European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 7, Issue 2, 2020

Neurophysiological Assessment And Cognition In Adult Females With Iron Deficiency Anaemia.

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Abstract: Background: Iron deficiency anemia (IDA) is the most prevalent form of anemia especially in developing countries. Females of child bearing age are vulnerable for developing IDA due to menstrual blood loss, dietary deficiency, pregnancy and lactation. IDA is diagnosed by low blood hemoglobin and serum ferritin levels which correlates with body iron stores. Of the various biological effects of iron, there are many evidences that iron effects normal neurological functioning and development. This forms the biological basis for the behavioral and cognitive developmental delays observed in iron-deficient anemic infants and children. Reduced levels of blood hemoglobin and serum ferritin has been positively correlated with low cognitive score and altered evoked potentials in females with IDA. Lack of research studies to know the effect of IDA on neurophysiological parameters like cognitive performance, event related potential and brainstem auditory response are little explored in Indian adult females.

Methods: Cognitive status is assessed using Montreal Cognitive Assessment (MoCA) scale which is validated testing scale for diagnosis of Mild Cognitive Impairment and neurophysiological assessment is done using auditory Event Related Potential (ERP / P300) and Brainstem Evoked Response Audiometry (BERA). MoCA score, ERP and BERA values will be compared in females with iron deficiency anemia and normal females and also associated with blood hemoglobin and serum ferritin levels. IDA may impair Cognitive performance and hearing in adult females which may have tremendous impact on understanding of speech and problem solving in them.

Hence, the aim of the study is to confirm a relationship between IDA and neuro-cognitive features using cognitive scale and evoked potentials in adult females.

Keywords: Iron Deficiency Anemia, Hemoglobin, Serum Ferritin, Cognition, MOCA, Event Related Potential (ERP), Brainstem Evoked Response Audiometry (BERA).

Background/ Rationale

Anemia is considered as a major public health issue throughout the world with Iron Deficiency Anemia (IDA) being the most prevalent and neglected type of anemia. According to estimation 25% of the world's population suffer from anemia among which iron deficiency contributes to 50% of cases.[1]

The WHO estimates that 30% of non-pregnant females and 42% of pregnant females are affected by IDA throughout the globe with females in developing countries being more susceptible.

In India, the National Family Health Survey (NFHS - 4) conducted during the year 2015-16 shows a prevalence of IDA in 53% of women belonging to age group of 15 – 49 years.

Iron is not only required during early developmental stages (in-utero, infancy and childhood) for normal brain growth but also plays an important role in later part of life for the maintenance of various metabolic activities and physiological functions.[1]

IDA impairs cognitive performance and functional integrity of CNS including attention, memory, skills and brainstem auditory transmission. Researchers have documented poor cognitive development and altered auditory transmission in infants and children with IDA by both observational and interventional studies, but similar measurements are lacking in adult females.

MoCA and P300 are tools useful for assessing cognitive status and BERA is used to assess hearing by detecting electrical activity in auditory pathway in brainstem. P300 and BERA are non-invasive neurophysiological methods which provide information regarding functional integrity of the central nervous system.

Observational and interventional studies have been performed in women of the reproductive age (18 – 35 years) to estimate global cognitive function using scales like Montreal Cognitive Assessment scale (MoCA), Mini Mental State Exam (MMSE) and Wechsler's memory scale (WMS). It has been found that IDA impairs the cognitive performance in them, inferring that iron plays an important role during non-developmental periods of life as well. Beard et al, Murray Kolb et al and Alecia J. L et al. found that low levels of hemoglobin and serum ferritin positively correlated with low cognitive scores.[2,3,4,5]

"The Montreal Cognitive Assessment (MoCA) was designed as a rapid screening instrument for Mild Cognitive Impairment (MCI). It assesses different cognitive domains: memory, naming, language, attention and concentration, executive functions, visuo-spatial abilities, calculations, conceptual thinking, verbal abstraction and orientation. Low score indicates cognitive decline." [6]

P300 is an electrophysiological tool used to assess the cognitive performance. It is a component of auditory Event Related Potential (ERP) and is a noninvasive procedure to assess memory and attention domains of cognition. The principle of this test is to assess the ability of the subject to discriminate between two different auditory stimuli (one is frequent - standard and the other is infrequent - target) and identify the infrequent target stimuli from the frequent standard stimuli.[7]

The amplitude and latency of the P300 wave reflects the information processing associated with immediate memory and attention. Cognitive decline is recognized by decreased amplitude and longer latency when compared to age matched controls, which was observed in individuals with IDA.[8]

BERA is used to assess the auditory transmission i.e generation and conduction of impulse in the auditory pathway. It is recorded as different wave forms (I - V) depending on the site of origin in the auditory pathway in the brainstem.

Increase in duration of waves, absolute and inter peak latencies along with decrease in amplitude of waves indicates asynchronisation or axonal dysmyelination at the axonal or synaptic levels. Auditory brainstem responses in various experimental studies revealed a direct relationship between iron deficiency and delayed responses indicating impaired conduction. [9,10]

The effect of IDA on cognitive performance and evoked potentials like P300 and BERA in adult females in comparison to normal females remains inadequately explored, especially in Indian population.

Research question: Does iron deficiency anemia in adult females have an effect on Cognitive score, P300 and BERA?

Research Hypothesis:

We hypothesize that iron deficiency anemia (IDA) in adult females will lead to decline in cognitive performance and alter the evoked potentials like P300 and BERA, resulting in impaired hearing and cognition. This may consequently lead to difficulty in understanding of speech and problem solving, when compared to the non-anemic adult females.

Objectives

1. To compare cognitive performance assessed using MoCA in females with iron deficiency anemia and non-anemic adult females
2. To compare evoked potentials – P300 and BERA in females with iron deficiency anemia and non-anemic adult females
3. To find the association between the Blood Hemoglobin levels and Serum Ferritin levels with Cognition score (assessed using MoCA) and evoked potentials (P300 and BERA) in adult females with iron deficiency anemia and non-anemic adult females.

Methodology

Study setting: The study will be conducted in the Central Neuro-Physiology lab of Acharya Vinoba Bhave Rural hospital and teaching institute, Jawaharlal Nehru Medical College, DMIMS Deemed University (NAAC Accredited Grade A), Wardha.

Study design: Analytical Cross-Sectional study and observational

Study population: The participants will be selected from adult females residing in Wardha district, in the child bearing age ranging between 18 – 30 years.

Inclusion criteria for study group (Females with IDA):

1. Females in the age group of 18 -30 years
2. Females whose Hemoglobin level is less than 12g/dl
3. Serum ferritin is less than 15 ng/mL

Inclusion criteria for comparison group (Normal females without IDA- non anemic):

1. Age matched females with normal Blood Hemoglobin and Serum Ferritin levels are considered as iron sufficient non anemic.

2. Hemoglobin levels are more than 12 g/dl.

3. Serum ferritin levels are more than 15 ng/mL

Exclusion criteria:

1. Age <18 or >30 years.

2. Body mass index <18 or >30 kg/m^2.

3. Current pregnancy or pregnancy within the previous year, current lactation, hormonal contraceptive use, irregular menses.

4. Current blood donation

5. Pre-existing ear diseases with clinical deafness

6. Endocrine disorders (e.g: Diabetes Mellitus, Thyroid Dysfunction)

7. Neurological diseases

8. Other types of anemia

9. Abnormal neuro-motor development

10. Use of medications (e.g. Major or minor tranquilizer) that may alter cognitive and neurophysiological measures.

Variables

Independent – Iron Deficiency Anemia, Normal (Non-Iron Deficient and non anemic) .

Dependent – Hemoglobin, Serum Ferritin, MoCA, P300, BERA parameters.

Definitions

Anemia - Anemia is defined as the hemoglobin of less than 12 g/dl in females (for Non-pregnant women, 15 years of age and above). No anemia is hemoglobin level of ≥12g/dl, mild anemia is hemoglobin level of 11–11.9 g/dl, moderate anemia is hemoglobin level of 8–10.9 g/dl and severe anemia is hemoglobin level of <8 g/dl
(Hemoglobin concentration for the diagnosis of anemia and assessment of severity. WHO)

Iron deficiency anemia - Serum Ferritin <15 ng/ml with hemoglobin <12 g/dl (WHO/UNICEF/UNU,2001).

Data collection tools

1. Height (mt) is measured using a stadiometer and Weight (kg) is measured using a weighing scale.

2. Blood sample to analyze – Blood Haemoglobin (Hb), Serum Ferritin.

Estimation of blood Hemoglobin concentration will be done using automatic Coulter method and estimation of Serum Ferritin concentration will be done by Immunoassay. The Standard Operating Procedure (SOP) given will be followed accordingly. Established age adjusted values for Blood Hemoglobin and Serum Ferritin will be used.

3. Cognition : Montreal Cognitive Assessment (MoCA)

MoCA is a 30 point assessment for diagnosing Mild Cognitive Impairment (MCI) that can be completed in a duration of 10 minutes. MoCA has been validated in identification of MCI. A score below 26 indicates MCI and above 26 is considered normal (http://www.mocatest/)

The scoring breakdown is as follows:

Visuospatial and executive functioning: 5 points

Naming: 3 points

Attention: 6 points

Language: 3 points

Abstraction: 2 points

Delayed recall (short-term memory): 5 points

Orientation: 6 points

Education level: 1 point is added to the test-taker's score if they have 12 years or less of formal education

4. Neurophysiological assessment using evoked potentials: P300 & BERA will be recorded using Neuron Spectrum-5, Neurosoft Chroma (Russia).

a) The Event Related Potential (ERP, P300 wave)

It is one of methods to assess human cognition directly reflecting cortical neuronal activity. It reflects information processing cascade which is associated with attention and memory mechanisms. It is recorded as a positive wave deflection in the Event Related Potential (ERP) at

a peak latency of 300ms. To record, the electrodes are placed over the vertex and mastoid then auditory stimulus is given through headphones. The P300 wave is commonly elicited in an Oddball paradigm, where a subject detects an occasional target stimulus in a regular train of standard stimuli. Its latency varies with the difficulty to discriminate the target with standard stimuli. In patients with decreased cognitive ability, the wave has longer latency and the amplitude is reduced when compared to age matched normal individuals

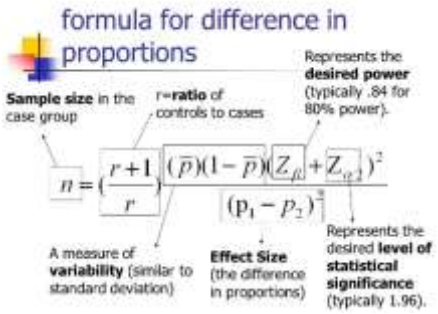
b) Brainstem Evoked Response Audiometry (BERA)

BERA represents the progressive activation of different levels of the auditory pathway consisting of I – V waves. Waves I & II arises from cochlear nerve, wave III from Cochlear Nucleus , wave IV rom Superior Olivary Nucleus and wave V from Lateral Lemniscus. Latency of Wave I indicates peripheral transmission and wave III and V indicates brainstem transmission of auditory impulses. The inter peak latency between wave I-V depicts conduction time and is an index of central nervous system development.

An evoked potential is generated by a brief click transmitted from an acoustic transducer in the form of an insert earphone or headphone. The elicited waveform response is measured by surface electrodes typically placed at the vertex of the scalp and mastoid. The amplitude (microvoltage) of the signal is averaged and charted against the time (millisecond). The waveform peaks are labeled I-VII. These waveforms normally occur within a 10-millisecond time period after a click stimulus presented at high intensities (70-90 dB normal hearing level [nHL]). It includes parameters like absolute latencies of wave I, II, III, IV and V with inter-peak latencies of I-III and III-V, I-V and amplitude of waves I, III and V

Sample size: 260 [130 females with IDA and 130 normal adult females]

Sample size was calculated using the formula for difference in proportions.



Prevalence – 43%, Odds Ratio – 2, Confidence Interval (CI) – 95%, Power – 80%

Sampling technique – Purposive sampling

Data collection process / Study approach

The study group will be recruited from Department Of General Medicine and Obstetrics and Gynecology, JNMC, diagnosed with Iron Deficiency Anemia (IDA), while Normal non-anemic subjects will be selected from healthy community volunteers on the basis of history, clinical examination and hematological assessment. Informed consent from each subject is obtained and the entire study is done with due permission from Institutional Ethics Committee following prescribed ethical standards.

At the baseline assessment, females with IDA and Normal females are subjected to history taking, complete clinical and neurological examination, with special attention to signs of IDA followed by Blood analyses, Cognition and Neurophysiological assessment using P300 & BERA. The collected data is analyzed followed by report writing.

Data entry : collected data will be entered in Microsoft excel .

Analysis Plan –

SPSS v.21 will be used for performing statistical analysis.

Statistical analysis will be done to find out mean, standard deviation and standard error of mean. All the values will be expressed as mean and 1 standard deviation.

Table 1. Comparison of baseline characteristics and hematological parameters. Test – unpaired 't' test. p-value < 0.05 is considered to be statistically significant.

Parameters	Females with IDA (Mean ± SD)	Normal Females (Mean ± SD)
Age		
Weight (kg)		
Height (mt)		
BMI		
Hemoglobin (g/dl)		
Serum ferritin (ng/mL)		

Table 2. Comparison of Neuro-physiological parameters – BERA and P300 . Test – unpaired 't' test. p value<0.05 is considered to be significant.

BERA Parameters	Females with IDA (Mean ± SD)	Normal Females (Mean ± SD)

Absolute latency(ms) I II III IV V		
Inter-peak latency(ms) I-III III-V I-V		
Amplitude (μV) I III V		
P300 Latency (ms) Amplitude (μV)		

Table 3. Comparison of cognitive performance – MoCA score.

Analytical test - ANOVA. p value <0.05 is considered as significant.

Cognitive performance	Females with IDA (Mean ± SD)	Normal Females (Mean ± SD)
MoCA score • Visuospatial and executive functioning 5 points • Animal Naming: 3points • Attention • Language • Abstraction • Delayed recall (short-term memory) • Orientation • Education level		

Table 4. Relationship of Hemoglobin and the Cognitive status. Test applied Chi square-p value <0.05 considered as significant

Anemia status/ grading	No. of subjects	Hemoglobin g/dl	MoCA score

No anemia			
Mild			
Moderate			
Severe			

Table 5. Association between Blood Hemoglobin, P300 and BERA.

Test applied – ANOVA. p value <0.05 considered as significant

Parameter	IDA(Mean ± SD)	Normal (Mean ± SD)
Blood Hemoglobin (g/dl)		
P300		
BERA		

Table 6. Association between Serum Ferritin, P300 and BERA.

Test applied – ANOVA. p value <0.05 considered as significant

Parameter	IDA(Mean ± SD)	Normal (Mean ± SD)
Serum Ferritin (ng/ml)		
P300		
BERA		

Expected outcome – Iron deficiency anemia in adult females may effect the normal neurophysiological functions which is reflected by cognitive decline and impaired evoked potentials like p300 and BERA.

Discussion- Research studies by many investigators revealed that IDA greatly influences cognitive functions in infants, children and adolescents. These clinical studies demonstrated significant positive correlation between blood hemoglobin levels and various cognitive aspects in anemic and non-anemic children and adolescents[11,12] . However, studies in adults on this topic are limited and controversial.

Eman Khedr et al. studied 28 young adults in the study titled - Iron states and cognitive abilities in young adults: Neuropsychological and Neurophysiological assessment, wherein they assessed the effect of IDA on cognition using different cognitive tests (MMSE, WMS-R, and P300). It was demonstrated that when compared to non- anemic control subjects, adults with IDA scored

less in various cognitive tests and had prolonged p300 latency and reduced amplitudes indicating decreased cognitive performance. Blood hemoglobin levels positively correlated with the cognitive scores i.e higher Hb levels resulted in better cognitive performance. P300 latency showed significant negative correlation with Hb levels, inferring delayed response in anemic subjects when compared to normal adults.[8]

In another cross sectional study, 1951 females aged between 10 -20 years residing in rural area of Haryana showed positive correlation between blood hemoglobin levels and MoCA scores [5]. Similar study done by H Kececi showed reduced brain electrogenesis reflected by altered P300 in adults with IDA.[13]
Hence, these studies concluded that IDA is an important factor strongly related to cognitive achievement in adults.

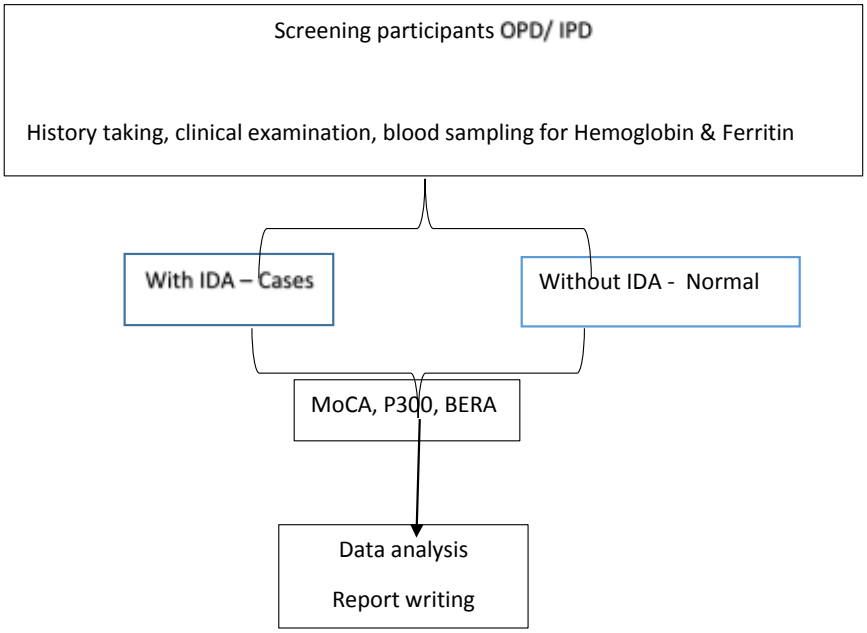
IDA also has an impact on transmission in the auditory pathway which is assessed using BERA, which is non-invasive method to detect neuro-deficit like sensory hearing impairment. Ramit Dey studied 40 subjects with IDA in the age group of 12-16 years and found increased absolute latencies in the waves I, III & V and also increased inter-peak latencies indicating delayed transmission in auditory pathway when compared to non-anemic subjects. The blood hemoglobin levels negatively correlated with the wave I latency suggesting severity of anemia may have direct association with impulse transmission and degree of hearing impairment.[10]

In a cohort study done in children the prevalence of hearing loss was 3.0% in children with IDA and 1.7% in those without IDA[14]. Similar studies in the U.S.A and Taiwan in adult population has suggested IDA may be an significant causal factor for sensorineural hearing loss.[15,16] Few articles related to this study were reported [17-20.]

The possible mechanisms explained for reduced cognitive performance and neuro-deficits in IDA are that since iron is an important cofactor required for DNA synthesis and neurotransmitter metabolism, its low levels may hamper normal physiological functioning; and low hemoglobin in anemia leads to reduced oxygen delivery leading to increased ischemia in these tissues.[4,14]

IDA in various studies is seen to independently effect cognition (MoCA) & P300) and auditory brainstem responses. In addition many studies have demonstrated that hearing impairment has negative consequences on cognition. As per our knowledge, there are no studies where an association has been established between IDA, Evoked Potentials and Cognitive Performance.

Since there is data mounting regarding the effects of IDA during developmental stages like infancy, childhood and adolescence; the present study focuses on non-developmental effects of IDA in adult females. Because of high prevalence of IDA in developing countries[21,22,23,24], these females are vulnerable to deleterious effects of IDA particularly on cognitive skills and auditory response. Impaired cognition and hearing have a huge impact on understanding speech and problem solving which may affect her daily activities. Lack of confidence, poor academic performance, less recognition at work place and home may be experienced due to decrease in intellect. Hence, it is important for clinician and policy makers to address the potential causative factors, routinely screen this age group for early detection and provide timely correction of the deficiency so as to improve the quality of life in these females.



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