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Original Research Article

Should visual evoked potential be an essential protocol for amblyopia management? - A novel protocol

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Abstract

Background: Amblyopia is a neurodevelopmental visual disorder caused by abnormal visual input during early childhood, leading to impaired visual acuity. Visual evoked potentials (VEP) serve as an objective measure of visual pathway integrity, with P100 latency often prolonged in amblyopic eyes. Vitamin B12, crucial for neural function, has been implicated in optic neuropathies, though its association with amblyopia remains uncertain.
Aim: This study aims to explore the association between Visual evoked potentials (VEP) and Vitamin B12 levels in different types and grades of amblyopia among children in North India.
Materials and Methods: A cross-sectional study was conducted involving 45 children aged 6-16 years diagnosed with either strabismic or anisometropic amblyopia. VEP P100 latency and N75-P100 amplitude were measured using the VEP machine (Nicolet AT2+6 Amplifier). Vitamin B12 levels were estimated using chemiluminescence immunoassay (CLIA). Data were analysed using t-tests and ANOVA.
Results: VEP P100 latency was significantly prolonged, and N75-P100 amplitude was significantly reduced in amblyopic eyes compared to fellow eyes ($p < 0.001$). No significant differences in VEP values were observed between different types or grades of amblyopia. Vitamin B12 levels also did not differ significantly in different types and grades of amblyopia.
Conclusion: VEP serves as an important diagnostic tool for detecting neurophysiological changes associated with amblyopia. Vitamin B12 levels may not significantly influence the condition's severity or type. Further research is needed to explore the role of nutritional factors in amblyopia.

Keywords: Amblyopia, Visual evoked potentials, Vitamin B12.

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1. Introduction

Amblyopia, commonly known as "lazy eye," is a leading cause of visual impairment in children, with a global prevalence ranging from 1% to 6%.[1] It typically develops due to abnormal visual experiences during early childhood, leading to reduced visual acuity that cannot be corrected with lenses alone. Many studies have underscored the importance of early detection and routine screening for amblyopia during childhood health checkups to prevent long-term visual impairment.[2-4] Amblyopia can be classified into several types, including strabismic, anisometropic, and deprivation amblyopia, each associated with different underlying mechanisms.[5]

Visual evoked potentials (VEP) provide a non-invasive method for assessing the functional integrity of the visual pathways, making them crucial for diagnosing amblyopia. The P100 component of VEP, in particular, is often delayed in amblyopic eyes, reflecting disruptions in the visual processing pathways.[6] Additionally, Vitamin B12 is essential for normal functioning of nervous system. However, deficiencies potentially lead to various neurological disorders, including optic neuropathy.[7] Moreover, the relationship between Vitamin B12 levels and amblyopia remains unclear.

This study aims to investigate the correlation between VEP and Vitamin B12 levels across different types and grades of amblyopia in a paediatric population from North

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India. Understanding this relationship could enhance the diagnostic and therapeutic strategies for amblyopia. To the best of our knowledge, this is the first study in North India where both VEP and Vitamin B12 were correlated with amblyopia management.

2. Materials and Methods

This cross-sectional study was conducted at the Institute of Ophthalmology, Jawaharlal Nehru Medical College, Aligarh Muslim University, from September 2022 to August 2024. The study included 45 children aged 6 to 16 years, diagnosed with unilateral amblyopia (strabismic or anisometropic). Ethical approval was obtained, and informed consent was secured from all participants' guardians. Exclusion criteria included patients with glaucoma, neurological diseases, or those who were uncooperative. Pattern VEPs were recorded using the Nicolet AT2+6 Amplifier, focusing on the P100 latency and N75-P100 amplitude. Electrodes were placed according to the international 10-20 system of the ISCEV (International Society for Clinical Electrophysiology of Vision) protocol, with recordings analysed to determine the latency and amplitude of the P100 wave. Vitamin B12 levels were measured using the chemiluminescence immunoassay (CLIA) method. Blood samples were collected, processed, and analysed using a Beckman Coulter analyser. Data were analysed using JASP software version 0.18.3.0. Categorical data were expressed as percentages, and continuous data as mean ± standard deviation. Statistical significance was determined using t-tests, ANOVA, and Pearson correlation, with a p-value of <0.05 considered significant.

3. Results

The study population comprised 28 males (62%) and 17 females (38%), with a mean age of 10.8 ± 3.4 years. Of the 45 children, 24 had anisometropic amblyopia and 21 had strabismic amblyopia. The severity of amblyopia was classified as mild (24.4%), moderate (51.1%), and severe (24.4%). The sample size of 45 participants was considered sufficient based on previous similar studies, ensuring adequate statistical power to detect significant differences and draw meaningful conclusions. Amblyopia severity is categorized based on the visual acuity of the affected eye, assessed through standard visual acuity testing. "Mild" amblyopia generally corresponds to visual acuity ranging from 6/9 (LogMAR 0.2) to 6/12 (LogMAR 0.3), "moderate" amblyopia falls between 6/12 (LogMAR 0.3) and 6/36 (LogMAR 0.8), while "severe" amblyopia is defined as visual acuity worse than 6/36 (LogMAR >0.8).

VEP P100 latency was significantly longer in amblyopic eyes compared to fellow eyes, with mean latencies of 128.244 ms and 108.844 ms, respectively ($p < 0.001$). In **Figure 1**, VEP waveforms shows delayed P100 latency and reduced N75-P100 amplitude in the left eye (amblyopic) compared to the right eye. In **Table 1** and **Figure 2**, we compare VEP P100 values in different grades of amblyopia in amblyopic eye.

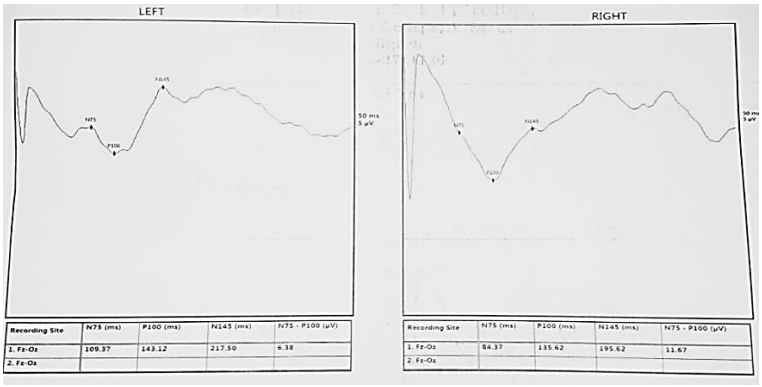

Figure 1: VEP waveforms showing delayed P100 latency and reduced N75-P100 amplitude in the left eye (amblyopic) compared to the right eye

Table 1: Comparing VEP P100 values in different grades of amblyopia in amblyopic eye

	P100 (ms)		
	Mild	**Moderate**	**Severe**
Number of patients	11	23	11
Mean	117.091	133.522	128.364
95% CI Mean (Upper limit)	128.173	152.740	140.951
95% CI Mean (Lower limit)	106.009	114.303	115.776
Std. Deviation	16.495	44.443	18.736
Range	54.000	237.000	57.000
Minimum	90.000	78.000	102.000
Maximum	144.000	315.000	159.000

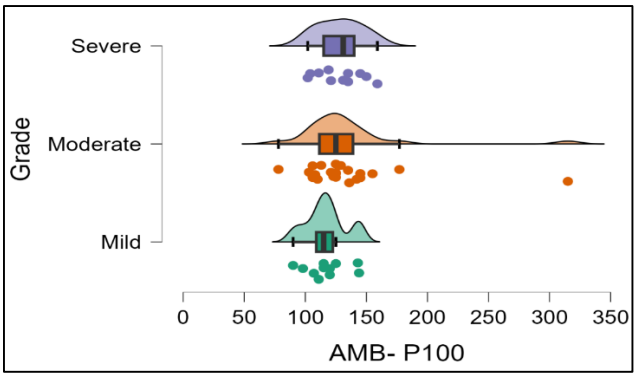

Figure 2: Raincloud plot showing the distribution of P100 latency value in amblyopic eye values across different grades of amblyopia

N75-P100 amplitude was also significantly lower in amblyopic eyes (9.000 µV) compared to fellow eyes (13.267 µV) ($p < 0.001$). In **Table 2** and **Figure 3**, we compare N75-P100 amplitude in different grades of amblyopia in amblyopic eye. No significant differences were observed in VEP values between strabismic and anisometropic amblyopia or across different grades of severity.

Table 2: Comparing N75-P100 amplitude values in amblyopic eye in different grades of amblyopia

	N75-P100 (µV)		
	Mild	**Moderate**	**Severe**
Number of patients	11	23	11
Mean	8.818	9.087	9.000
Std. Deviation	2.359	5.169	5.495
95% CI Std. Dev. (Upper limit)	3.459	7.101	7.867
95% CI Std. Dev. (Lower limit)	0.820	2.938	1.293
Minimum	6.000	4.000	5.000
Maximum	15.000	26.000	23.000

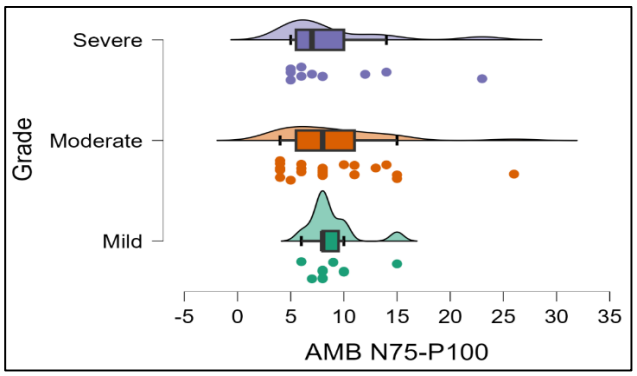

Figure 3: Raincloud plot showing the distribution of AMB N75-P100 amplitude values in amblyopic eye across different grades of amblyopia

The mean vitamin B12 level in the study population was 123.644 pg/mL, with no significant differences observed between strabismic and anisometropic amblyopia ($p = 0.504$) or across different grades of severity ($p = 0.716$). In **Table 3** and **Figure 4**, we compare vitamin B12 in different grades of amblyopia.

Table 3: Vitamin B12 in different grades of amblyopia

	Vit B12 (pg/ml)		
	Mild	**Moderate**	**Severe**
Number of patients	11	23	11
Mean	133.273	116.739	128.455
Std. Deviation	78.833	42.450	67.889
Minimum	51.000	84.000	41.000
Maximum	338.000	279.000	250.000

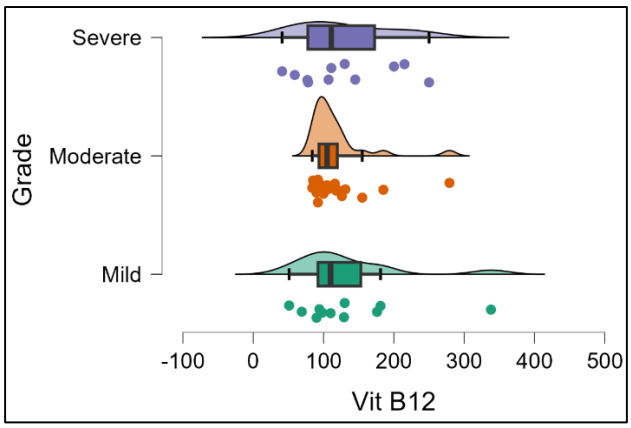

Figure 4: Raincloud plot illustrating the distribution of Vitamin B12 levels across different grades of amblyopia

4. Discussion

Amblyopia is a significant cause of visual impairment in children and presents a complex interplay of neurological and nutritional factors that warrant thorough exploration.[8] The present study aimed to bridge the gap in understanding the relationship between VEP and vitamin B12 levels in different types and grades of amblyopia. The present study findings align with previous studies. M. Gitanjali et al. reported that amblyopic patients had increased VEP latency and decreased amplitude before treatment, which improved following occlusion therapy. They demonstrated that the VEP changes reflect improvements in the visual processing capabilities of the brain as a response to treatment. In the present study also, there was statistically significant difference in VEP P100 latency values and N75-P100 amplitude in between the amblyopic and fellow eye of children.[9]

The present study results indicated no statistically significant difference in VEP P100 latency between strabismic and anisometropic amblyopia. The mean latency for strabismic amblyopia was 135.238 ms (SD = 43.793), while for anisometropic amblyopia, it was 122.125 ms (SD = 22.276) ($p = 0.204$), suggesting that the type of amblyopia did not significantly affect P100 latency. The N75-P100 amplitude showed no significant difference, with means of

7.714 µV for strabismic and 10.125 µV for anisometropic amblyopia (p = 0.082). Similarly, Kalaivaazhi & Kumar highlighted that P100 value significantly improved during occlusion therapy in patients with strabismus, and this improvement correlated positively with Snellen visual acuity.[10] In another study, Halfeld Furtado de Mendonca et al. explored 65 amblyopic children with pattern-reversal VEPs elicited by checkerboard stimuli with large, medium and small checks. The children were classified into three groups: Group A, Anisometropic amblyopia; Group B, Exotropic strabismic amblyopia; and Group C, Esotropic strabismic amblyopia. No statistically significant difference was found between the amblyopic and sound eye of amblyopic children in the 3 groups for VEP P1 amplitude and latencies for any check sizes.[11] These findings did not align with the present study where we found significant difference in VEP P100 latency and amplitude between amblyopic and fellow eye of same subject. The difference could due to a combination of factors, including differences in study populations, methodologies, statistical analysis, and the interpretation of clinical and neurophysiological data. Shawkat et al. in their study also found significant differences between amblyopic and fellow eyes in amblyopes with respect to VEP pattern-onset components. The reversal P100 and offset P110 and Nl65components showed significant differences. These findings concurred with the present study findings.[12]

When comparing VEP P100 latency values across different grades of amblyopia (mild, moderate, and severe) in the amblyopic eye, the present study found no significant differences. The mean VEP P100 values were 117.091 ms for mild amblyopia, 133.522 ms for moderate amblyopia, and 128.364 ms for severe amblyopia. The p-value of 0.435 from the ANOVA test indicates that the variations in VEP P100 values among these grades are not statistically significant. For N75-P100 amplitude in amblyopic eye, no significant differences were found across different grades of amblyopia, with mean N75-P100 amplitudes of 8.818 µV for mild, 9.087 µV for moderate, and 9.000 µV for severe amblyopia. The p-value of 0.988 shows no significant statistical difference. To the best of our knowledge, there are no studies correlating VEP in different grades of amblyopia especially in North Indian population.

In the present study, vitamin B12 levels did not show significant differences between strabismic and anisometropic amblyopia. The mean vitamin B12 level for strabismic patients was 129.952 pg/mL, and for anisometropic patients, it was 118.125 pg/mL, with no statistically significant difference (p = 0.504). Vitamin B12 levels below 150 pg/mL were considered deficient. Additionally, when analysing vitamin B12 levels by the severity of amblyopia (mild, moderate, severe), the differences were not statistically significant (p = 0.716). These findings suggest that vitamin B12 levels may not be a key differentiating factor in the types or severities of amblyopia. A similar study by Subasi et al. evaluated amblyopic children aged 5 to 18 years (n=46) and age-matched controls (n=32). They found lower vitamin B12 and folate levels and higher body mass index in children with severe amblyopia. Vitamin B12 levels were significantly lower in children with severe amblyopia (205.90 pmol/L) compared to the control group (306.27 pmol/L), aligning with our findings, as our study population also exhibited deficient mean vitamin B12 levels.[13] The study by Foulds et al. found that 40% of patients with tobacco amblyopia, a condition marked by vision loss due to tobacco smoke's toxic effects, had serum vitamin B12 levels below 150 µg/mL. Additionally, 45.7% of these patients exhibited defective vitamin B12 absorption, linking B12 deficiency to tobacco amblyopia. This suggests that low B12 levels, combined with tobacco use, may heighten the risk of vision impairment.[14] Similarly, Heaton et al. found that patients with tobacco amblyopia had significantly lower serum vitamin B12 levels (mean 218 µg/mL) compared to healthy controls (mean 538 µg/mL).[15]

5. Limitations

This study's relatively small sample size and cross-sectional design limit the generalizability of the findings. Future research should include larger, longitudinal studies to better understand the role of VEP and Vitamin B12 in amblyopia.

6. Novelty of the Study

This study contributes to the understanding of VEP changes in amblyopia and highlights the potential role of Vitamin B12. The findings support the use of VEP in diagnosing and predicting amblyopia, advocating for early detection and comprehensive diagnostic approaches. Future research should investigate the use of Pattern VEPs to evaluate visual processing, providing a more nuanced understanding of how amblyopia impacts localized visual function.

7. Conclusion

1. VEP showed significantly prolonged P100 latency and reduced N75-P100 amplitude in amblyopic eyes compared to fellow eyes, highlighting its effectiveness as a diagnostic tool.
2. No significant differences in VEP parameters were found between anisometropic and strabismic amblyopia, indicating similar neurophysiological patterns across these types.
3. VEP values did not vary significantly across different grades of amblyopia suggesting that the severity of amblyopia may not influence VEP results.
4. Pattern VEP should be made an essential armamentarium both for amblyopia diagnosis and prognosis.
5. Vitamin B12 levels were generally low in the study population, though there were no significant

differences between the types or grades of amblyopia.

8. Source of Funding

None.

9. Conflict of Interest

None.

10. Ethical

Ethical No.: IECJNMC/876.

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