

Research Article

Hyperhomocysteinemia, Cognitive Deficits, and Delayed P300 Latencies in Malnourished Children from Northern India

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ABSTRACT

Background: Malnutrition during childhood can adversely affect behaviour and cognitive performance. Vitamin B deficiencies in particular have been linked to cognitive impairment and hyperhomocysteinemia, a risk factor for cardiovascular and neurological disorders. The objective of this study was to assess the effects of malnourishment on cognition and plasma vitamin B12, folate, and homocysteine levels in children in Northern India.

Methods: 825 school going children were screened following which 75 children were included in the malnourished group and 50 apparently healthy children in the control group. Attention span, long and short-term memory, and latenciesof the brain event related potential P300 were assessed as the cognitive measures. 1.0 ml blood (fasting) was collected from each subject in EDTA tubes and centrifuged (2000 rpm) at 4 °C for 20 minutes,separate the plasma. Fasting plasma levels of homocysteine, vitamin B12, and folate were also determined by ELISA.

Results: Significantly lower scores in the attention span and memory tests were observed in the malnourished group as compared to the control group ($P < 0.001$). These differences were further exemplified by delayed P300 latencies observed in the malnourished group as compared to controls ($P < 0.001$). Elevated plasma homocysteine along with lower folate, and vitamin B12 levels were also observed in the malnourished group when compared to controls($P<0.001$).

Discussion: These results encourage pilot studies to establish whether interventions to treat vitamin B12 folate and iron deficiencies in the malnourished children studied here could help improve cognitive performance and ameliorate hyperhomocysteinemia.

MeSH Headings/Keywords: Child malnutrition; Attention span and memory; Cognition; Folate and vitamin B12; B vitamins; Homocysteine; Event related potentials; P300

Introduction

Nutrients play an important role in the development and functioning of the brain. The importance of nutrients in neurotransmission and for the maintenance of neuronal integrity and brain structure have been the topic of several reviews [1-7]. Malnutrition during childhood can adversely affect behavior and cognitive performance and is also associated with a greater susceptibility to infections [8-10]. Best et al. [11], following a comprehensive review of the literature, highlighted malnutrition in school-aged children as a public health issue for the developing world and also pointed out the relatively limited information available on the micronutrient status in this sub-population [12]. It is therefore of great importance to monitor the prevalence of malnutrition within this age-group along with their micronutrient status. This would enable targeted interventions to address various etiologies and thereby disorders, associated with malnutrition. For example, B vitamin deficiency is associated with cognitive impairment as well as hyperhomocysteinemia, a condition that is associated with a range of cardiovascular and neurological diseases. Significantly, intervention with appropriate supplements can ameliorate hyperhomocysteinemia [13-19].

There is a growing need for sensitive, objective, and culturally sensitive tools to assess cognitive function in order to monitor the adverse neurological effects associated with malnutrition. Such tools will also help evaluate the effectiveness of dietary or other interventions towards countering these adverse effects. Additionally, the consistent application of these tools will facilitate cross-study comparisons by researchers and policy makers and thereby enable informed decision making when formulating regional or national health education and food fortification programmes [20]. P300, a positive going brain event-related potential (ERP) with a peak amplitude at approximately 300ms (hence the name), has the potential to emerge as the basis of such tools which importantly, can be applicable to different age groups [21]. In clinical and laboratory settings P300 is commonly elicited and detected during signal detection tasks (e.g. auditory or visual) as a component of a characteristic ERP waveform which also consists of preceding positive going P100 and P200 and negative going N100 and N200 components. Although P300 has not been conclusively linked to an exact cognitive process, it has been associated with cognitive information processing which includes attention, memory, and decision-making and is therefore a useful tool to assess cognitive function [21,22]. Delayed P300 latencies (time between stimulus and detection of peak amplitude) are indicative of cognitive impairment/dysfunction and have been associated with neurological disorders such as Alzheimer's and Parkinson's disease [23-29]. P300 latencies have also been used as a cognitive measure in children [30,31].

The objective of this study was to assess the effects of malnutrition on cognition in school-going children in Northern India using P300 latencies as well as attention span and long and short-term memory scores as cognitive measures. Fasting plasma levels of homocysteine, vitamin B12 and folate were also determined due to their association with cognitive impairment.

Methods

Subjects

The study was approved by the Institutional Ethics Committee, Institute of Medical Sciences, Banaras Hindu University, Varanasi (vide Ref. no. Dean/2012-13/376 and dated 17-12-2012). The children were selected from Sonbhadra, Chandowali, and Varanasi in Eastern Uttar Pradesh, India.The parents were approached through teachers at the respective schools and informed consent from the parents of the children was obtained prior to their participation in the study. 825 school going children aged 5 to 12 years old of both genders were initially screened for malnutrition on the basis of body mass index (BMI) [weight(kg)/height^2(m^2)] following the World Health Organizations parameters which specifies normal values for 5 to 12 year olds as 15.26-17.53 and 15.24-18.00 for boys and girls respectively. Children whose body mass index values were <2 times lower than the standard deviation of the reference values were classified as malnourished [32]. BMI were measured by taking a children's weight and dividing by their height squared with help of scales and stadiometer. Children previously diagnosed with auditory canal infections, the presence of risk factors for hearing loss, malformation in the ear auricle, or neurological/behavioural pathologies were excluded from the study. Additionally, anamnesis, inspection of the external auditory canal, conventional pure tone audiometry, and acoustic immittance testing were conducted to exclude children with hearing abnormalities. After the screening procedures75 children diagnosed with malnutrition were included in the study.50 apparently healthy children with adequate nutritional status were also included in the study as controls.

Nutritional values

The dietary habits of the children were recorded by the 24 hour dietary recall method and a food-frequency questionnaire was used to collect information on daily calorie and nutrient intake of the children [33]. This diagnosis was performed by a nutritionist.

P300 latency measurement and cognitive tests

P300 ERPs were elicited and measured as auditory ERPs using an odd ball paradigm as previously described with minor modifications [30]. Recording of P300 ERPs were conducted using the Medigl+ NDE-2 computerized neuron density evaluation system (investigational product) and the P300 component was defined as the highest amplitude positive peak occurring between 250-550 ms in response to the rare stimulus. These values were selected to encompass the range of latency values observed in a recent meta-analysis [21]. Memory and attention span tests were performed using electronic devices (Medicaid, India).

Collection of blood and biochemical assessments

1.0 ml blood (fasting) was collected from each subjectin EDTA tubes and centrifuged (2000 rpm) at 4°C for 20 minutes. After centrifugation, plasma was collected and dispensed into 1.5 ml eppendorf tubes and stored at –80°C until assay. Plasma folic acid, vitamin B12, and total homocysteine levels were determined using commercially available ELISA kits (ENZO Life sciences).

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences version 16 software. All data are presented as mean ± standard error mean (SEM). Group means were compared using the student t-test. P values < 0.05 were considered as significant.

Results

75 malnourished (35 males, 40 females; age 8.33 ± 0.13 years) and 50 apparently healthy (28 males, 22 females; age 8.32 ± 0.26 years) childrenwere included in the study.The daily nutrition values recorded forthe children using the 24 hour recall method are presented in Table 1. Significantly lower protein, calcium, iron, vitamin A, vitamin C and calories (energy) were recorded in the malnourished group when compared to the controls. The malnourished group also had a significantly lower body mass index indicating a negative impact of the dietary and nutritional deficiencies on growth in these children (Table 1; P < 0.001).

Folate and vitamin B12 deficiencies have been linked with cognitive impairment [15] and their plasma levels were therefore determined in this study. Plasma levels of these vitamins were found to be significantly lower in the malnourished children (Table 2; P <0.001). Folate and vitamin B12 deficiencies can also result in hyperhomocysteinemia, a condition associated with various cardiovascular and neurological disorders [16,19,34].

Table 1: Daily nutrition values and body mass index recorded in the control and malnourished children.

	Control	Malnourished	t-value	P value
Protein (gm)	22 ± 0.31	10 ± 0.14	40.0	<0.001
Calcium (gm)	569 ± 5.9	347 ± 4.6	29.09	<0.001
Iron (gm)	14 ± 0.19	6 ± 0.14	35.39	<0.001
Vitamin A (iu)	1333 ± 27.5	655± 12.8	24.47	<0.001
Vitamin C (mg)	25 ± 0.53	10 ± 0.12	33.01	<0.001
Energy (kcal)	2200 ± 31.2	1100 ± 28.7	25.29	<0.001
Body mass index	14.62 ± 0.10	10.09 ± 0.12	27.17	<0.001

Values are expressed as mean ± SEM

Table 2: Biochemical parameters measured in the control and malnourished children.

	Control	Malnourished	t-value	P-value
Folate (nmol/L)	28.42 ± 0.28	15.97 ± 0.60	16.04	<0.001
Vitamin B12 (pg/ml)	348.64 ± 10.28	236.98 ± 6.15	9.90	<0.001
homocysteine (µmol/L)	6.82 ± 0.12	28.69 ± 0.31	56.06	<0.001

The results obtained here revealed dramatically higher plasma homocysteine levels in the malnourished children (>4 fold) when compared to the control group (Table 2; P < 0.001).

In order to identify potential adverse effects of malnutrition on cognition in this study population, short and long term memory and attention span tests were conducted. Significantly lower scores on these cognitive measures were observed in the malnourished group compared to the controls (Table 3; P < 0.001) indicating potential adverse neurological effects of the nutritional deficiencies (and elevated homocysteine levels) in the malnourished children. To further assess the effects of malnutrition on cognition, the latencies of the auditory P300 ERP were recorded in both groups as a measure of information processing [21]. Significantly higher P300 latencies were observed in the malnourished children compared to the control group (Table 3; P < 0.001), which provides further evidence towards the adverse effects of malnutrition on cognition in these children.

In addition, malnourished children had significantly lower folic acid levels(15.97 ± 5.23 nmo/L) versus 15.97 ± 5.23nmol/L; P < 0.01) as well as a significantly lower BMI (P < 0.001) than healthy children (Table 2). A non-significant negative correlation between folic acid and other biological paremerters were observed in malnourished children (Table 4).

Discussion

Nutrientsare quantitatively and qualitativelyessential for proper growth and development and play an important role in cognitive functions via their involvement in numerous processes such as energy metabolism andneurotransmission [3]. It is therefore unsurprising that malnourished children are prone towards developing neurological abnormalities involving behaviour (e.g. attention deficit hyperactivity disorders) and cognition [8,12]. The importance of monitoring

Table 3: Cognitive parameters and P300 latencies measured in the control and malnourished children.

	Control	Malnourished	t- value	P-value
Attention span score	9.28 ± 0.18	6.93 ± 0.13	10.98	<0.001
Short term memory score	7.96 ± 0.21	4.45 ± 0.12	15.57	<0.001
Long term memory score	18.62 ± 0.29	13.92 ± 0.22	12.92	<0.001
P300 latency (ms)	271.4± 1.35	421.8± 2.34	49.16	<0.001

Values are expressed as mean ± SEM

and alleviating child malnutrition can be summarised using the following excerpt from a World Bank report–"Malnutrition slows economic growth and perpetuates poverty throughthree routes-direct losses in productivity from poor physical status; indirectlosses from poor cognitive function and deficits in schooling; and lossesowing to increased health care costs" [35]. Indeed, a recent comprehensive review of the literature highlighted malnutrition in school-aged childrenas a public health issue for the developing world [12].

Owing to various social, economic, and cultural factors, child malnutrition is of particular concern within India

[3,36,37]. The present study was conducted to assess the impact of malnutrition on cognition in school-aged children in a region in Northern India and found both memory and attention deficits in the malnourished children. These malnutrition associated cognitive deficits were further underpinned by the delayed auditory P300 latencies observed in the malnourished children when compared to healthy controls. This was in agreement with a previous study conducted in Brazil which also observed delayed P300 latencies associated with child malnutrition.[30] Additionally, an Indian study reported delayed P300 latencies in children with poor academic performance compared to good performers although the nutritional status of the subjects was not investigated [31]. P300 latencies, a putative measure for information processing, have been used by numerous researchers and clinicians as a measure of cognitive function in both non-diseased subjects and in clinical conditions such as Alzheimer's and Parkinson's disease [21,23-28]. The employment of simple auditory tones to elicit P300 ERPs and the portable nature of the equipment required to measure P300 latencies makes this evoked potential a particularly useful, objective, and culturally sensitive cognitive parameter. This parameter could prove to be especially useful to determine adverse effects of malnutrition in children from the economically disadvantaged, illiterate, uneducated, and often neglected population in rural India.

Significantly lower levels of daily nutrient (including vitamin A, vitamin C, iron and calcium) intake in the malnourished children studied here may have contributed towards the cognitive deficits observed in them. Vitamin A plays an important role in the regulation of neural plasticity [38], whereas Vitamin C, besides its role as an anti-oxidant, is involved in processes such as myelin formation and synaptic potentiation [39]. Iron, an essential element, is required for

Table 4: Correlation between folic acid and biological parameters of the both group.

Biological parameters	Correlation coefficient value (r value)		P value	
	Control	Malnourished	Control	Malnourished
tHcy (µmol/L)	-0.006	0.064	0.968	0.586
Vitamin B12 (pg/ml)	0.174	-0.252*	0.228	0.029
Vitamin A (iu)	0.141	-0.169	0.327	0.148
Vitamin C (mg)	-0.128	0.001	0.375	0.991
Attention span (Score)	0.075	-0.100	0.605	0.394
BMI	-0.143	-0.089	0.323	0.447
Short term memory (Score)	-0.025	-0.073	0.865	0.536
Long term memory (Score)	-0.016	-0.080	0.910	0.496
Protein (gm)	-0.120	0.005	0.408	0.964
Calcium (gm)	0.194	-0.172	0.176	0.140
Iron (mg)	0.042	-0.179	0.774	0.125
Energy kcal	-0.077	0.076	0.593	0.517

[f]r value is Pearson Correlation co-relation; NS: Non-Significant

oxygen and electron transport and myelin and neurotransmitter synthesis [40,41]. Calcium acts as an intracellular messenger in nerve cell signalling pathways and as a cofactor in the activation of enzymes and is involved in the release of neurotransmitters at the synapse [42,43]. Therefore deficiencies of these nutrients can affect a multitude of processes in the central nervous system including intra and extra cellular signalling, neurotransmission, and energy metabolism and thereby affect cognitive function.

The neurological functions of B vitamins (folate and vitamin B12) in particular have been the topic of extensive research owing to the key roles they play in virtually all levels of brain function and epidemiological studies linking their deficiencies to cognitive impairment and . hyperhomocysteinemia [15,44]. In the present study lower levels of vitamin B12 and folate were found in the malnourished children when compared to the control group. Of particular concern was the degree (>4 fold) to which homocysteine levels were elevated in the malnourished children when compared to the control group. Hyperhomocysteinemia has been associated with cardiovascular and neurological disorders including dementia [15,16,19]. Although a causal role for homocysteine in the onset of dementia is still a matter of debate [15,34,45], some key studies have pointed out the risk that elevated levels of this non-protein amino acid may pose [14]. Notably, elevated homocysteine levels are associated with greater rates of brain atrophy which can be retarded by interventions involving homocysteine lowering B-vitamins [46,47]. Another study reported that elevated homocysteine levels in midlife also increases the risk of dementia in later life [17]. Therefore, managing homocysteine levels in these malnourished children via interventions (dietary management or supplements) that increase the intake of vitamin B12 and folate may not only concurrently improve cognitive performances but also lower the risk of a multitude of diseases in later life.

Considering the lower daily intake of other nutrients by the malnourished children, it is possible that interventions involving other micro or macro nutrients (other than folate and vitamin B12) may also be required to address the cognitive deficits in these children. Comprehensive micronutrient profiling in these children will help determine whether more comprehensive intervention plans will be required. Encouragingly, various interventions have proven to be effective in addressing the adverse effects of malnutrition on both growth and cognition [12,20].

Conclusion

In conclusion, the elevated plasma levels of homocysteine coupled with lower folate and vitamin B12 levels and the lower attention span and memory scores coupled with delayed P300 latencies observed in the malnourished children studied here encourage pilot studies involving homocysteine lowering interventions in these children. In addition, iron is the most important nutrient for normal haemoglobin levels,seem to correlate to cognitive performance. Its deficiency is directly altered neurotransmitter in the brain and affect behaviour and cognitive performance in the malnourished children, has a negative impact on learning, motor skills, cognitive, behaviour and also socioeconomic status. Therefore, iron supplements seem to be beneficial in the malnourished children. Such interventions could not only improve behaviour and cognitive performance but also potentially lower the risk of neurological and cardiovascular disorders in later life.

Funding

The study was supported by the Department of Science and Technology, Ministry of Health, Government of India.

Conflict of Interest

The authors declare no conflicts of interest.

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Submitted Mar 04, 2016
Accepted Apr 19, 2016