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 latencies of 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
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].

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 included in the study. The daily 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 subject 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) children were included in the study. The daily nutrition values recorded for the 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].

50 apparently healthy children with adequate nutritional status were also included in the study as controls.
Hypermethionemia, Cognitive Deficits, and Delayed P300 Latencies in Malnourished Children from Northern India

Values are expressed as mean ± SEM

<table>
<thead>
<tr>
<th>Table 1: Daily nutrition values and body mass index recorded in the control and malnourished children.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>Protein (gm)</td>
</tr>
<tr>
<td>Calcium (gm)</td>
</tr>
<tr>
<td>Iron (gm)</td>
</tr>
<tr>
<td>Vitamin A (iu)</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
</tr>
<tr>
<td>Energy (kcal)</td>
</tr>
<tr>
<td>Body mass index</td>
</tr>
</tbody>
</table>

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 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 and alleviating child malnutrition can be summarised using the following excerpt from a World Bank report—“Malnutrition slows economic growth and perpetuates poverty through three routes-direct losses in productivity from poor physical status; indirect losses from poor cognitive function and deficits in schooling; and losses owing to increased health care costs” [35]. Indeed, a recent comprehensive review of the literature highlighted malnutrition in school-aged children as 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.

<table>
<thead>
<tr>
<th>Table 2: Biochemical parameters measured in the control and malnourished children.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
</tr>
<tr>
<td>homocysteine (µmol/L)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3: Cognitive parameters and P300 latencies measured in the control and malnourished children.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>Attention span score</td>
</tr>
<tr>
<td>Short term memory score</td>
</tr>
<tr>
<td>Long term memory score</td>
</tr>
<tr>
<td>P300 latency (ms)</td>
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</tbody>
</table>

Values are expressed as mean ± SEM

Discussion

Nutrients are quantitatively and qualitatively essential for proper growth and development and play an important role in cognitive functions via their involvement in numerous processes such as energy metabolism and neurotransmission [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
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.

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

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

*r value is Pearson Correlation co-relation; NS: Non-Significant

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Conflict of Interest

The authors declare no conflicts of interest.

REFERENCES


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