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Research Article

Estimation of Serum Neurokinin A Level in Correlation to Visual Evoked Potentials and Morphological Brain Changes in Malnourished Children: A Follow-Up Case-Control Study

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ABSTRACT

Aim: *To investigate the effects of protein energy malnutrition (PEM) on the developing brain and to show the association between these changes and neurokinin A (NKA) levels .Re-evaluation after treatment was done.*

Methods: *A prospective case control study including 30 children with PEM plus 30 healthy age and sex matched controls was planned. Clinical examination along with laboratory measurement of albumin, iron, ferritin and NKA levels were done for all participants. Visual evoked potential (VEPs) and brain MRI were performed. Investigations were repeated after treatment and compared with previous data.*

Results: *Significant reduction in serum NKA levels of malnourished children was reported. NKA levels were associated negatively with significant latencies in VEPs at P2-N2 in Oz and Cz but positively at P2-N2 amplitudes inO_1, Oz and Cz within PEM group compared to controls. Malnourished children mostly showed grade 2 brain atrophy, with significant negative correlation between NKA levels and degree of brain atrophy. Nutritional rehabilitation was associated with increased NKA levels and significant improvement in both VEPs and brain atrophy.*

Conclusion: *PEM during early infancy significantly affects the developing central nervous system (CNS) in the form of morphological brain atrophy and altered neurophysiological function. These PEM-induced brain changes seem to be reversible thus malnutrition should be detected and treated early to prevent permanent CNS damage. As well, we shed new lights on NKA deficiency within these patients that may play a potential role in the etiological basis for PEM-induced brain atrophy in addition to the resultant functional disorders. Further extended researches are required to validate this tachykinin as a new alarm in early screening for the development of brain harms in PEM children within poor districts. Also, this may be helpful in predicting the prognosis of such patients.*

Keywords: *protein energy malnutrition (PEM); neurokinin A (NKA); visual evoked potential (VEPs); brain magnetic resonance imaging (MRI).*

INTRODUCTION

Protein energy malnutrition (PEM) is the most common leading cause of death in children within developing countries. The World Health Organization (WHO) reported that malnourished children numbered 181.9 million (32%) in developing countries and severe acute malnutrition affects nearly 20 million children in preschool-age, mostly from the African and south-east Asia regions [1]. As well, about 149.6 million children younger than 5 years are defined as malnourished in these regions when measured in terms of weight for age. This figure is 5 times the prevalence in the western world [2].

PEM develops in children whose consumption of protein and energy is insufficient to satisfy their nutritional needs. At early ages, malnutrition has an important impact on the central nervous system and adversely affects brain development. Thus, the longer the PEM, the younger the child, the poorer the maternal health and literacy are associated with more adverse effects of PEM on the nervous system [3,4]. Studies in experimental animals in the west and children within developing countries have revealed many adverse effects of PEM on the biochemistry of developing brain. These in turn lead to tissue damage, developmental growth and differentiation arrest, synaptic myelination and transmission reduction as well as retardation in the overall development of dendrites activity [4].

Visual Evoked Potentials (VEPs) are used primarily as an indicator for the sensory brain function. They are employed to measure the performing integrity of the visual pathways from retina via the optic nerves to the visual cortex of the brain. Also, they better quantify functional integrity of the optic pathways than scanning techniques such as magnetic resonance imaging (MRI) [5]. There is electrophysiological evidence that chronic malnutrition during infancy causes abnormal development of sensory processes via both human and animal studies which have shown abnormalities in VEPs [6].

Tachykinins are neuropeptides which are present in the central and peripheral nervous system where they act as neurotransmitters and/or neuromodulators [7,8]. On top, tachykinins have a number of neuroprotective physiological roles in medical conditions [9]. The mammalian tachykinin ligand-receptor system is considered an emerging target for central neurological disorders [9].

Neurokinin A (NKA), formerly known as Substance K, is one of the tachykinins which is highly homologous to substance P (SP). It is a neurologically active peptide translated from the pre-protachykinin gene A [10]. NKA has many excitatory effects on mammalian nervous systems and is also influential on the mammalian inflammatory and pain responses as well [11]. Additionally, it is involved in many stress induced neurological disorders such as depression, schizophrenia and epilepsy [12]. NKA is localized in frontal cortex, striatum, septum, hippocampus, substantia nigra, ventral tegmental area in addition to distinct subsets of amacrine cells in the retina of experimental animal [13]. However, the expression of NKA in humans brain and if it has any influence on the onset of PEM-induced neurological abnormalities in children or not is not entirely investigated thus far. The exact underlying basis of PEM-induced deficits in developing nervous system as well as the pathways that link malnutrition with brain maturity are not well established. Furthermore, the reversibility of this detected deterioration following treatment regimens and whether the brain damage is permanent or not is still not fully inspected.

The ultimate goal was to study the effect of PEM on the developing CNS by assessment of the levels of NKA, gVEPs and brain MRI. On top, we aimed to study the effect of nutritional rehabilitation on these parameters.

METHODS

Participants

A total of 60 children were included in this case-control study. Among those 60 children, 30

children with protein energy malnutrition (PEM) were categorized as a patient group (group I) and included 9 males (30%) and 21 females (70%) with their ages ranged from 2-14 months. Diagnosis of protein energy malnutrition was done according to the WHO classification of PEM using Z score [14]. The study comprised30 apparently healthy children who had no clinical manifestations suggestive of protein energy malnutrition or any risk factor of visual affection as a control group (group II). The children in the control group were 11 males (36.7%) and 19 females (63.3%) and their ages ranged from 2-14 months. The patients were recruited from the pediatric inpatient department at Maternity and Children University Hospital, Minia University, Egypt. The control subjects were collected from infants with history of febrile convlusions while performing electroencephalography at the neurophysiological unit, in Neurology department, Minia University Hospital after signing written consents due to refusal of the parents of healthy infants to give them chloral hydrate to do gVEPs.

The study was conducted during the period from January 2014 to June 2015.The patient's group was further followed up after the next 3 months from the recovery of malnutrition concerning their demographic data, clinical signs, visual evoked potential (VEP) test, radiological findings as well as laboratory findings. Children with mental retardation, history of head trauma, stroke, epilepsy, medical illness, ocular diseases like congenital glaucoma, cataract, uveitis or any risk factor for visual affection (Hyperbilirubinemia at serum level requiring exchange transfusion, perinatal asphyxia as indicated by low Apgar score, birth weight<1500 grams, congenital infections or bacterial meningitis) were excluded from the study.

The children were considered to have severe acute malnutrition if they had; weight for age < -3 SD, or weight-for-height/length < -3 Z-score from the median (z-scores) of normal child, mid upper arm circumference (MUAC) measurement under 11.5 cm and/or bilateral pitting oedema in both feet and legs. All the children were evaluated and examined for presence of any complications as gastro-enteritis (GE), pneumonia or sepsis. Our patients were treated according to recent updates of WHO standardized protocol for management of severe malnutrition [1]. Patients were considered for hospital discharge when their weight for height reached 90% of median.

Clinical Data Collection

All included children were subjected to thorough prenatal, natal and postnatal history taking. This history was involving sex and age of children, vaccination history, history of present illness, nutritional history, plus family history in particular history of childhood visual loss. Also, all children were subjected to careful clinical examination including anthropometric measurements. Their measurements were plotted on WHO Growth Charts to determine the percentiles for each parameter [14]. Brain MRI had been requested to all children. Also, VEP test was done to all subjects. All these examinations and investigations were performed to patients during PEM and 3 months from hospital discharge after their clinical improvement.

Blood Samples Collection

Blood samples (5 ml) were collected under complete sterile condition from all subjects. Samples from patients were withdrawn during their admission for treatment of PEM and 3 months after their improvement. Complete blood counts (CBC) samples (1 ml) were collected in anti- coagulants EDTA tubes. Serum was separated from the residual blood following sample clotting in plain tubes by centrifugation and analyzed for blood urea, serum creatinine, serum albumin, random blood sugar, serum ironand serum electrolytes including Na^{+}, $K^{+}$and Ca^{++}. The remaining serum was stored at -70 °C for further evaluation of NKA and serum ferritin.

Laboratory methods

Blood urea, serum creatinine and RBS were analyzed using automated colorimetric method (Mindray BS-800) while CBC was evaluated by automated blood counter (Sysmex KX-21N). Serum electrolytes including Na^{+}, $K^{+}$and Ca^{++} were measured with Audicom Automatic Electrolyte Analyzer (AC9900) and serum iron was assayed by Iron Colorimetric Assay Kit (Bio Vision, Inc., CA, USA).Ferritin and neurokinin A (NKA) levels were determined in the serum of all subjects. Serum ferritin was assayed by using enzyme immunosorbent assay (EIA) kit (Ferritin Human ELISA Kit, ab108837, Abcam, Cambridge, USA). The assay was performed according to the manufacturer's instructions. Final serum ferritin values were expressed in nanogram per milli (ng/ml). As well, NKA levels were detected in serum using a human NKA EIA kit (Kamiya Biomedical Company, Seattle, WA, USA) with cat. No. KT-53271.The assay was performed according to the manufacturer's instructions. Final NKA values were expressed in picograms per milli (pg/ml).

Radiological Examination

All patients were referred to Radiology Department for Magnetic Resonance Imaging (MRI) examination of their brains on admission and 3 months after hospital discharge. The patients were examined on 0.5 T MRI scanners (GE Bright Speed). The examination was done while infants laid in a supine position with their heads immobilized by molded foam placed around the head throughout the imaging procedure. Pediatric head coil was used. Most of infants were sedated by receiving chloral hydrate, 15-30 mg/kg before imaging [15].

Conventional T1WI and T2WI pulse sequences were acquired in different planes (axial, sagittal and coronal planes). These were including 2 image acquisitions including T1WI (TR/TE 450/15 msec, slice thickness 4 mm with inter-slice gap of 8 mm and matrix: 256 x168) in addition to T2WI (TR/TE 4750/110 msec, slice thickness 4 mm with inter-slice gap of 8 mm and matrix: 224 x 224). The conventional MR Images were assessed for the presence of features of focal/diffuse atrophic brain changes. These changes were involving global parenchymal volume loss; dilated sub-arachnoid spaces with prominent cortical sulci and deepened gyri along with widened Sylvian fissures (indicating cortical atrophy) as well as ventriculomegaly (indicating central atrophy).

Koedam atrophy score has been used to enable visual rating of cerebral atrophy on MRI. Cerebral atrophy has been graded as (grade 0) with closed sulci and no gyral atrophy, (grade 1) with mild sulci widening and mild gyral atrophy, (grade 2) with moderate sulci widening and moderate gyral atrophy in addition to (grade 3) with marked sulci widening and knife-blade gyral atrophy [16].

Goggles Visual Evoked Potential Test

VEP was performed in the neurophysiology unit in Neurology department, Minia university Hospital using Neuropack Manager (NIHON-KOHDEN) version 08-14, copyright 1997-2008 after Gaining Consent of the parents. VEP was done under chloral hydrate sedation in a dose of 15-30 mg/kg [15]. During the examination, the infants were lying on their parent's lap in a semi-dark and quiet room. Recordings were made from t (OZ), below (O1), (O2) and (CZ) according to the international 10-20 system. Impedance for each electrode was usually below 5-10 kΩ. The stimulus was a red flash delivered by the Neuropack Manager (NIHON-KOHDEN) light emitting diode goggles at a frequency of 2 Hz. Each trial consisted of 200 responses with excessive artifacts were automatically rejected. The trials were repeated two or more times to ensure reproducibility of the response. The second prominent positive wave was called P2 and the preceding prominent negative wave was labeled N2 according to visual evoked potential standards (2004). Measurements of P2 amplitude were made from the P2 peak to the preceding N2 peak.

Statistical Analysis

The collected data were statistically analyzed using statistical package for social sciences (SPSS) program version 20.0(SPSS Inc., Chicago, IL, USA). Quantitative results were presented as mean and standard deviation (SD) while qualitative data were presented as percents (%). Results were expressed as tables and figures. Student independant-test was used to compare results between groups as regards quantitative variable. p-values equal to or less than 0.05 are statistically significant. Correlation was performed by using Pearson correlation coefficient (r).

RESULTS

The malnourished showed statistically significant lower levels of potassium (p=0.036), calcium (p=0.006), serum iron (p=0.000), serum ferritin (p=0.003) and NKA (p=0.000) than apparently healthy infants. The degree of brain atrophy measured by MRI was grade 2 in 15 (50%), grade 1 in 7(23%) and more severe i.e. grade 3 in 6(20%) of malnourished cases. The random blood sugar and albumin showed no significant difference between both groups.

The malnourished infants showed P1 which present in 3 (10%), N1 in 14(46%) and N3 in 6(20%) when recording from both eyes. The malnourished patients showed significant delayed of P2 and N2 in both eyes at Oz (p=0.035 & 0.004) and Cz (p=0.027) respectively than controls. There was no significant difference of P2-N2 amplitude between right, left and both eyes between both groups. The latencies of P2 and N2 were significantly prolonged in right (p=0.008 & 0.014), left (p=0.049 & 0.47)and both eyes (p=0.048 & 0.026 respectively) between patients and controls with increasing in delay with increasing severity of malnutrition. There was no significant difference in gVEPs amplitude (difference between P2-N2) between patients and controls at Oz and Cz points of recordings as well as right and left eye in both groups. The latencies of P2 and N2 showed partially significant negative correlation with serum iron and ferritin at the Cz point (for iron r=-0.22; p=0,214; r=-o.31 p=0,047 and for ferritin r=-0.29, p=0.034; r=-o.42 p=0,019 respectively). The right eye P2 and N2 latencies were negatively correlated with serum iron and ferritin (for iron r=-0.24, p=0.034; r=-0.35 p=0.049 and for ferritin r=- 0.28, p=0.025; r=-0.37 p=0027 respectively) while the left eye were not.

The albumin level were positively correlated with the P2 and N2 latencies in both eyes (r=0.36, p=0.037 and r=0.40, p=0.032 respectively) however there was no correlation with right or left eyes.

The NKA was statistically negatively correlated with P2 and N2 in both eyes at Oz (r=-0.48, p=0.030; r=-0.472, p=0.036) and Cz (r=-0.434, p=0.05; r=-0.430, p=0.05) respectively without significant correlation in either right or left eye. However, the NKA was positively correlated with the amplitude between P2-N2 in left (r=0.686, p=0.001) and both eyes (at Oz r=0.465, p=0.039 and at Cz r=0.451, p=0.046). The NKA was positively correlated with age, weight (r=0.66, p= 0.001), serum albumin respectively as well as length (r=0.71, p=0.000) and head circumference(r=0.47, p=0.036). While was negatively correlated with oedema (r=-0.514, p=0.021) and degree of brain atrophy (r=-0.93, p=0.000).

After nutritional rehabilitation, significant improvement of serum iron, ferritin, NKA and latencies by VEP were documented (table VIII).

Table I: Comparison of demographic and clinical data between patients and control groups

Variable		Groups		*p*-value
		Group (I) PEM Patients (30 children)	Group (II) Control (30 children)	
Age (Months) Range Mean (SD)		(2-14) 6.65 (4.11)	(4-14) 7.86 (2.64)	0.290
Gender	Male	9 (30%)	11 (36.7%)	0.122
	Female	21 (70%)	19 (63.3%)	
Weight (kg) Range Mean (SD)		(2-9) 4.40 (1.8)	(6.75-10.5) 8.68 (1.5)	**0.000****
Length (cm) Range Mean (SD)		(52.0-80.0) 64.2 (8.1)	(64.0-82.0) 71.9 (5.4)	**0.005***
Head circumference (cm) Range Mean (SD)		(37.0-46.0) 40.6 (2.7)	(40.0-46.5) 42.8 (1.6)	**0.020***
Z- score	Normal	-	30 (100%)	**0.000****
	Moderate PEM	9 (30.0%)	-	
	Severe PEM	21 (70.0%)	-	
Oedema	Yes	7 (23.3%)	-	
	No	23 (76.7%)	30 (100%)	

SD= standard deviation; PEM= protein energy malnutrition
* Significant (p≤0.05) ** Significant (p≤0.001)

Table II: Comparison between patients and control groups regarding laboratory data and MRI findings

Variable		Groups		*p*-value
		Group (I) PEM Patients (30 children)	Group (II) Control (30 children)	
Serum albumin (g/dL) Range Mean (SD)		(2.00-4.00) 3.24(0.68)	(3.00-4.10) 3.47 (0.50)	0.295
Potassium (mmol/L) Range Mean (SD)		(3.10-4.22) 3.86 (0.29)	(3.21-5.70) 4.51 (0.88)	**0.036***
Calcium (mg/dL) Range Mean (SD)		(7.0-9.7) 8.78 (0.65)	(8.90-10.0) 9.36(0.36)	**0.006***
Random blood sugar (mg/dL) Range Mean(SD)		(70.0-150.0) 103.1 (7.8)	(75.0-130.0) 99.7 (3.1)	0.796
Serum Iron (µg/dL) Range Mean (SD)		(32.5-69.7) 48.4 (14.4)	(57.9-96.5) 82.5 (19.6)	**0.000****
Serum ferritin (ng/ml) Range Mean (SD)		(34.7-68.4) 51.7 (16.1)	(46.7-89.4) 74.1 (21.8)	**0.003***
NKA (pg/ml) Range Mean (SD)		(9.0-36.0) 19.2 (8.5)	(36.0-42.0) 38.6 (1.68)	**0.000****
Brain atrophy grading (MRI)	Grade 0	2 (6.7%)	30 (100%)	**0.000****
	Grade 1	7 (23.3)	-	
	Grade 2	15 (50%)	-	
	Grade 3	6 (20%)	-	

SD= standard deviation; PEM= protein energy malnutrition; NKA= neurokinin A; MRI= Magnetic Resonance Imaging
* Significant (p≤0.05) ** Significant (p≤0.001)

Table III: Comparison of gVEPs latencies between patients and controls at Oz and Cz points of recording

gVEPs Oz & Cz (m. sec)		Groups		p-value
		Group (I) Patients (30 children)	Group (II) Control (30 children)	
Oz	P2	108.6 (24.5)	91.5(25.7)	**0.035***
	N2	157.7 (34.6)	129.8 (25.3)	**0.004****
Cz	P1	84.9 (30.2)	60.0 (24.9)	**0.032***
	N1	89.2 (42.7)	68.2 (21.5)	0.506
	P2	107.5 (40.3)	79.9 (17.1)	**0.027***
	N2	153.6 (56.3)	113.8 (20.9)	**0.027***
	P3	235.5 (54.3)	159.7(48.3)	**0.048***
	N3	244.3(25.4)	158.0 (26.4)	**0.010***

gVEPs= goggles Visual Evoked Potentials; Oz= mid occiput; Cz= mid central
* Significant (p≤0.05) ** Significant (p≤0.001)

Table IV: Comparison of P2 and N2 latencies among grades of malnutrition according to Z- score

gVEPs latencies (m. sec)		Z- score			*p*-value
		Normal	Moderate malnutrition	Severe Malnutrition	
O_2	P2	95.3 (18.8)	111.9(16.5)	123.2 (33.1)	**0.008***
	N2	131.9(37.9)	153.4(18.8)	183.1 (37.6)	**0.014***
O_1	P2	94.4 (26.6)	132.1(25.7)	119.4 (32.3)	**0.049***
	N2	131.2(25.6)	139.8(36.4)	165.1 (36.7)	**0.047***
Oz	P2	91.5(23.7)	121.5 (17.9)	103.2 (30.2)	**0.048***
	N2	129.8(29.4)	173.2(17.9)	151.1 (38.8)	**0.026***
Cz	P2	87.7(27.1)	95.2(16.9)	106.2(45.1)	0.365
	N2	85.1(38.5)	57.3(10.3)	92.1 (44.2)	0.439

gVEPs= goggles Visual Evoked Potentials; Oz= mid occiput; Cz= mid central;
O1= Left occipital point recording;O2= Right occipital point recording

Table V: Correlation between gVEPs latencies and serum iron, ferritin plus albumin levels in malnourished children

gVEPs latencies (m. sec)		Iron		Ferritin		Albumin	
		r	*p*	r	*P*	r	*P*
O_2	P2	-0.24	**0.034***	-0.28	**0.025***	0.12	0.487
	N2	-0.35	**0.049***	-0.37	**0.027***	0.15	0.385
O_1	P2	-0.27	**0.031***	-0.24	0.147	0.17	0.331
	N2	-0.17	0.321	-0.19	0.324	0.24	0.180
Oz	P2	-0.11	0.521	-0.14	0.495	0.36	**0.037***
	N2	-0.14	0.442	-0.17	0.374	0.40	**0.032***
Cz	P2	-0.22	0.214	-0.29	**0.034***	0.31	0.078
	N2	-0.31	**0.047***	-0.42	**0.019***	0.19	0.280

r= Correlation coefficient; r= 0.75 - 1(strong correlation); r= 0.5 - 0.74(moderate correlation);
r= 0.25 - 0.49(fair correlation); r= 0.1 - 0.24(weak correlation)
**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table VI: Correlation between NKA levels and demographic data, clinical features as well as degree of brain atrophy in malnourished children group

Correlations	NKA	
	R	*p*-value
Age (months)	0.663	**0.001****
Weight (kg)	0.665	**0.001****
Length (cm)	0.719	**0.000****
Head circumference (cm)	0.472	**0.036***
Oedema	-0.514	**0.021***
Serum albumin (g/dL)	0.668	**0.001****
Potassium (mmol/L)	-0.058	0.810
Calcium (mg/dL)	-0.302	0.196
Random blood sugar (mg/dL)	0.051	0.832
Serum Iron (µg/dL)	0.321	0.167
Serum ferritin (ng/ml)	0.135	0.570
Degree of brain atrophy (MRI)	-0.93	**0.000****

r= Correlation coefficient; r= 0.75 - 1(strong correlation); r= 0.5 - 0.74(moderate correlation); r= 0.25 - 0.49(fair correlation); r= 0.1 - 0.24(weak correlation)
**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed).

Table VII: Correlation between NKA levels of malnourished children and goggle visual evoked potential latencies and amplitudes

Correlations	NKA	
	r	*p*-value
Oz P2 latency (m. sec)	-0.485	**0.030***
N2 latency (m. sec)	-0.472	**0.036***
Cz P2 latency (m. sec)	-0.434	**0.05***
N2 latency (m. sec)	-0.430	**0.05***
O_2 P2-N2 amplitude (uv)	0.279	0.234
O_1 P2-N2 amplitude (uv)	0.686	**0.001****
Oz P2-N2 amplitude (uv)	0.465	**0.039***
Cz P2-N2 amplitude (uv)	0.451	**0.046***

r= Correlation coefficient; r= 0.75 - 1(strong correlation); r= 0.5 - 0.74(moderate correlation); r= 0.25 - 0.49(fair correlation); r= 0.1 - 0.24(weak correlation)
**Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed).

Table VIII: Comparison between PEM cases at onset and 3 months after discharge from hospital as regards NKA, laboratory data, MRI findings and visual evokes

Variable		PEM children		*p*-value
		At admission Mean (SD)	Follow up (after 3 months) Mean (SD)	
NKA (pg/ml)		19.2 (8.50)	34.0(6.7)	**0.000****
Albumin (g/dL)		3.24 (0.68)	3.5 (0.9)	**0.47***
Iron (µg/dL)		48.4 (14.4)	85.7(10.1)	**0.001****
ferritin (ng/ml)		51.7 (16.1)	128.2 (13.30)	**0.005****
Latencies (m. sec)				
Oz	**P2**	108.6(24.5)	90.1(15.0)	**0.049***
	N2	157.7 (34.6)	122.1(16.10)	**0.001****
Cz	**P2**	107.5 (40.3)	82.1 (18.5)	**0.046***
	N2	153.6 (56.3)	116.3 (17.60)	**0.023***
O_2	**P2**	118.3 (29.8)	94.8(18.0)	**0.036***
	N2	156.8 (45.5)	124.3 (15.80)	**0.000****
O_1	**P2**	119.6 (31.0)	94.7(11.5)	**0.045***
	N2	146.2 (39.2)	123.1 (12.50)	**0.002***

NKA= neurokinin A; Oz= mid occiput; Cz= mid central; O1= Left occipital point recording; O2= Right occipital point recording
*Significant ($p \leq 0.05$) ** Significant ($p \leq 0.001$)

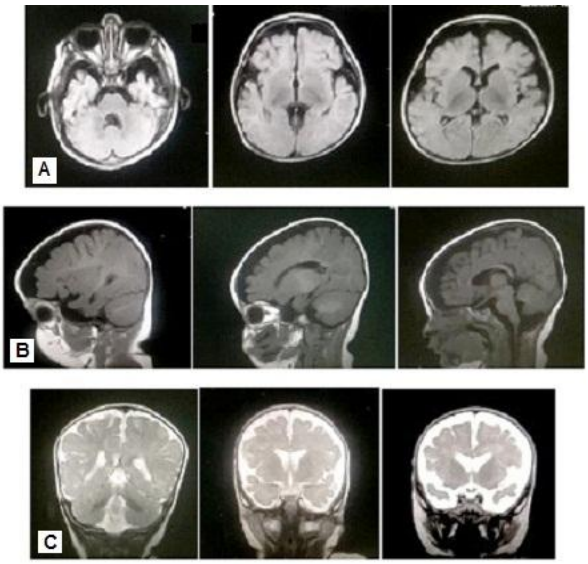

Figure (1): Brain MRI for PEM infant. Three months old infant presented by severe PEM.(A)Axial T1WI.(B) Sagittal T1WI.(C)Coronal T2WI. Moderate degree of cortical atrophic brain changes (grade II) in the form of prominent cortical sulci, widened gyri deepened inter-hemispheric fissure as well as Sylvian fissures.

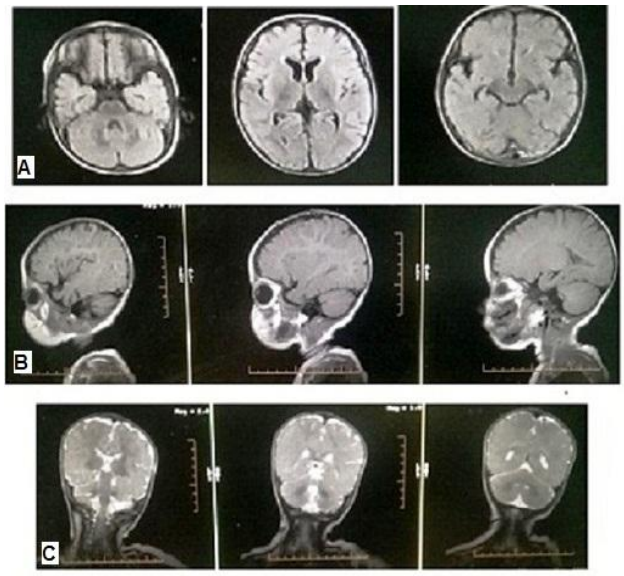

Figure (2): MRI for follow up of PEM infant. Follow-up of the same child in fig. 1 after three months of nutritional rehabilitation. (A) Axial T1WI. (B) Sagittal T1WI. (C) Coronal T2WI. Brain showed improvement of previously seen cortical atrophic brain changes with unremarkable brain MRI.

DISCUSSION

Protein energy malnutrition (PEM) is a major problem predominantly in developing countries. About 6% of children under age of five years are underweight for their age [17]. Miller, (2013) reported that malnutrition impairs the mental and cognitive development of the child thus can lower their IQ by up to 9 points [18]. However, the exact underlying base of PEM-induced brain disorders is yet mysterious. The present study aimed to investigate the effects of malnutrition on the developing brain of malnourished children by measurement of their visual evoked potentials (VEP) and detecting PEM-related morphological brain changes via MRI. Another complementary goal of our study was to correlate between both VEPs and MRI findings from PEM children and NKA as our proposed potential trigger for the changes that affects their developing brain.

Based on Z score, 30% of malnourished children in the present study were suffering from moderate PEM while 70% were severely malnourished. Also, 23.35% of children among those severely malnourished ones were edematous. In our study, we found that the head circumference was significantly lower in PEM children compared to the control group. Elizabeth et al., (2014) reported that brain weight is reduced in severely malnourished children and this deficit in weight of the brain is accompanied by a deficit in total number of brain cells [19]. This is supported by MRI findings in our study. Here, brain atrophy measured by MRI was grade 2 in 15 (50%), grade 1 in 7 (23%) and grade 3 in 6(20%) of malnourished cases. This was in agreement with El-Sherif et al study, 2012 who found that cerebral atrophy and a dilated ventricle were the commonest MRI findings seen in the children suffering from severe malnutrition and also comes in concordance with the study carried by Atalabi et al., 2010 [20,21]. Odabaş et al., 2005 showed that all malnourished children had cerebral atrophy and 75% of the children had cerebral atrophy plus ventricular dilatation but none of the children had abnormality in the brain stem or cerebellum [22]

Moreover, we found insignificant difference regarding serum albumin between malnourished children and healthy controls. This finding disagrees with the previous study of Mahaman et al., 2014 who found that the malnourished children had significantly lower serum concentrations of albumin than controls [23]. This could be

referred to the low percentage of edematous children in ours study in comparison to these others studies. On the other hand, the serum levels of calcium, potassium, iron and ferritin were significantly decreased in malnourished children. The reduced levels in malnourished children could be attributed to dietary deficiency of these nutrients or because of infections owing to increased metabolic losses. This finding agrees with previous ones reported by Adegbusi and Sule 2011 and Rahman and Begum 2005 who found that serum total calcium was markedly decreased in correlation to the type of PEM [24,25]. Additionally, the studies of Mahaman et al., 2014, Muller and Krawinkel, 2005 and Shaaban et al., 2005 stated a decrease in serum iron and ferritin and hence anemia within experimental malnutrition. They found a decrease in iron incorporation, in the number of reticulocytes and furthermore an interruption in the maturation process of erythroblasts [23], [26-27].

Neuronal tissue can be severely damaged either through physical trauma or intracellular stresses, either chronic or acute. Either of these scenarios can result in calcium overload, protein degradation, unfolded protein response or an accumulation of DNA damage [9]**.** Endogenous cellular responses are activated within nerve tissue in response to damage in order to protect cellular proteins plus its nucleic acid integrity and from here the role of tachykinins emerge and could be significantly apparent. It has been established that tachykinins have a number of neuroprotective physiological roles in medical conditions [9]**.** As one of the TK peptides, NKA is a major excitatory neurotransmitter in the peripheral nervous system. Furthermore, NKA plays different roles not only as neurotransmitter but also as a local factor involved in almost all regulatory aspects of neuro-physiological functions beside brain patho-physiological processe [28].

To the best of our knowledge, there were no reported previous studies which were carried on NKA in PEM or between NKA and VEPs; only some studies were done on NKA in patients with depression, schizopherina and epilepsy [12]. Also, most of the studies that concerned about NKA were conducted on experimental animals not on humans. Krock et al., 2016 and Burbach et al., 2001 reported that Neurokinin A can directly induce nerve growth factor mRNA expression and the secretion of bioactive nerve growth factor protein in both human and murine keratinocyte nerve growth factor [29,30]**.** Moreover, it has been shown that neurokinin-1 tachykinin receptor plays a role in the synaptic plasticity associated with morphological and functional development of the mammalian CNS in rat brain. SP is the preferential endogenous ligand for this receptor type. However, it does not exclude the possibility that NKA can also stimulate NK-1 sites. In fact, various studies have clearly shown that all three mammalian tachykinins can interact with the various NK receptor classes [31]. Likewise, studies of receptor trafficking and synaptic transmission provided evidence for neuronal transmission. According to these studies, neuronal tachykinins stimulate neurokinin receptors (NKRs)on enteric nerves. Activation of enteric neurons persuades endocytosis of the NK_1R which requires neuronal conduction, SP and NKA release and thus NK_1R activation [32,33]. Tachykinins make excitation of postsynaptic potentials in enteric neurons by activating NK_1R and NK_3R [34]. Therefore, our hypothesis was that NKA may have a possible impact on PEM-induced brain aberrations and we aimed to accomplish a real life study to explore the role of NKA in PEM-induced brain deficits within children. In this study, there was a significant decrease in NKA levels in malnourished children which had positive correlations with age, weight, length and head circumference in malnourished children. On the contrary, negative correlations with the degree of brain atrophy was demonstrated. Regarding serum albumin levels and the degree of oedema, there were significant correlations between both of them and NKA levels. Moreover, these low levels of NKA may indicate the presence of brain damage.

Schäffer et al., 2005 had shown that substance K is localized to distinct subsets of amacrine cells in the anuran retina [11]. Similarly, Teuchner et al., 2010 revealed that NKA features neuroprotective properties in the rat retina [35]. However, these studies were performed on experimental animals only and no further studies were performed to support these conclusions regarding NKA retinal localization or its visual impacts in humans. Our study correlates between serum NKA levels and VEPs in PEM children. VEPs are used primarily to measure the functional integrity or disruption of the visual pathways in neurologic diseases, causing central nervous system pathology [5].

Our study showed a significant negative correlation between NKA with P2 and N2 latencies in both eyes at Oz and Cz but positive correlation with the amplitude at P2-N2 in left eyes. There were no significant differences between right and left eyes in latencies of all VEPs in both patients and control. In addition, N1, P2, N2(at Oz point of recording) were significantly delayed in patients than control. Also, P2 and N2 (at Cz point of recording) showed significant statistical delay in patients than controls in both eyes. The present results agreed with McDonald et al., 2007and dos Santo and Alencer 2010 who reported that malnourished children had a significant delay in VEPs and are less sensitive to the frequencies of the waves than the normal healthy children [36,37]. Furthermore, the present findings showed that there were insignificant decrease in O2, O1 and Cz amplitudes between both control and patient groups. These results partly agreed with Colon and Visser, 2012who reported that the amplitude decreased in comparison to the normal control eyes and on recovery it was shown that the amplitude of the pattern VEP was significantly correlated with the visual acuity [38]. In this study, there was a significant ($p<0.05$) delay in P2 and N2 of both eyes in children with severe and moderate malnutrition. Also, N2 and P2 of Oz latencies in the moderate and severe malnutrition children were delayed significantly ($p<0.05$) than the normal children. These results are in line with McDonald et al., 2007; Durmaz et al., 1999and Faldella et al., 1996 who found that children with PEM had obvious delay in visual evoked potentials [36],[39-40]. Significant reduction in nerve conduction velocity may be due to nutritional deficiency affecting myelination of peripheral nerves. Severe and chronic malnutrition affects motor & sensory nerve conduction velocity but mild to moderate malnutrition alters sensory nerve conduction velocity which is more sensitive than motor nerve conduction study in detecting early or mild demyelinating diseases.

Follow up of our patients after their discharge from hospital were done on regular basis in nutritional rehabilitation clinic, complete improvement of PEM were attained in most of our patients within 3 months from their discharge, at this time the previous laboratory investigations mainly NKA, ferritin and albumin in addition to gVEPs and brain MRI were repeated to detect the impact of nutritional rehabilitation on these parameters concerned with brain functions and myelination. A Follow up assessment of our patients is considered a great advantage of this study which adds to it a clinical significance. In our follow up, we found significant elevation of serum iron,ferritin,albumin and NKA in children after recovery from malnutrition. Also, follow up MRI revealed improvement of previous atrophic changes in 90% of cases and this comes in concordance with Elsherif et al study 2012 [20] Gunston et al., suggested that loss of myelin lipid accounts for the cerebral shrinkage seen in their patients and restoration of lipid to the myelin membrane with re-feeding accounts for the reversal of cerebral shrinkage [41]. Additionally, we found significant improvement of latencies detected by VEPs after nutritional rehabilitation. This was in disagreement with McDonald et al., 2007 who performed fVEPs which continued to show abnormality at discharge. They suggested the possibility that nutritional rehabilitation did not fully eliminate the physiological deficit [36]

The limitations to the current literature involve: 1) The relatively small numbers of participants which are due to financial issues. 2) The lack of association between NKA levels and IQ of PEM subjects after recovery. Larger multi-centers studies as well as longer follow up ones are necessary in order to seek deeper into the detailed impact of NKA along with its connecting neuronal pathways with exploration of other possible involved mediators. Furthermore, seeking for any connection between NKA levels and both cognitive functions as well as child IQ of subjects who had history of PEM after nutritional rehabilitation should be considered in future directions. Finally, measuring the levels of NKA in the serum of PEM children mother's plus recognizing if NKA is secreted in their breast milk or not could be valuable later on.

CONCLUSION

From our findings, we can conclude that a reduction in energy and/or essential nutrient supply riming the first stages of life have profound effects on somatic growth and organ structural plus functional development for the growing brain. Thus, it will affect the future of our developing country. These changes are hopefully reversible with proper nutritional rehabilitation. Additionally, we ascertained that NKA could play a role in the occurrence of these PEM-related brain harms which needs to be extensively researched. Prevention of early childhood malnutrition as well as early recognition and management must be a major public health goal, especially in our developing countries.

REFERENCES

1. WHO Guideline: updates on the management of severe acute malnutrition in infants and children. Geneva: World Health Organization; 2013.
2. Agozie C, Ngozi S, Chika, Chinyeaka M and Chinelo A. Under-five Protein Energy Malnutrition Admitted at the University of In Nigeria Teaching Hospital, Enugu: a 10 year retrospective review. Nutrition Journal 2012: 11-43.
3. Udani PM. Protein energy malnutrition (PEM), brain and various facets of child development. Indian J Pediatr1992; 59(2):165-86.
4. Labadarios, D, Mchiza Z.J.R., Steyn NP et al. Food security in South Africa: a review of national surveys. Bulletin of the World Health Organization 2011; 89(12):891-899.
5. Hemalatha G, Anuradha B, and Adinarayana R. Processing of Visual Evoked Potentials using Mode Deviation. International Journal of Engineering. Trends and Technology (IJETT) 2015.
6. Chaudhary R, Chugh M, Darokhan Z, Katreddi R, Ramachandra R and Rema V. Physiological Slowing and Upregulation of Inhibition in Cortex are Correlated with Behavioral Deficits in Protein Malnourished Rats. PLoS one 2013; 8(10).
7. Vaudry H, Conlon JM. Identification of a peptide arising from the specific post translation processing of secretogranin II. FEBS Lett 1991;284:31–3.
8. Kirchmair R, Hogue-Angeletti R, Gutierrez J, Fischer-Colbrie R, Winkler H. Secretoneurin — a neuropeptide generated in brain, adrenal medulla and other endocrine tissues by proteolytical processsing of secretogranin II (chromogranin C). Neuroscience 1993;53:359–65.
9. Maudsley, Stuart; et al. "The Mammalian Tachykinin Ligand-Receptor System: An Emerging Target For Central Neurological Disorders". CNS & Neurological Disorders - Drug Targets2010; 9 (5): 627–635.
10. Nakanishi, Shigetada. "Molecular Mechanisms Of Intercellular Communication In The Hormonal And Neural Systems". IUBMB Life 2006; 58 (5/6): 349–357.
11. Schäffer DA, Gábriel R. Two major tachykinins, substance P and substance K, are localized to distinct subsets of

amacrine cells in the anuran retina. NeurosciLett.2005; 7;386(3):194-8.
12. Nagano, Masatoshi;Oishi, Takao; Suzuki, Hidenori. "Distribution And Pharmacological Characterization Of Primate NK-2 Tachykinin Receptor In The Central Nervous System Of The Rhesus Monkey". NeurosciLett. 2011;503 (1): 23–26.
13. Martin S. Steinhoff, Bengt von Mentzer, PierangeloGeppetti, CharalabosPothoulakis, and Nigel W. Bunnett. Tachykinins and Their Receptors: Contributions to Physiological Control and the Mechanisms of Disease. Physiol Rev, 2014; 94(1):265-301.
14. WHO. Child growth standards and the identification of severe acute malnutritionin infants and children.World Health Organization and the United NationsChildren's Fund1995.
15. Baranak C, Marsh R and PotsicW .Sedation in brainstem response audiometry. International Journal of Pediatric Otorhinolaryngology,1984; 8: 55-59.
16. Koedam EL, Lehmann M, Van der FlierWM et al.Visual assessment of posterior atrophy development of a MRI rating scale. Eur Radiol 2011;21:2618-2625.
17. El-Zanaty, Fatma and Ann Way. Egypt Demographic and Health Survey 2008. Cairo, Egypt: Ministry of Health, El-Zanaty and Associates, and Macro International.2009
18. Miller J. Iron Deficiency Anemia: A Common and Curable Disease. Cold SpringHarbPerspect Med 2013;3.
19. Elizabeth L Prado and Kathryn G Dewey. Nutrition and brain development in early life. 2014; (72) 4, 267–284.
20. El-Sherif A ,Babrs G and Ismail A. Cranial Magnetic Resonance Imaging (MRI) Changes in Severely Malnourished Children before and after Treatment.Life science journal 2012;9.
21. Atalabi OM, Lagunju IA, Tongo OO, Akinyinka OO.Cranial magnetic imaging findings in Kwashiorkor". Int J Neurosci2010; 120(1):23-7.
22. Odabaş D, Caksen H, Sar S et al. Cranial MRI findings in children with protein energy malnutrition. Int J Neurosci. 2005;115(6):829-37
23. Mahaman Y., Akuyam S., Danborno B.,Galadima O., Belemsigri M. and Moussa SM. Evaluation of some Laboratory Parameters of Malnourished Children in Magaria District, Zinder, Niger Republic. Sub-Saharan African Journal of Medicine 2014; 1(2).
24. Adegbusi H., Sule M. Anthropometric and biochemical assessment among under five children in Kusada Local Government Area, Katsina State, Nigeria.Bayero J Pure Appl Sci 2011;4:137-40.
25. Rahman M. and Begum BA. Serum total protein, albumin and A/G ratio in different grades of protein energy malnutrition. Mymensingh Med J 2005;l:38-40.
26. Muller O. and Krawinkel M. Malnutrition and health in developing countries.Can Med Ass J 2005;171:279-93.
27. Shaaban S., Marzouk D, Nassar M., Ezzat N., Mohamed I. Early detection of protein energy malnutrition in Sharkia Governorate. J Egypt Public Health Assoc. 2005; 80(5-6):665-85.
28. TakenoriOnaga. Tachykinin: recent developments and novel roles in health and disease.Biomolecular Concepts.2014;3(5) : 225–243.
29. Emerson Krock, J. Brooke Currie, Michael H. Weber, Jean A. Ouellet, Laura S. Stone, Derek H. Rosenzweig and Lisbet Haglund. Nerve Growth Factor Is Regulated by Toll-Like Receptor 2 in Human Intervertebral Discs. The Journal of Biological Chemistry 2016; 291:3541-3551.
30. Burbach, Guido J; Kyu; Zivony, Adam S; Kim, Amy; Aranda, Jennifer; Wright,

Stacey; Naik, Shubhada M; Caughman, S Wright; Ansel, John C; Armstrong, Cheryl A. The Neurosensory Tachykinins Substance P and Neurokinin A Directly Induce Keratinocyte Nerve Growth Factor". Journal of Investigative Dermatology. 2001; 117 (5): 1075–1082

31. Taoka M, Song SY, Kubota M, Minegishi A, Yamakuni T, Konishi S. Increased level of neurokinin-1 tachykinin receptor gene expression during early postnatal development of rat brain. Neuroscience 1996; 74(3):845-53.
32. Component analysis. Clin Neurophy-siol.2007 ;118(4):896-900.
33. Grady EF, Gamp PD, Jones E, Baluk P, McDonald DM, Payan DG, BunnettNW. Endocytosis and recycling of neurokinin 1 receptors in enteric neurons. Neuroscience 1996;75: 1239–1254.
34. Southwell BR, Seybold VS, Woodman HL, Jenkinson KM, Furness JB. Quantitation of neurokinin 1 receptor internalization and recycling in guinea-pig myenteric neurons. Neuroscience 1998;87: 925–931.
35. Alex G, Kunze WA, Furness JB, ClercN. Comparison of the effects of neurokinin-3 receptor blockade on two forms of slow synaptic transmission in myenteric AH neurons. Neuroscience 2001; 104: 263–269, 2001.
36. Barbara Teuchner, Andreas Dimmer, Josef Troger et al. Secretoneurin and the tachykinins substance P and neurokinin-A/B in NMDA-induced excitotoxicity in the rat retina. Regulatory Peptides 165 (2010) 123–127
37. McDonald CG, Joffe CL, Barnet AB, Flinn JM. Abnormal flash visual evoked potentials in malnourished infants: an evaluation using principal dos Santos Natanael Antonio ; Caroline Costa Gomes Alencar. Early malnutrition diffusely affects children contrast sensitivity to sine-wave gratings of different spatial frequencies. 2010;4(13), 189-194
38. Colon E, VisserSL . Evoked Potential Manual: A Practical Guide to Clinical Applications (2012).
39. Durmaz S, Karagöl U, Deda G, OnalMz. Brainstem auditory and visual evoked potentials in children with protein-energy malnutrition. Pediatr Int. 1999 ;41(6):615-9.
40. Faldella G., Marina G., Rosina A., et al. Visual evoked potentials and dietary long chain polyunsaturated fatty acids in preterm infants. Archives of Disease in Childhood 1996;75:108-1 12.
41. Gunston GD, Burkimsher D, Malan H, Sive AA.Reversible cerebral shrinkage in kwashiorkor: an MRI study". Arch Dis Child1992;67(8):1030–2.