A Study of Event Related Potentials in Vitamin B12 Deficient Patients without Neuropsychiatric Symptoms
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Abstract: The aim of the study was to evaluate Event Related Potentials (ERPs) in patients with Vitamin B12 deficiency without neuropsychiatric symptoms and effect of Vitamin B12 supplementation on ERPs. The patients were divided into 3 groups according to their Serum Vitamin B12 levels. The serum levels of Vitamin B12 in the groups were- Group 1 - < 211pgm/ml (deficient), Group 2 - 211-350 pgm/ml (insufficient) and Group 3 - > 350pgm/ml (sufficient). Standardized Mini Mental State Examination (SMMSE) was done in all subjects at the time of entry point. ERPs study and SMMSE were repeated in subjects of group 1 after 3 months of Vitamin B12 supplementation. Latency of N200 and P300 of group 1 showed statistically significant difference when compared to group 2 and 3 before treatment (p<0.05) and the difference disappeared after 3 months of Vitamin B12 supplementation. The study concludes that ERP parameters are deranged prior to clinical manifestation of neuropsychiatric features in Vitamin B12 deficiency and can be used to assess efficacy of Vitamin B12 supplementation.

Keywords: SMMSE; ERPs; Vitamin B12 deficiency; P200; P300; N200.

INTRODUCTION
The ERPs have garnered much attention as simple yet powerful tool to access the eponymous human brain and its functioning. The ERPs technique can be used to assess brain activity at some particular moment and hence has immense utility as they are direct instantaneous reflection of neurotransmitter mediated neural activity. ERPs can be easily assessed in hospital setting with minimal cost and have the potential to be used as biomarkers for disease screening, risk stratification and as indices of progression.

The ERP components strongly associated with cognitive process, selective attention and conscious discrimination in human being are N200 negativity and P300 positivity appearing 200ms and 300ms post stimulus respectively. Shorter P300 latencies indicate superior cognitive processing while P300 amplitude is a reflection of stimulus information and attention. The more attentive a patient is, the larger the P300 amplitude [1, 2]. Prolonged P300 latencies have been reported in various disorders associated with abnormal cognition like Alzheimer’s disease, Parkinson’s disease or other neurodegenerative diseases, psychiatric disorders, traumatic brain injury, various nutritional, toxic and metabolic disorders. Vitamin B12 deficiency with neurobehavioural abnormalities has been shown to be associated with abnormal P300 latencies in various studies, which improves after Vitamin B12 supplementation [3, 4].

The aim of our study was to assess ERP parameters in subjects with Vitamin B12 deficiency without neuropsychiatric symptom and normal SMMSE scores and change in ERP parameters after Vitamin B12 supplementation.

MATERIALS AND METHODS
Subjects
Patients aged between 18-65 years, who were incidentally found to be Vitamin B12 deficient but without neuropsychological symptoms in neurology OPD or wards were included in this study between June 2016 and December 2017. The subjects were divided into 3 groups according to their Serum Vitamin B12 levels. Group 1 included 21 patients with serum Vitamin B12 level below 211pgm/ml. Group 2 patients had Serum Vitamin B12 level 211-350 pgm/ml and Group 3 patients had serum Vitamin B12 level more than 350 pgm/ml. Exclusion criteria included history of focal neurological deficit, head injury, CNS infection, cerebrovascular disease, endocrinopathies like diabetes mellitus, thyroid dysfunction or any disorder with
potential to affect cognitive process and symptomatic memory impairment.

Procedure
All the participants were subjected to systemic and neurological examination and were investigated for hemogram, biochemistry including fasting blood sugar, renal and liver function test, HIV and serum thyroid levels. Vitamin B12 levels were assayed in serum sample using ADVIA Centaur Assay (sensitivity 45-2000 pg/ml, normal range 211-911 pg/ml). All the participants underwent ERPs studies. Participants of Group 1 received Vitamin B12 supplementation in the form of six intramuscular (IM) injections of 1000 mcgm of cyanocobalamin every alternate day after sensitivity testing and 1000 mcgm IM monthly injections for maintenance. ERPs studies were recorded after 3 months of Vitamin B12 supplementation in Group 1 participants only.

Apparatus and running the test
The P300 parameters were elicited by standard oddball paradigm with auditory stimuli on Recorders and Medicare System machine (Chandigarh). Two types of auditory stimuli, target (infrequent) and non target (frequent) with frequency 1000 Hz and 500 Hz respectively were presented in a pseudo-random manner, so that no two non target stimuli appeared consecutively. The target and non target stimuli comprised 20% and 80% respectively of the total auditory stimuli presented during the test. Auditory stimuli of intensity 80-90 dB Sound Pressure Level (SPL) were delivered binaurally at the frequency of 1 Hz with inter stimulus interval of 1-2 seconds. Participants were seated in an acoustically shielded room and were asked to remain alert during the test. They were asked to count the number of target (infrequent) stimuli throughout the test.

Recordings were done at Cz with ground electrode placed at Fpz and earlobes used as reference. Filter bandpass frequency was 0.01 -100 Hz with epoch length 1000ms and electrode impedance was kept below 5 KOhm. 36 artifact free responses were included for analysis.

The latency windows for N100, P200, N200 and P300 was 75-175, 150-260, 190-360 and 300-500 msec respectively. P300 amplitude was measured from pre stimulus base to peak.

STATISTICAL ANALYSIS
The ERPs study measured peak latencies and base to peak amplitude of waves P200, N200 and P300 in every subject. ADD ON package of Microsoft Excel method was utilized for statistical analysis of ERPs waves, SMMSE and patient characteristics like age and sex. Probability values of <0.05 were considered statistically significant.

RESULTS
The neurological and systemic examinations of participants were within normal limits. Blood investigations including hemogram, biochemistry and thyroid profile were normal in all subjects. The mean ages of groups 1, 2, 3 were 34.19 ± 12.6, 35.46 ± 13.77 and 25.13 ± 5.48 respectively without any statistically significant difference (p>0.05) (Table 3). SMMSE scores of groups 1, 2 and 3 showed no significant difference before or after treatment. (p>0.05) (Table 2).

Latency of N200 and P300 of group 1 showed statistically significant difference when compared to group 2 and 3 before treatment (p<0.05) and the difference disappeared after 3 months of Vitamin B12 supplementation (Table 4). Other ERP parameters including P300 amplitude didn’t reveal significant difference on comparison between groups 1, 2 and 3.
A. Before treatment  
B. after treatment

**Fig-1:** ERP waveforms recorded from a Vitamin B12 deficient patient after presentation of rare stimulus

**Table-1: Profile of patients**

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of patients</th>
<th>Percentage of patient</th>
<th>No (Percentage) of patient in group 1</th>
<th>No. (Percentage) of patient in group 2</th>
<th>No. (Percentage) of patient in group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32</td>
<td>60.37%</td>
<td>10 (47.62%)</td>
<td>12 (70.59%)</td>
<td>10 (66.67%)</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td>39.62%</td>
<td>11 (52.38%)</td>
<td>5 (29.41%)</td>
<td>5 (33.33%)</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 to 30 years</td>
<td>30</td>
<td>56.6</td>
<td>8 (38.09%)</td>
<td>8 (47.05%)</td>
<td>14 (93.33%)</td>
</tr>
<tr>
<td>31 to 45 years</td>
<td>13</td>
<td>26.41</td>
<td>8 (38.09%)</td>
<td>4 (23.52)</td>
<td>1 (6.67%)</td>
</tr>
<tr>
<td>46 and above</td>
<td>10</td>
<td>18.87</td>
<td>5 (23.80%)</td>
<td>5 (29.41)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td></td>
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</tbody>
</table>

**Table-2: SMMSE score and analysis of SMMSE score comparisons between groups**

<table>
<thead>
<tr>
<th>Category</th>
<th>SMMS score Mean ±SD</th>
<th>Group1 before treatment vs. Group 1 after treatment (p value)</th>
<th>Group1 BT vs. Group 2 (p value)</th>
<th>Group1 BT vs. Group 3 (p value)</th>
<th>Group2 vs. Group3 (p value)</th>
<th>Group1 AT vs. Group2 (p value)</th>
<th>Group1 AT vs. Group3 (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1BT</td>
<td>26.39 ±4.2</td>
<td>0.07</td>
<td>0.63</td>
<td>0.18</td>
<td>0.32</td>
<td>0.93</td>
<td>0.12</td>
</tr>
<tr>
<td>AT</td>
<td>26.43 ±2.9</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Group2</td>
<td>26.98 ±2.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group3</td>
<td>28.33 ±4.6</td>
<td></td>
<td></td>
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</tbody>
</table>

BT: Before treatment, AT: after treatment

**Table-3: Average age and comparison between the group ages**

<table>
<thead>
<tr>
<th>Group Name</th>
<th>No. of patients</th>
<th>Mean age ±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>21</td>
<td>35.33±10.75</td>
<td>0.511</td>
</tr>
<tr>
<td>Group 2</td>
<td>17</td>
<td>37.11±12.15</td>
<td>0.52</td>
</tr>
<tr>
<td>Group 3</td>
<td>15</td>
<td>25.66±5.48</td>
<td>0.35</td>
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DISCUSSION
The aim of this study was to assess the ERPs in patients of Vitamin B12 deficiency without neuropsychiatric manifestations and change in ERP parameters with Vitamin B12 supplementation.

Vitamin B12 plays a crucial role in the synthesis of neurotransmitters like Dopamine, Acetylcholine, RNA membrane phospholipids and myelin by functioning as a coenzyme for various enzymes. MUT(Methylmalonyl-CoA mutase) an isomerase uses adenosylcobalamin form of Vitamin B12 to convert Methylmalonic CoA to Succinyl CoA which is essential for myelin synthesis. Failure of this reaction due to Vitamin B12 deficiency leads to elevated levels of Methylmalonic acid - a myelin destabilizer. Methionine synthase uses Methylcobalamin form of Vitamin B12 to transfer a methyl group from 5 Methyl tetrahydrofolate to homocysteine generating Tetrahydrofolate and methionine. Vitamin B12 deficiency leads to hyperhomocysteinemia and increased level of 5 Methyl tetrahydrofolate, thus decreasing TH and methionine availability for DNA synthesis and methylation of myelin sheath phospholipids. Thus, Vitamin B12 deficiency adversely affects myelin and neurotransmitter synthesis .There is no universal marker for Vitamin B12 status and estimation of serum Vitamin B12 levels is a cost-effective parameter of choice [5].

There are varied neurocognitive consequences of Vitamin B12 deficiency. The dominant feature is subacute cognitive impairment and psychiatric manifestations. Prompt recognition, diagnosis and replacement is essential as delayed replacement may cause irreversible changes [6].

A majority of cross sectional studies have established association between Vitamin B12 status and sensitive neuropsychological test performance although the evidence is weaker than that for folate .Low Vitamin B12 status has been associated with accelerated decrease in SMMSE scores, poor memory function and cognitive decline in geriatric population.
but a clear association remains unsubstantiated and we lack compelling evidence to recommend widespread Vitamin B12 supplementation in non-deficient population. The mixed results of various studies could be due to different study designs and hypotheses [5].

Our study classified the subjects according to their serum Vitamin B12 levels after exclusion of factors which might affect cognition ad ERP parameters, thus confounding the results. The subjects were divided in three groups-deficient (<211pgm/ml), insufficient (211-350pgm/ml) and sufficient (>350pgm/ml) according to their serum Vitamin B12 levels. The subjects in our study had no neuropsychiatric symptoms and their SMMSE scores were within normal range. ERP studies in group 2 & 3 were within normal ranges. Our study also concluded that patients in Group 1 with Vitamin B12 deficiency (<211pgm/ml) had deranged ERP parameters as compared to group 2 & 3 which improved after 3 months of Vitamin B12 supplementation. There was improvement in latencies of N200 and P300 after 3 months of Vitamin B12 supplementation without any significant change in P300 amplitude and SMMSE scores. Of these ERP parameters P300 is the most consistent and useful parameter which can be used to assess the effectiveness of Vitamin B12 replacement therapy.

Prior studies have established relationship between reversibility of cognitive impairment and deranged P300 parameters in Vitamin B12 deficient subjects with Vitamin B12 supplementation [7-11].

In our study we concluded that ERPs, especially N200 and P300 latencies are deranged in subjects with Vitamin B12 deficiency without neuropsychiatric symptoms and normal SMMSE scores. These ERP parameters improve after Vitamin B12 supplementation. ERPs are cost effective tool for screening of early subclinical cognitive dysfunction in patients with Vitamin B12 deficiency. ERPs may also serve as useful index for reversibility of cognitive dysfunction after Vitamin B12 supplementation. Such patients should receive prompt Vitamin B12 supplementation as they might be having subclinical cognitive dysfunction which may progress to frank cognitive impairment and the reversibility of cognitive dysfunction can be monitored by ERPs.

In literature we could not find the study of ERPs in asymptomatic (neuropsychiatric symptoms) patients of Vitamin B12 deficiency, we conclude that ERPs especially P300 latency may be deranged prior to neuropsychiatric manifestations. This necessitates early and prompt Vitamin B12 supplementation in such patients. Further studies using more sensitive neuropsychiatric assessment batteries are needed as SMMSE score alone is rather insensitive for assessing early cognitive dysfunction. Serum Folate levels assay should also included in further studies as isolated Folate deficiency might also cause neuropsychiatric symptoms.

REFERENCES