.05	> 0.05	> 0.05

* = Mann Whitney U test

Table 2: The mean values of plasma AA and DHA levels, MDI and PDI scores and AA/DHA ratio in PUFA +ve patients (before and after nutritional rehabilitation) and controls

| Parameters | Controls (A) | PUFA +ve patients | | p-value | | |
		Before rehabilitation (B)	After rehabilitation (c)	A vs B	A vs C	B vs C
AA (µg mL^{-1})	3.61±1.03	2.45±0.73	3.17±0.81	<0.001	>0.05	<0.001
DHA (µg mL^{-1})	1.29±0.41	0.77±0.11	0.95±0.13	<0.001	<0.05	<0.05
MDI score	97±10.92	79.33±8.33	86.85±9.69	<0.001	<0.05	<0.001
PDI score	97.13±12.38	70.23±10.57	87.23±9.40	0.001	<0.05	<0.001
AA/DHA ratio	2.97±1.01	3.08±0.78	2.66±0.44	>0.05	>0.05	< 0.05

Table 3: The mean values of plasma AA and DHA levels, MDI and PDI scores and AA/DHA ratio in PUFA -ve patients (before and after nutritional rehabilitation) and controls

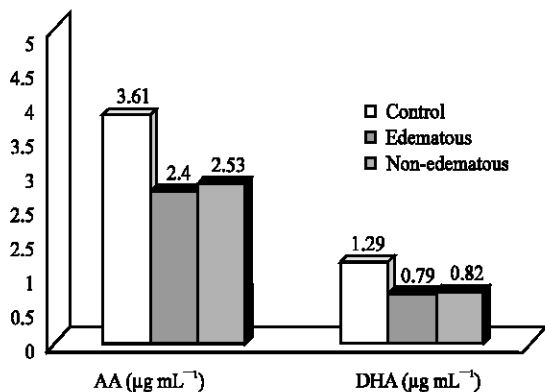| | | PUFA -ve patients | | p-value | | |
Parameters	Controls (A)	Before rehabilitation (B)	After rehabilitation (c)	A vs B	A vs C	B vs C
AA (μg mL^{-1})	3.61±1.03	2.52±0.64	2.76±0.70	<0.001	<0.05	<0.05
DHA (μg mL^{-1})	1.29±0.41	0.82±0.13	0.93±0.20	<0.001	<0.05	<0.05
MDI score	97±10.92	76.3± 8.78	88.19±8.93	<0.001	<0.05	<0.001
PDI score	97.13±12.38	75.28±11.62	87.47±8.43	<0.001	<0.05	<0.001
AA/DHA ratio	2.97±1.01	3.21±0.70	3.14±0.81	> 0.05	> 0.05	> 0.05

Fig. 1: The mean plasma AA and DHA levels in edematous and non-edematous patients before nutritional rehabilitation and controls
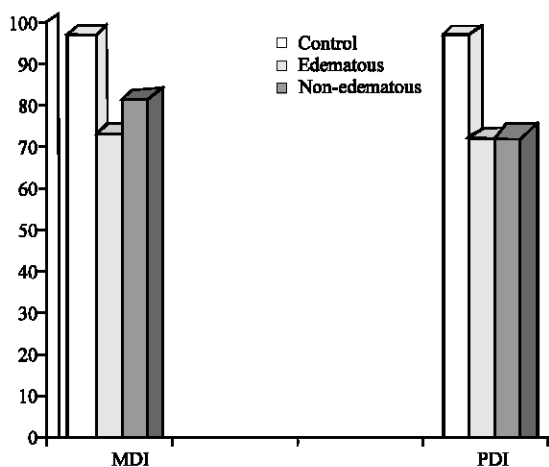

Fig. 2: The mean values of MDI and PDI scores among controls and both edematous and non-edematous patients before nutritional rehabilitation
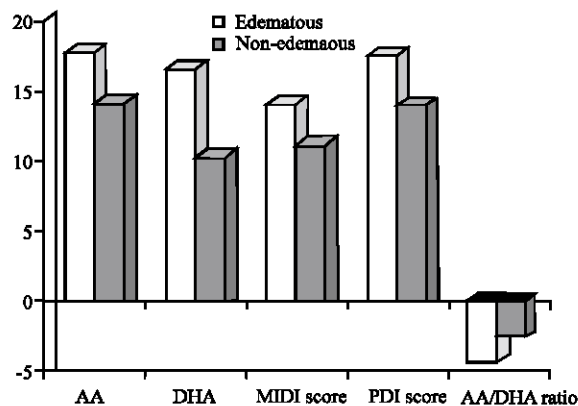

Fig. 3: The mean rate of change of AA and DHA plasma levels, MDI and PDI scores and AA/DHA ratio among edematous and non-edematous patients after nutritional rehabilitation
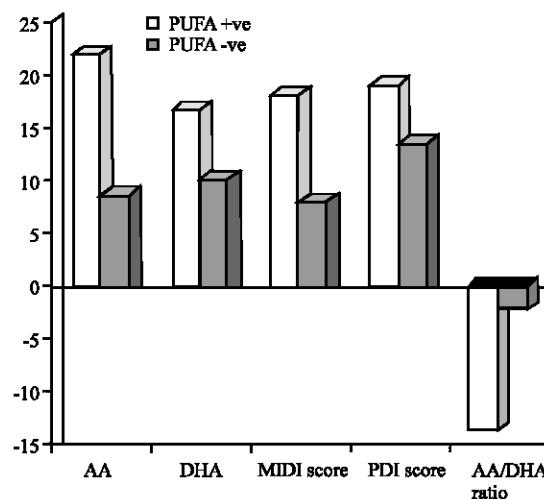

Fig. 4: The mean rate of change of AA and DHA plasma levels, MDI and PDI scores and AA/DHA ratio among PUFA +ve and PUFA Bve patients after nutritional rehabilitation

subgroups compared to controls, except for the mean value of plasma AA level which was still significantly lower only in PUFA -ve subgroup compared to controls. Meanwhile, there was significant decrease in AA/DHA ratio only in PUFA +ve group after nutritional rehabilitation (Table 2-3).

The mean rate of change of plasma AA and DHA levels, MDI and PDI scores as well as the AA/DHA ratio

were significantly higher in PUFA +ve patients compared to PUFA Bve ones after nutritional rehabilitation (Fig. 4). Both AA and DHA levels revealed significant positive correlations with both MDI and PDI scores (Table 4).

Table 4: Correlations between MDI and PDI scores and the plasma levels of both AA and DHA in the studied infants

Parameters	MDI	PDI
AA	0.54*	0.53*
DHA	0.52*	0.50*

* Significant value ($p<0.05$)

DISCUSSION

In this study, the mean values of plasma LC-PUFAs; AA (ω-6) and DHA (ω-3) were significantly lower in all PEM patients whether edematous or not at the start of the study compared to controls. This is in agreement with the study of Smit *et al.* (1997) who found that, children diagnosed as grades 2 and 3 of malnutrition had decreased erythrocyte Omega-6 and to lesser extent Omega-3 fatty acids. Similarly, Decsi *et al.* (1996) found significantly lower levels of both AA and DHA in plasma phospholipids of severely malnourished children compared to controls. Moreover, Decsi and Koletzko (2000) concluded that, the contribution of different Essential Fatty Acids (EFAs) and LC-PUFAs to the fatty acid composition of plasma and erythrocyte membrane lipids were reduced in children with PEM in comparison with well-nourished children. Smit *et al.* (2000 a) and Tichelaar (2000) explained the poor PUFA status in malnourished children on the basis of low intake, malabsorption, impaired desaturation and elongation enzymes, peroxidation of PUFAs and the use of PUFAs as an energy source via β oxidation.

The mean values of plasma LC-PUFAs; AA and DHA were lower in edematous than in non-edematous subgroups but the differences were statistically insignificant. However, Leichsenring *et al.* (1995) found that, children with KWO had a significant reduction of PUFAs in plasma cholesterol esters and plasma phospholipids compared to marasmic children and controls. Moreover, Franco *et al.* (1999) found low Linoleic Acid (LA) levels in the three groups of malnourished infants; marasmus, Kwo and marasmic Kwo with lower levels in marasmic than in KWO patients while the major metabolite of LA; AA was lower in Kwo group than in marasmic one. These results were explained by the deficient dietary intake of EFAs (LA and α- Linoleic acid) in marasmic patients, while impairment of LC-PUFA synthesis due to defective desaturase and elongase activities as well as the increased rate of PUFA peroxidation were more likely to occur in Kwo and marasmic KWO patients (Haussaini *et al.*, 2001).

In this study, the mean values of plasma LC-PUFAs showed highly significant increase after 8 weeks of nutritional rehabilitation in all malnourished patients whether given PUFA supplemented or unsupplemented formula. However, the rate of change in DHA levels was significantly higher in edematous than non-edematous patients. This indicates that, with adequate management of PEM, there is restoration of the enzymatic activities involved in fatty acid metabolism, so Linoleic acid and α- Linoleic acid (α- LA) can be readily converted to their LC-PUFA derivatives. Meanwhile, the mean rate of change in plasma AA and DHA were significantly higher in PUFA +ve group than in PUFA -ve one. This confirms that, LC-PUFA supplementation in malnourished children leads to a better improvement in their plasma LC- PUFA status. These results are in agreement with those of Smit *et al.* (2000 b) who supplemented 10 malnourished children aged 8-30 months with Fish Oil (FO) capsules for nine weeks. These children and controls did not have significantly different RBC fatty acid compositions at enrolment, whereas at the study end, FO supplemented children had higher red blood cell DHA and EPA compared to controls. However, no significant changes in RBC ω-6 fatty acids were found. They concluded that FO supplementation improves the DHA status of malnourished children and the supplement is apparently well absorbed and not exclusively used as a source of energy.

Similar results were obtained from the study of Marin *et al.* (2001) who studied plasma and erythrocyte phospholipids fatty acid composition in three groups of full term malnourished infants who were selected according to their prior feeding. Two groups had received commercial formulas, one of them supplied with LA and α-LA and the other supplied in addition with LC-PUFA from ω-3 series. Reference group of breast-fed infants was also enrolled. Infants who received formulas showed increased values of total saturated fatty acids and decreased values of PUFA from both ω-6 and ω-3 series compared to that of breast-fed infants. These differences were more remarkable in case of infants who received formula without PUFA. They concluded that, in malnourished infants, a nutrient formula enriched with LC-PUFA could be helpful to achieve an erythrocyte and plasma fatty acid pattern similar to that obtained in breast-fed infants.

In this study, the mean values of AA/DHA ratio were non-significantly higher in PEM patients whether edematous or non-edematous than controls. These values showed significant decrease only in patients received LC-PUFA supplemented formula according to the ratios recommended by ESPGHAN (2005). It was proved that high AA/DHA ratio promotes the pathogenesis of many diseases and negatively affects brain functions (Simopoulos, 2002).

In this study, the cognitive function of PEM patients was assessed by using BSID-II which is one of the most widely used tools to study infant global development. At the start of the study the mean values of MDI and PDI scores were significantly lower in malnourished patients whether edematous or non-edematous compared to controls. Similar results were obtained from the study of Grantham-Mc Gregor (1995) who found that during the acute stage of PEM, children's developmental levels were much poorer than adequately nourished children who were in hospital for other diseases. They also showed altered behavior, apathy and reduced activity levels. Further, Mohamed (1996) reported that malnourished infants had lower scores regarding MDI and Infant Behavior Recording (IBR) when compared to controls. In fact, the mechanism by which malnutrition could affect cognition are many including the direct insult to the brain which may be irreversible if occurred during the period of rapid brain growth, also, the undernourished children functionally isolate themselves from the environment and explore less and have reduced activity levels, so they acquire fewer skills (Levitsky and Strupp, 1995). Another explanation is that inadequate supply of a number of essential micronutrients can compromise brain function (Meck and Williams, 2003). Moreover, the deficiency of AA and DHA which are the major components of brain phospholipids and play a role in maintaining structural and functional integrity of membranes (Garcia *et al.*, 2005).

In this study, the mean value of MDI was significantly lower in edematous compared to non-edematous subgroup of PEM patients at presentation. This may be explained by mental changes which are common features during the acute stage of Kwashiorkor that may interfere with cognitive response (Brewster *et al.*, 1997).

After 8 weeks of nutritional rehabilitation, there was a significant increase in MDI and PDI scores of BSID-II in all PEM patients. However, they were still significantly lower when compared to controls. In agreement to these results, Mohamed (1996) found that the mean value of MDI in malnourished infants was significantly lower than that in another group of previously malnourished infants who have been studied 6-9 months after nutritional rehabilitation. Meanwhile, this previously malnourished group had lower scores compared to healthy controls. This may indicate that the negative effect of malnutrition on the cognitive function may be prolonged for uncertain period of time after nutritional rehabilitation and it may be influenced by the environmental deprivation which usually accompanies malnutrition that is more difficult to be changed.

Despite the significant increase in all indices of BSID-II in both edematous and non-edematous patients. Yet, the mean rate of change was higher in edematous subgroup compared to non-edematous one. However, differences did not reach significant values. So, inspite of the poor cognitive performance in KWO and M.KWO (edematous) patients during the acute stage of disease, they seem to catch up more rapidly than marasmic (non-edematous) patients if they receive an early nutritional rehabilitation and interventional program. These findings agree with those of Mendez and Adair (1999) who concluded that moderate undernutrition of long term duration may have poorer cognitive outcome than transient severe under nutrition because the severity of malnutrition and the stage of brain development also play a role in modifying its effect on the neurodevelopmental function.

In this study, the mean rate of change in BSID-II scores after nutritional rehabilitation in PUFA +ve subgroup were higher than in PUFA -ve one, but the difference was statistically significant only for MDI. Meanwhile, the levels of MDI and PDI scores had significant +ve correlations with the levels of both AA and DHA. Similar results were obtained in the studies done by Agostoni *et al.* (1995) and Lucas *et al.* (1999) who had assessed the neurodevelopmental outcome of healthy term and/or preterm infants after supplementation with LC-PUFA enriched formulas. Also, Birch *et al.* (2000) found significantly higher values of MDI in infants fed LC-PUFA enriched formula than in those fed placebo formula. However, PDI showed non-significant differences among diet groups in their study. Moreover, Bouwstra *et al.* (2003) also in a study of term infants, reported positive effects of LCPUFA supplementation at 3 months of age by using a test that assessed the quality of general movements considered to be an indicator of brain function and Fewtrell *et al.* (2004) reported significant enhancement effects in 18 months old children fed fish oil-supplemented formula as preterm infants in the MDI component of the Bayley Scales. On the other hand, Makrides *et al.* (2000) has found similar values of Bayley MDI and PDI in infants fed DHA supplemented, DHA + AA supplemented and placebo formulas at 1 and 2 years of age. Also, Auestad *et al.* (2003) in a 39-mo follow-up study of term infants, did not observe a significant difference in performance on several mental and motor development tests.

These findings may be due to that AA and DHA along with other smart micronutrients like vitamin B complex, vitamin C, vitamin E, iodine, iron, zinc, copper, taurine etc. are crucial for brain development and its

integrity and functionality (Singh, 2003). Also, AA and DHA are selectively incorporated, retained and highly concentrated in the phospholipids bilayer of biologically active brain and retinal neural membranes and are accumulated in large amounts during infancy (Winston *et al.*, 2003). The decrease in plasma DHA and/or AA may lead to defective neuronal functions due to modulation of neural membranes, dopamine and serotonin release, synthesis of biologically active derivatives and the nuclear mediated transcription of lipid responsive genes (Wainwright, 2002). Hence, researches had shown that LC-PUFAs may contribute to improved cognitive development (Heird, 2001).

The significant decrease of AA/DHA ratio in LC-PUFA supplemented patients in our study might be a contributing factor in the significant improvement of cognitive functions among this group (Straarup *et al.*, 2006). Moreover, the significant increase in both MDI and PDI in our patients may be in part due to the performed cognitive stimulation in addition to the improved nutritional levels which had been proved to make children to perform at substantially higher levels than those provided with only nutritional intervention (Angelsen *et al.*, 2001).

From the course of this study, we can conclude that malnourished infants and children had substantially decreased plasma levels of LC-PUFAs as well as impaired cognition as evidenced by lower scores of BSID-II. Supplementation of malnourished patients with LC-PUFAs enriched formula; for 8 weeks, produced a significant improvement in both plasma LC-PUFA status and neurodevelopmental function. Thus, LC-PUFA supplementation should be considered in dietary management of PEM because of its crucial effect in brain development. Further, environmental stimulation should be stressed in the global program of PEM rehabilitation.

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