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EVENT RELATED POTENTIALS IN ANEMIC SCHOOL – GOING GIRLS OF AGE GROUP 8 TO 10 YEARS

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Abstract : In the present study the effects of anemia on cognitive functions were studied in school going girls aged 8-10 years. The cognitive functions were assessed by Event Related Potentials (P300) and by the psychometric tests, i.e., Raven's progressive matrices test and Digit span attention test. The girls with Hb < 12 g/dl were classified into anemic and Hb > 12 g/dl into control group. Hematological values of the control group were significantly better than anemic group. P300 latency in the anemic girls was delayed as compared to control group but, no statistically significant difference was observed for P300 latency and P300 amplitude between the control group and the anemic group. The psychometric test scores for intelligence quotient and transformed quotient were also better but not statistically significant in control group of girls as compared to anemic girls. However, the hematocrit values showed a significant correlation with the P300 wave latency showing that the hematological status is associated with some effects on cognition.

Key words : anemia iron deficiency cognition

INTRODUCTION

There is a very high prevalence of anemia, especially the iron deficiency anemia (IDA) in the world particularly the developing countries (1). Prevalence levels in India are very high with IDA affecting almost 60-90% of the population, particularly the pregnant women, young children and adolescents (2, 3). A large number of studies have found that iron deficient (ID) children are lagging behind the iron replete children in the cognitive tests measuring the global intelligence scores, learning and memory (3, 4) and the cognitive tests measuring the specific cognitive deficiencies such as fine and gross motor skills, visual motor skills and language and various preschool skills (5, 6, 7, 8). The reversibility of the harmful effects of the IDA on the cognition has been studied after iron supplementation and the effects have been found to be partially reversible in some studies (4, 6, 7) and to be irreversible and persistent in other studies

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(5, 9). Extensive reviews of most of the trials have shown that in all these trials, at the best a weak correlation is seen between the ID or IDA and cognition. Moreover, the effects of the potential confounding factors like the socioeconomic status and deficiencies of the other micronutrients cannot be clearly ruled out in any of the studies (10, 11). Electrophysiological tests have been considered to be more specific regarding the demonstration of any effects of IDA/ID on the cognition (10, 11, 12). In our own laboratory we demonstrated the delayed latencies of the auditory brainstem responses (ABRs) of the IDA children versus the control children in a group enrolled from the outpatient department of our hospital (13). Similar findings in the ABRs have been observed in the IDA infants (12). ABRs are an index of the myelination of the auditory pathways. Iron has been implicated in the myelination and the development of various neurotransmitter systems in the brain (14, 15). The brain regions specifically affected by the ID/IDA include the striatum, nucleus accumbens, substantia nigra, hippocampus and the frontal lobes (15). These areas are also important in generation of the waves of the event related potentials (ERPs), particularly the P300 which is the most prominent wave. ERPs are associated with cognition and information processing, and are used as a measure of recognition memory. The latency and amplitude of P300 can be used as an index for the assessment of the neural processing time and the neural activity (16, 17). Anemia patients on hemodialysis have shown delayed latencies of the P300 (18). In our previous study, which was conducted on the anemic boys from a school, we were able to find significant delay in the P300 latency of the anemic group as compared to non anemic group. Moreover, the delay in the P300 latency persisted despite the vigorous iron therapy for 3 months (19). Studies on infants from hospital outpatient department with age less than 12 months and having IDA have shown delayed developmental patterns of ERP activity (20); and also a negative correlation has been seen between the severity of anemia as assessed by the hemoglobin levels and the VEP Latency (21). However, in a study by Otero et al in school children in the age group of 8 to 10 years, no significant differences in reaction times and P300 Latency were observed between control and ID/IDA groups (22).

MATERIAL AND METHODS

Following our work in anemic boys (19), we planned another study to assess the effects of anemia on the cognition in the girls using the P300 as an index for assessment of cognition along with the Ravens progressive matrices test (RPMT) and Digit span attention test (DST).

Forty-two school-going girls of 8-10 years age, from lower socioeconomic status and living in the same community were subjects of this study. They were selected after obtaining detailed history and a thorough physical examination. Those having any chronic/acute disease or infection, hearing and neurological abnormality, hematological disorder, genetic disorders, jaundice, long term drug therapy, h/o hospitalization and h/o hematinic therapy were excluded. Parents of subjects were informed about the nature of the study following which a written consent was obtained for conducting hematological, psychometric and neuro-

physiological tests. Malnutrition was ruled out using specific anthropometric criteria of Body Mass Index (BMI) and ratio of mid-arm circumference to the head circumference.

The hematological assessment included measurements of Hemoglobin (Hb), hematocrit (Hct), MCV (Mean corpuscular volume) and MCH (Mean corpuscular hemoglobin); and were detected by electrical impedance method using Coulter Hematological Particle Counter Model T-890, Coulter Electronics, UK. Serum Iron (SI), total iron binding capacity (TIBC) and %age saturation were measured using the spectrophotometry. Peripheral blood smear examination was done to study RBC morphology, particularly size and chromicity of the RBCs. Presence of microcytosis along with anisocytosis was also taken as an index of iron deficiency.

The groups were divided into control (C) and anemic (A) on the basis of their hemoglobin values with the cutoff Hb value for anemia taken as < 12 g/dl (5).

For P300 component of auditory events related potentials (AERP), the standard procedure for recording the auditory response to 'odd ball paradigm' being used in our laboratory was performed. SMP 4100 auditory/visual stimulator and MEB 5200 Neuropack II evoked potential recorder, Nihon Kohden, Japan, were used for AERP recording. The P300 waves recorded are actually an average of large number of individual waves in each subject (16, 23).

Ravens progressive matrices test (RPMT) is a test of the person's capacity to apprehend complex figures presented for her observation, see the logical system of relationship between them and choose the figure [from many choices provided] to complete each system of relationship presented. In the whole process the subject uses a systematic method of reasoning (24). RPMT scores were converted into Wechsler's equivalent IQ score [RPMT-IQ].

Digit span test (DSAT) measures immediate auditory recall, attention, short term memory and freedom from distractibility (25). Raw scores were converted into transformed quotient (TQ) using Indian norms available [DSAT-TQ].

Data was analyzed using unpaired t test. Correlation analysis was done between various hematological parameters and the P300 latency and amplitude using Graph Pad Insat Version 3.10'.

RESULTS

When the girls were analyzed, the following findings could be made:

1) Control girls (CG) vs. anemic girls (AG) (Table I): The hematological parameters of control group were better than anemic group especially for Hb/hematocrit and MCH. The P300 latency and the RPMT and DSAT scores for the control group were higher (better) but not significant statistically as compared to anemic group. Serum iron profile was not very different in both groups. No significant differences were observed in the P300 latency, P300 amplitude, RPMT IQ scores, and the DST scores (Table I).

2) No correlation between the Hb and P300

TABLE I: Statistical significance between control girls (CG) and anemic girls (AG) using unpaired t-test.

Variable	*CG (n=19) Mean±SD*	*AG (n=23) Mean±SD*
Age (Years)	9.1±0.59	8.96±0.498
BMI	16.1±0.66	15.8±0.49
Hematological		
Hb (g/dl)	12.7±0.44	10.1±1.96**
Hct (l/l)	0.38±0.01	0.30±0.06**
MCV (fl)	91.0±8.74	86.2±9.99
MCH (g/dl)	31.0±3.43	28.8±3.52*
SI (µg/dl)	78.0±30.0	68.0±35.00
TIBC (µg/dl)	326.0±50.0	308.0±38.00
%saturation	24.9±11.66	22.1±10.54
P300		
P300 Latency (ms)	351.5±27.13	366.0±29.63
P300 Amplitude (µV)	10.6±5.10	10.9±4.66
Psychometric		
RPMT Derived IQ Score	101.4±9.76	96.2±9.52
DSAT Transformed Quotient	98.0±15.28	94.2±12.84

P<0.001**; P<0.05*

values, the RPMT IQ scores and DST TQ scores was observed.

3) Similarly, no correlation between the hematocrit and P300 values was observed when the anemic group and control group data was analyzed individually. However, when the data from the anemic girls and control girls was pooled, a significant correlation between hematocrit and P300 latency was observed: 'Graph Pad Insat Version 3.10' was used and non parametric correlation applied [Spearman Rank Correlation]: Spearman r = –0.3097 (corrected for ties) 95% confidence interval: –0.5672 to 0.003011 . The two-tailed P value = 0.0460, considered significant (Fig. 1).

4) There was no significant correlation between other hematological parameters

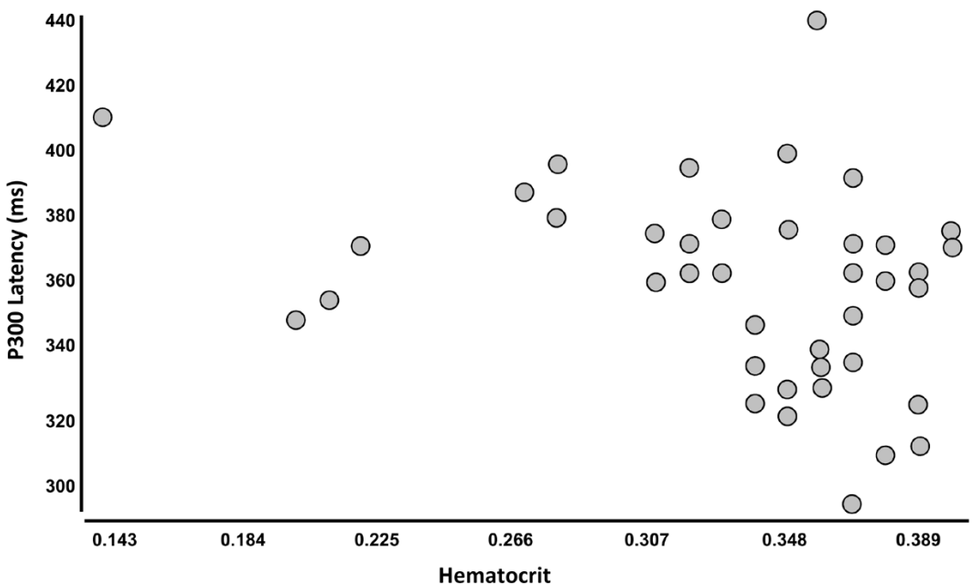

Fig. 1: Correlation (Spearman r = –0.3097; P=0.04) between hematocrit and P300 latency for all control and anemic group values (done on Graph Pad Insat Version 3.10).

and the P300 parameters, the RPMTIQ scores and DST TQ scores.

DISCUSSION

In the present study, the Hb < 12 g/dl has been taken as the main criteria for initial classification into the anemic and control groups. In population with high incidence of anemia, the Hb values are an important criteria for detecting iron deficiency taking Hb < 12 g/dl as the dividing line. Moreover, even in non iron deficient anemic groups, the Hb remains a critical deciding factor so that the values below cut off of Hb < 12 g/dl are associated with documented cognitive deficits and the improvement in Hb results in improvement of the neurophysiological parameters in anemic groups (18, 26, 27). The results show that the anemic school going girls have a clearly lower value for Hb, hematocrit and the MCH. The latency values of the P300 waves in the anemic groups were delayed but, the significant difference with respect to control group of girls was not present.

In a similar study done by Otero et al in children in the age group of 8 to 10 years and from a school going population, the P300 latency and amplitude values as well as the reaction times were observed and compared in the control and iron deficient anemic groups. There was no significant difference observed with respect to the P300 latency and amplitude values between the control and the anemic groups, however, the iron supplementation in the anemic children brought up the P300 wave forms to normal shapes in central (Cz) and parietal (Pz) regions (22).

In the present study the hematocrit has shown a significant correlation with the P300 latency so that the greater hematocrit values are associated with lesser P300 latency. This shows that the hematological status is correlated with the P300 latency, but its effect/association with P300 latency in the studied age group remains weak. Similar correlation between the hematocrit and the P300 latency and the EEG wave patterns has been observed by Pickett et al (26). Moreover, the improvement in hematocrit resulted in substantial improvement in the P300 latency values in patients suffering from chronic renal failure as shown by the decrease in P300 latency values, and reduced slowing of the EEG waves (26, 27).

Additionally, in a few studies done on infants with ID and IDA it has been shown that these are not associated with any delay or difference in the latencies of the BAEPs when compared with control iron replete groups (28, 29) It has been proposed by various reviews of all the studies on ID/IDA and cognition done till date that it may not be the ID/IDA alone but a comprehensive effect of several macro and micronutrient deficiencies, environmental and social deprivations that causes the deficits in cognition and that the association between ID/IDA and cognitive deficits is not very strong (10, 11). In our similar study on boys, significant differences between the IDA and control groups for P300 latency values was observed (19); however, differences observed in the current study are not significant. The subjects of the current and previous studies were carefully chosen from similar background and socioeconomic status to minimize the confounding effects of these

on the study and most importantly were from the normal population of school going children instead of the hospital admissions/ out patients. A possible explanation of the differential effects observed in the present study as compared to the previous study could be due to gender differences in the brain development, as the neuro-anatomical development and functional connectivity has been found to be different in the males and females (30). A further study with a greater sample size is required to establish the role of anemia along with other nutritional, environmental and social factors in causing the observed cognitive deficits in the affected populations.

REFERENCES

1. Beard J, Stoltzfus R. Iron-deficiency anemia: reexamining the nature and magnitude of the public health problem. *J Nutr* 2001; 131: 563S–703S.
2. Indian Council Medical Research. Evaluation of the national nutritional anaemia prophylaxis programme. An Indian Council Medical Research (ICMR) Task Force Study, New Delhi, India 1989; 1–107.
3. World Health Organization (2008) Vitamin and Mineral Nutrition Information System (VMNIS). WHO Global Database on Anaemia [INDIA], 30 January 2008.
4. Seshadri S, Gopaldas T. Impact of iron supplementation on cognitive functions in pre-school and school-aged children: the Indian experience. *Am J Clin Nutr* 1989; 50: 675–686.
5. Lozoff B, Jimenez E, Smith JB. Double burden of iron deficiency in infancy and low socio economic status: a longitudinal analysis of cognitive test scores to 19 years. *Arch Pediatr Adolesc Med* 2006; 160: 1108–1113.
6. Soewondo *S,* Husaini M, Pollitt E. Effects of iron deficiency on attention and learning processes in pre-school children: Bandung, Indonesia. *Am J Clin Nutr* 1989; 50: 667–674.
7. Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. *Lancet* 1996; 348: 992–996.
8. Sen A, Kanani SJ. Deleterious Functional Impact of Anemia on Young Adolescent School Girls. *Indian Pediatrics* 2006; 43: 219–226.
9. Lozoff B, Jimenez E, Wolf AW. Long-term developmental outcome of infants with iron deficiency. *N Engl J Med* 1991; 325: 687–694.
10. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr* 2007; 85: 931–945.
11. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. *J Nutr* 2001; 131: 649S–668S.
12. Roncagliolo M, Garrido M, Walter T, Peirano P, Lozoff B. Evidence of altered central nervous system development in infants with iron deficiency anemia at 6 mo: delayed maturation of auditory brainstem responses. *Am J Clin Nutr* 1998; 68: 683–690.
13. Shankar N, Tandon OP, Bandhu R, Madan N, Gomber S. Brainstem auditory evoked potential responses in iron deficient anemic children. *Indian J Physiol Pharmacol* 2000; 44: 297–303.
14. Beard J. Iron Deficiency Alters Brain Development and Functioning. *J Nutr* 2003; 133: 1468S–1472S.
15. DeUngria M, Rao R, Wobken JD, et al. Perinatal iron deficiency decreases cytochrome c oxidase (CytOx) activity in selected regions of neonatal rat brain. *Pediatr Res* 2000; 48: 169–176.
16. Goodin DS. Event related (endogenous) potentials. In: Aminoff MJ eds. Electrodiagnosis in clinical neurology, 3rd edition. New York, Churchill livingstone 1993; 575–595.
17. Polich J. Updating P300: An Integrative Theory of P3a and P3b. *Clin Neurophysiol* 2007; 118: 2128–2148.

18. Temple RM, Deary IJ, Winney RJ. Recombinant erythropoietin improves cognitive function in patients maintained on chronic ambulatory peritoneal dialysis. *Nephrol Dial Transplant* 1995; 10: 1733–1738.

19. Bandhu R, Shankar N, Tandon OP, Madan N. Effects of iron therapy on cognition in anemic school going boys. *Indian J Physiol Pharmacol* 2003; 47: 301–310.

20. Burden JM, Westerlund AJ, Armony-Sivan R, Nelson CA, Jacobson SW, Lozoff B, Angelilli ML, Jacobson JL. An Event-Related Potential Study of Attention and Recognition Memory in Infants with Iron-Deficiency Anemia. *Pediatrics* 2007; 120(2): e336–e345.

21. Monga M, Walia V, Gandhi A, Chandra J, Sharma S. Effect of iron deficiency anemia on visual evoked potential of growing children. *Brain & Development* 2010; 32: 213–216.

22. Otero GA, Pliego-Rivero FB, Contreras G, Ricardo J, Fernandez T. Iron supplementation brings up a lacking P300 in iron deficient children. *Clin Neurophysiol* 2004; 115: 2259–2266.

23. Tandon OP. P3 event related potentials in young adults. *Indian J Physiol Pharmacol* 1990; 34: 191–194.

24. Raven JC. Instructions for using progressive matrices sets A, B, C, D & E. In: Raven JC eds. Guide to the standard progressive matrices sets A, B, C, D & E. London, HK Lewis & Co. Ltd 1960; 1–26.

25. Malin AJ. Intelligence scale for Indian children. In: Malin AJ eds. Indian Adaptation of Weschsler's intelligence scale for children. *India* 1969; 21–31.

26. Pickett JL, Theberge DC, Brown WS, Schweitzer SU, Nissenson AR. Normalizing hematocrit in dialysis patients improves brain function. *Am J Kidney Dis* 1999; 33: 1122–1130.

27. Singh NP, Sahni V, Wadhwa A, Garg S, Bajaj SK, Kohli R, et al. Effect of improvement in anemia on electroneurophysiological markers (P300) of cognitive dysfunction in chronic kidney disease. *Hemodial Int* 2006; 10: 267–273.

28. Kürekc_i AE, Sanci SÜ, Karaoğlu A, Ulas_ÜH, Atay AA, Serdar MA, et al. Effects of iron deficiency versus iron deficiency anemia on brainstem auditory evoked potentials in infancy. *Turk J Pediatr* 2006; 48: 334–339.

29. Sarici SÜ, Serdar MA, Dündaröz MR, Ünay B, Akin R, Deda G, Gökçay E. Brainstem auditory-evoked potentials in iron-deficiency anemia. *Pediatr Neurol* 2001; 24: 205–208.

30. De Bellis MD, Keshavan MS, Beers SR, Hall J, Frustaci K, Masalehdan A, Noll J, Boring AM. Sex Differences in Brain Maturation during Childhood and Adolescence. *Cerebral Cortex* 2001; 11: 552–557.