Temporal ordering and auditory attention related with hemoglobin concentrations in adolescents

Ordenação temporal e atenção auditiva e sua relação com concentrações de hemoglobina de adolescentes

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Abstract

Background: *Anemia can cause changes in temporal ordering as well as in auditory attention, important factors in the process of learning process and language acquisition.* ***Purpose:*** *To evaluate the association between concentrations of hemoglobin and Temporal Ordering and Auditory Attention in adolescents.* ***Methods:*** *Series study, involving 17 adolescents with inadequate hemoglobin concentrations compared with a control group of 17 adolescents with adequate hemoglobin concentrations, aged 13-18 years and 11 months, of both sexes, attending public schools. It was evaluated hearing abilities of use pitch pattern test and* $P_{300,}$ *hemoglobin concentrations, nutritional status and socioeconomic and demographic characteristics.* ***Results:*** *The inadequate hemoglobin concentrations were not associated with auditory skills, when adjusted for nutritional status and socioeconomic and demographic characteristics. The*

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concentrations of hemoglobin were found to be at low levels, considered as mild anemia. Temporal ordering presented were more changed in the adolescents with inadequate hemoglobin concentration. The average latency of the P_3 component in adolescents with inadequate hemoglobin levels was lower in those with higher age, higher educational level, higher socioeconomic status and registration of repetition. ***Conclusion:*** *Mild anemia does not cause deleterious effects on abilities of temporal ordering and auditory attention. Investigations with larger numbers of cases and other levels of anemia are recommended to test this association.*

Keywords: *Electrophysiology; Attention; Adolescent Nutrition; Hearing; Hemoglobins.*

Resumo

Introdução: *A anemia poderá provocar alteração nas habilidades de ordenação temporal, assim como na atenção auditiva, fatores importantes no processo de aprendizado e aquisição da linguagem.* ***Objetivo:*** *Avaliar a associação entre as concentrações de hemoglobina, a ordenação temporal e a atenção auditiva em adolescentes.* ***Método:*** *Estudos de casos, envolvendo 17 adolescentes com concentrações inadequadas de hemoglobina comparado com grupo controle com 17 escolares com concentrações adequadas de hemoglobina, de 13 a 18 anos e 11 meses de idade, de ambos os sexos, de escolas públicas. Foram analisadas as habilidades auditivas com uso de teste padrão de frequência e P_{300} de concentrações de hemoglobina, estado nutricional e características socioeconômico-demográficas.* ***Resultados:*** *As concentrações inadequadas de hemoglobina não mostraram associação com as habilidades auditivas, quando ajustadas para estado nutricional e características socioeconômico-demográficas. As concentrações de hemoglobina situaram-se em patamares que configuram um grau de anemia leve. A habilidade de ordenação temporal apresentou-se mais alterada para os adolescentes com concentração inadequada de hemoglobina. A média de latência do componente P_{300} nos adolescentes com concentrações inadequadas de hemoglobina foi menor naqueles com maior idade, melhor escolaridade, melhor nível socioeconômico e com registro de repetência.* ***Conclusão:*** *A anemia leve não provoca efeitos deletérios nas habilidades de ordenação temporal e atenção auditiva. Investigações com maior número de casos e outros níveis de anemia são recomendáveis para testar essa associação.*

Palavras-chave: *Eletrofisiologia; Atenção; Nutrição do Adolescente; Audição; Hemoglobinas.*

Resumen

Introducción: *La anemia puede causar alteraciones en las habilidades de organización temporal, así como en la atención auditiva, factores importantes en el proceso de aprendizaje y la adquisición del lenguaje.* ***Objetivo:*** *Evaluar la asociación entre las concentraciones de hemoglobina, la ordenación temporal y la atención auditiva en adolescentes.* ***Método:*** *Estudio de casos con 17 adolescentes con concentraciones inadecuadas de hemoglobina en comparación con un grupo control de 17 estudiantes con concentraciones adecuadas de hemoglobina, de 13 a 18 años y 11 meses de edade, de ambos sexos, de escuelas públicas. Fueron analizadas las habilidades auditivas con uso de test estándar de frecuencias y P_{300} de concentraciones de hemoglobina, estado nutricional y características socioeconómico-demográficas.* ***Resultados:*** *Las concentraciones de hemoglobina inadecuados no mostraron asociación con las habilidades auditiva, cuando se las ajusta a estado nutricional y características socioeconómicas y demográficas. Las concentraciones de hemoglobina se mantuvieron en niveles que representan un grado de anemia leve. El pormédio de latencia del componente P_{300} en los adolescentes con concentraciones inadecuadas de hemoglobina fue menor en aquellos con mayor edad, mejor educación, mejor nivel socioeconómico y con registro de repetición escolar.* ***Conclusión:*** *La anemia leve no causa efectos nocivos en las habilidades de ordenación temporal y atención auditiva. Investigaciones con mayor número de casos y otros niveles de anemia son recomendables para probar esta asociación.*

Palabras claves: *Electrofisiología; Atención; Nutrición del Adolescente; Audición, Hemoglobinas.*

Introduction

Nutritional anemia is a public health issue, and may be defined as the condition whereby the hemoglobin (Hb) content in the blood is below levels considered normal, chiefly due to iron deficiency, which accounts for 90% of the nutritional anemia etiology[1].

The groups most at risk for nutritional anemias are pregnant women, children under two years of age, and adolescents[2]. Iron deficiency may have harmful consequences for cognitive development, but this role is difficult to quantify, as it is determined in conjunction with socioeconomic and environmental factors. However, studies have described that anemic adolescents present daytime sleepiness[3], which has a negative impact on their auditory attention (AA), given that it leads to a slowing of information processing.

Auditory processing (AP) involves the behavioral phenomena of sound localization and lateralization, auditory discrimination and auditory pattern recognition, temporal aspects of hearing (resolution, masking, integration, and ordering), among others[4]. An auditory processing disorder (APD) is characterized by an impairment in the ability to analyze and/or interpret sound patterns.

AP may be assessed by behavioral and electrophysiological tests (long latency auditory evoked potentials), which have been proven increasingly powerful as a diagnostic method for issues related to the central auditory system[5-7].

Temporal ordering (TO) involves the perception and the processing of two or more auditory stimuli. It is thanks to this skill that an individual is able to correctly distinguish the order in which sounds occur and process speech sounds, thus being able to understand them[9]. A study has shown that anemia may alter this ability[10]. This skill may be assessed by employing a behavioral auditory test called frequency pattern test (FPT)[8,11].

The structures involved in tonal pattern tests are presumably centered in each of the hemispheres, and the corpus callosum is presumably the structure responsible for the connection between them. The right hemisphere is activated for the recognition of the acoustic contour (intonation, stress and rhythm), and the left hemisphere is responsible for ordering the sequence of stimuli and naming what was heard[8], i.e., both brain hemispheres are involved when performing this test, each with an different function, but working in conjunction, regardless of the ear being stimulated.

Another skill approached in this article, and an important one for auditory processing, is auditory attention (AA), which in turn may be assessed by the long latency auditory evoked potential called $P_{300.}$

This test involved cortical areas of auditory perception, attention, and memory[12]. Its results may be evaluated by the latency (time elapsed until a response is obtained) and/or the amplitude (the value attained by measuring the interval between a positive polarity peak and its closest negative polarity peak). Amplitude does not seem to be a very useful parameter due to its reduced stability for the interpretation of this potential's results[13], given its great variability in clinical groups; therefore, latency has been the parameter of choice in the analysis of this type of data[14].

The P_{300}'s most important response, the component (positive polarity peak) called P_3, takes place when the individual consciously recognizes a change in the acoustic stimulus, which may be either tone burst or speech. In order to elicit the P_3 the oddball paradigm is used, whereby the presentation of a frequent stimulus is randomly interrupted by a rare stimulus[15-17].

This potential has components such as P_1, N_1, P_2, and N_2, which precede the P_3, but latencies, both of the N_2 and the $P_{3,}$ are known to vary according to the difficulty distinguishing a rare stimulus among the frequent ones [13,16]. These latencies are associated with attention, given that the response improves, then the individual is paying attention to the task of detecting the target (rare) stimuli[5]. There are no studies analyzing the temporal ordering and auditory attention through FTP and P_{300} among adolescents according to their nutritional status and socioeconomic and demographic factors.

Methods

Study design and casuistry

This study's sample derived from a cohort of adolescents, designed to investigate the influence of excess body weight in the occurrence of dyslipidemia which began in 2007. From the 256 adolescents found, 26 had inadequate hemoglobin concentrations.

From this sampling, a study of the case series type was conducted involving 17 adolescents with inadequate hemoglobin concentrations, which were compared to a control group composed of 17 students with adequate hemoglobin concentrations, paired according to sex and age. Adolescents between 13 and 18 years of age, of both sexes, regularly enrolled in municipal and state public schools between June 2011 and September 2012, were considered eligible for the study. Those adolescents who were not found at school at the time of data gathering, but whose addresses were on record, were contacted and evaluated in their homes.

Adolescents who presented with an acute illness on the date of collecting biological material for testing in laboratory, or who had any physical impairment that would compromise their anthropometric assessments, were excluded from the study, as were those who were making use of polyvitamins or undergoing treatment for anemia during the two months that preceded the data gathering, those who had musical knowledge, and those with cognitive difficulties that prevented them from understanding the tests, such as Down syndrome or intellectual disabilities, and those who were taking antipsychotic medication.

The following aspects were evaluated: temporal ordering and auditory attention skills, Hb concentrations, nutritional status and the socioeconomic and demographic characteristics of the adolescents. One of the adolescents did not take the electrophysiological test (P_{300}).

Methods and assessment techniques

In order to ensure the participants' normal hearing, a condition for inclusion in the study, tonal and vocal audiometries were performed. In tonal audiometry, auditory thresholds equal to or lower than 20 dB HL were considered normal for the frequencies studied (500 Hz, 1000 Hz, 2000 Hz and 4000 Hz), and in the vocal audiometry, normal outcomes were determined by speech reception thresholds (SRT) equal to or close to the arithmetic mean of the 500 to 2000 Hz frequencies per ear (admitting a variation of +5 or -5 dB HL).

The aforementioned test was conducted inside an acoustic booth, by employing the AC 40 audiometer [*Interacoustics, Kansas City, MI, USA*], with TDH-39 earphones [*Telephonics, Huntington, NY, USA*], calibrated in accordance with the international ISO 8253-1(1989) standard and Resolution no. 364/09 of the Brazilian Federal Board of Speech, Language and Hearing Sciences.

In addition to the audiometry, imitanciometry was also used to rule out middle ear alterations. In this procedure, type-A tympanograms were considered normal, with either present or partially absent acoustic reflexes. For this test, the AZ 7 imitanciometer [*Interacoustic, Kansas City, MI, USA*] was used.

Both audiometry and imitanciometry (tympanogram and reflexes) were only performed after any pathology in the outer ear had been ruled out by an otoscope [*Welch Allin Inc., Skaneateles Falls, NY, USA*] examination conducted by an otorhinolaryngologist. These procedures lasted 10 minutes.

To assess the temporal ordering (TO) auditory skills the previously mentioned audiometer was used, and for reproducing the FPT an Ipad 2 [*Apple, Cupertino, CA, USA*] was connected to the audiometer, with the purpose of sending the sound stimuli recorded and already balanced by the manufacturer [*Auditec Inc., Saint Louis, MI, USA*]. Initially, at that stage, a training session was conducted in order to familiarize the participant with the acoustic stimulus, by explaining the presentation of the two tones (one high-pitched and one low-pitched), combined in a random manner. After this training session and once the participant's comprehension was ensured, thirty frequency patterns were presented, composed of three tones, two of which were the same, monaurally (one ear at a time). This procedure lasted 10 minutes. The data were analyzed based on the mean of the values obtained in the binaural evaluation.

The participant was then instructed to identify the patterns heard using the term "pi" for the high-pitched tone and "po" for the low-pitched tone, a task which is called imitation. The sequences were presented randomly (high/high/low, low/high/high, low/low/high, and low/high/high) at 50 dB SL. Percentages below 83% were considered inadequate[18]. The test results were recorded as a percentage of correct responses.

The AA was assessed through the P_{300} test by employing the equipment called Smart Ep [Intelligent Hearing Systems (IHS), Miami, FL, USA], composed of a signal mediator (which adds up and gives the means of the recorded electrical activities), an amplifier (including a pre-amplifier), an attached notebook (for wave visualization), and a signal generator, duly calibrated by the manufacturer and standardized for record acquisition and analysis.

The tests were conducted with the adolescents lying down on a stretcher located in a semidark room, and disposable electrodes [*Meditrace*, São Paulo, SP, Brazil] were attached to the skin with an electrolyte paste placed in the center for a better conductivity of the electrical current, and with ER-3A earphones [*Acústica Orlandi* Indústria, Comércio e Serviços Audiológicos Ltda., Bauru, SP, Brazil].

Before attaching the electrodes, the skin was rubbed with alcohol at 70% and an abrasive paste [*Nuprep*, Aurora, CO, USA]. The electrodes were placed in the following sequence: Fpz (forehead) for the ground electrode, Fz (vertex) for the inverted electrode, M_1 (mastoid) for the reference electrode on the left ear, and M_2 (mastoid) for the reference electrode on the right ear, and these were then connected to the pre-amplifier.

After the initial preparation, the participant was instructed to remain awake and very relaxed during the test. Before the test began, the earphones were inserted (the blue phone in the left ear, and the red phone in the right ear).

To obtain the response curve, 200 stimuli were presented at 70 dB HL (70 decibels Hearing Level) at 1000 Hz for rare stimuli (RS), and 80 dB HL (80 decibels Hearing Level) at 4000 Hz for frequent stimuli (FS), and the oddball paradigm was used, wherein 80% were FS and 20% were RS. It was also requested that the adolescents count mentally the number of RS presented, raising their fingers whenever they identified these RS and reporting their total number at the end of the test.

Controlling the artifacts was possible due to the acoustic isolation of the room where the test was conducted, with fluorescent lights turned off so as to avoid any interference that might have been caused by this sort of light. Another control factor was the impedance between the electrode and the participants' skin, which should be smaller than 3000 ohm.

The following equipment parameters were used: filter between 0.5 and 30 Hz, monaural stimuli, tone burst with a 20 ms plateau time (5 ms rise/fall time), with an interval between stimuli of 1.1 ms, analysis time of 500 ms, sensitivity of 160 micro volts, alternate polarity, a 30 Hz high-pass filter and a 1 Hz low-pass filter, and a 512 ms window. The procedure lasted for 45 minutes in average, between the placement of electrodes and the test itself.

The latencies of the P_1, N_1, P_2, N_2 and P_3 were marked, following the appearance of the waves, in the *positive – negative – positive* polarities, respectively, when they appeared, occurring in the replication of the "frequent" and "rare" tracing between 80 and 200 ms. The tracing was made on the touchpad of the notebook itself. The peaks of N_2 and P_3 waves were recorded in the device's memory as they appeared, being marked and monitored throughout the test, following the standards of normality for the latency of the N_2 component, responses in the 180 to 250 ms interval, and, for the P_3 component, between 220 and 380 ms[15], following the consensus between two speech-language therapists.

After the preliminary analysis of the results obtained for the N_2 and P_3 components, the arithmetic mean was used as a reference for the analysis of auditory attention.

The analysis of the Hb concentrations in the blood was conducted with the drawing, either in the schools or in participants' homes, of 2 ml of blood through venipuncture, after which the blood was placed in Vacuntainer tubes along with Ethylenediamine tetraacetic acid (EDTA) as an anticoagulant. The samples were sent to the Clinical Laboratory, where the blood was analyzed in the LH 750 hematology analyzer [*Beckman Coulter*, Brea, CA, USA].

Hb was measured in grams per deciliter (g/dL), and anemia was found to be present whenever concentrations were lower than 12 g/dL for female adolescents who were not pregnant and male adolescents from 12 to 14 years of age. For adolescents aged 15 or over, anemia was considered to be present when Hb concentrations were lower than 13 g/dL[1].

Anthropometry was conducted either in the schools or in participants' homes, by weighing and measuring the adolescents twice and working out the mean of the figures. Values with differences of more than 100g for weight or 0.5 cm for height were discarded. Body weight was obtained by using an electronic digital scale [*Omron*, Kyoto, Japan], with maximum capacity of 150Kg and precision of 100g. Participants were weighed barefoot, without objects in their hands or pockets, and without head accessories. Their height was measured by using a portable stadiometer [*Alturexata*, Belo Horizonte, MG, Brazil], with precision of 1mm and exactitude of 0.5cm. The adolescents were placed in an erect position, barefoot, with the arms at their sides, their heels, back and head touching the wall, and looking straight ahead. The procedures for measuring weight and height followed the recommendations in Lohman et al.[19]. The body mass index according to age (BMI-for-age) was used to assess the participants' nutritional status, expressed in z-score values, in accordance with recommendations of the World Health Organization[20].

The socioeconomic and demographic data were acquired via interviews, to determine the sex, the age – divided into two age brackets: between 13 years and 15 years and 11 months, and between 16 years and 18 years and 11 months –, the schooling – classified into elementary school (from the 6th to the 9th grade) and high school (from the 1st to the 3rd grade) –, and whether or not the adolescent had a history of school retention. The criteria used to determine the socioeconomic group to which the participants' families belonged were those of the Brazilian Economic Classification, established by the Brazilian Association of Research Enterprises[21]. The values adopted were: for B_2 and C_1 groups, family incomes of R$2,327.00 and R$1,391.00, respectively, and for the C_2 and D groups, family incomes of R$933.00 and R$618.00, respectively.

Data analysis

The data were transcribed via a double entry using the *Validate*, a module of the Epi-info Program, version 6.04 (*WHO/CDC, Atlanta, GE, USA*), to identify any typing errors. The statistical analysis was conducted by the Statistical Package for Social Science *(SPSS)* for Windows, version 13.0 (*SPSS Inc.* Chicago, IL, USA). The continuous variables were tested for the normality of distribution by the Kolmogorov Smirnov test. The N_2 and P_3 variables and Hb concentrations presented a normal distribution, and were expressed as mean and standard deviation. Pearson's chi-squared test was used to compare the proportions. To describe the proportions, the binomial distribution was approximated to the normal distribution by the confidence interval of 95%. Levene's test for equality of variances and Student's *t*-test for unpaired data were used to compare the means. A significance level of 5% was adopted for rejection of the null hypothesis.

Ethical aspects

The research protocol was approved by the Research Ethics Committee (no. 02/2010). All the adolescents who participated in the study were previously informed of its objectives, as well as of the methods to be adopted so that, with their permission, each father or person responsible would sign a free and informed agreement. All those involved were given copies of the tests and received the necessary guidance, depending on each case.

Results

The case series (n = 17 students) with inadequate Hb concentrations (11.8 ± 0.3 g/dL) has a control group (n = 17) with adequate Hb concentrations (13.3 ± 0.7 g/dL) (p value = 0.00). Regarding the TO and AA behavior for the groups studied, it bears pointing out that, in proportional terms, the percentage of altered responses was higher among the adolescents with inadequate Hb concentrations than among those without the nutritional disorder (Table 1).

Table 1. Temporal ordering (FPT) and auditory attention (N_2 e P_3) skills according to hemoglobin concentrations among adolescents ranging from 13 to 18 years of age

	Hemoglobin concentrations (g/dL)				
	Inadequate[1]		Adequate		p* value
	n	Mean [SD]	n	Mean [SD]	
FPT (%AR) [2]	10/17	58.8 [32.9 - 81.5][a]	4/17	23.5 [6.8 - 49.9][a]	0.08[b]
N_2	16	263.8 ± 23.4	16	260.4 ± 39.2	0.76
P_3	16	332.5 ± 30.9	16	342.7 ± 31.4	0.36

[1]Hb<12g/dL from 12 to 14 years of age and for females. From 15 years of age on, the Hb<13g/dL in males (OMS, 2001), [2] Frequency Pattern Test and % AR = percentage of responses with percentages < 83%.

* statistically significant values (p≤ 0.05) - t-student test for unpaired data

[a] Confidence Interval 95%, [b] Pearson's chi-squared test

Legend: n= number of adolescents whose test results were altered/total number of participants; SD= standard deviation.

For the temporal ordering skill, the number of altered results, i.e., those with means below 80%, was higher among participants with anemia. The statistical analysis did not show a statistically significant difference in the comparison between the groups, even though the probability was close to the significance level.

The TO assessment, when considered under the viewpoint of socioeconomic and demographic characteristics, did not show any association between the students who had the nutritional disorder and those who did not (Table 2).

Table 2. Temporal ordering (FPT) according to hemoglobin concentrations, adjusted for the nutritional status and the socioeconomic and demografic characteristics in adolescents from 13 to 18 years of age

VARIABLES	Hemoglobin concentrations (g/dL)							
	Inadequate[1]				Adequate			
	FPT				FPT			
	n	AR	%	$IC_{95\%}$	n	AR	%	$IC_{95\%}$
Nutritional Status[2]								
Eutrophy	14	10/14	71.4	[41.9–91.6]	13	4/13	30.8	[9.1–61.4]
Excess weight	3	0/3	0	[0 –70.5]	4	0/4	0	[0– 60.2]
Sex								
Male	3	2/3	66.6	[9.4 –99.1]	3	1/3	33.3	[0.8 – 90.6]
Female	14	8/14	57.1	[28.9–82.3]	14	11/14	78.6	[49.2 – 95.3]
Age (years)								
13 ├┤ 15 y 11 m	10	5/10	50	[18.7 –81.3]	10	0/10	0	[0– 30.8]
16 ├┤ 18 y 11 m	7	5/7	71.4	[29.0– 96.3]	7	4/7	57.1	[18.4 – 90.1]
School Year*								
Elementary school	8	5/8	62.5	[24.5– 91.5]	11	2/11	18.2	[2.3 – 51.7]
High school	8	4/8	50	[15.7– 84.3]	6	1/6	16.7	[0.4 – 64.1]
Retention								
Yes	10	7/10	70	[34.7– 93.3]	7	1/7	14.3	[0.4 – 57.9]
No	7	3/7	42.8	[9.9 – 81.6]	10	3/10	30	[6.7 – 65.3]
Socioeconomic status[3]**								
B_2 and C_1	10	8/10	80	[44.4– 97.5]	8	0/8	0	[0 – 36.9]
C_2 and D	7	5/7	71.4	[29 – 96.3]	6	2/6	33.3	[4.3 – 77.7]

[1]Hb<12g/dL from 12 to 14 years of age and for females. From 15 years of age on, Hb<13g/dL in males (OMS, 2001), [2] Eutrophy: ≥ Z Score -2 and ≤ 1; Excess weight: > Z Score +1, [3] Brazilian Association of Research Enterprises, 2010: B_2 and C_1 groups - R$2,327.00 and R$1,391.00, respectively, C_2 and D groups – R$933.00 and R$618.00, respectively.

*01(one) case with no record of the information within the group with inadequate Hb concentrations, ** 03(three) cases with no record of the information within the group with adequate Hb concentrations.

Legend: n= number of adolescents that fit the category studied; AR = number of adolescents whose responses were altered, i.e., with percentages <83% in the FPT/ total number of participants in the category studied.

As to AA, the means of the latencies in the N_2 components showed a similar distribution among the students of both groups when adjusted for the nutritional status and the socioeconomic and demographic characteristics (Table 3).

On the other hand, in the series of students with inadequate Hb concentrations, the AA assessed by the P_3 component showed a more prolonged latency among the younger students, enrolled in elementary school, with no history of school retention, and who belonged to the least privileged socioeconomic group (Table 4).

Table 3. Auditory attention assessed by the N_2 component according to the hemoglobin concentrations adjusted for the nutritional status and the socioeconomic and demografic characteristics in adolescents from 13 to 18 years of age

VARIABLES	Hemoglobin concentrations (g/dL)									
	Inadequate [1]					Adequate				
	N_2					N_2				
	n	Mean		SD	p[a]	n	Mean		SD	p[a]
Nutritional Status[2]										
Eutrophy	13	265.8	±	24.4	0.49	12	257.1	±	41.4	0.58
Excess weight	3	255.0	±	20.4		4	270.1	±	34.8	
Sex										
Male	3	261.2	±	29.5	0.83	3	248.5	±	23.8	0.57
Female	13	264.4	±	23.2		13	263.1	±	42.18	
Age (years)										
13 ├┤15 y 11 m	9	268.0	±	26.5	0.43	9	250.6	±	30.6	0.27
16 ├┤18 y 11 m	7	258.3	±	19.3		7	272.9	±	47.5	
School year										
Elementary school	8	263.7	±	29.4	0.99	10	250.9	±	27.7	0.22
High school	8	263.9	±	17.7		6	276.1	±	52.4	
Retention										
Yes	10	256.5	±	16.6	0.10	7	263.8	±	54.1	0.77
No	6	276.4	±	29.1			257.7	±	25.7	
Socioeconomic status[3*]										
B_2 and C_1	9	264.6	±	21.5	0.88		250.6	±	34.9	0.21
C_2 and D	7	262.8	±	27.4			282.2	±	51.9	

[1]Hb<12g/dL from 12 to 14 years of age and for females. From 15 years of age on, Hb<13g/dL in males (OMS, 2001), [2] Eutrophy: ≥ Z Score -2 and ≤ 1; Excess weight: > Z Score +1, [3] Brazilian Association of Research Enterprises, 2010: B_2 and C_1 groups - R$2,327.00 and R$1,391.00, respectively, C_2 and D groups – R$933.00 and R$618.00, respectively.
[a] - statistically significant values ($p \leq 0.05$) - t-student test for unpaired data
* 03(three) cases with no record of the information within the group with adequate Hb concentrations.
Legend: n= number of adolescents that fit the category studied; SD = standard deviation

Table 4. auditory attention assessed by the P_3 component according to the hemoglobin concentrations adjusted for the nutritional status and the socioeconomic and demografic characteristics in adolescents from 13 to 18 years of age

VARIABLES	Hemoglobin concentrations (g/dL)									
	Inadequate[1]					Adequate				
	P_3					P_3				
	n	Mean		SD	p[a]	n	Mean		SD	p[a]
Nutritional Status[2]										
Eutrophy	13	332.5	±	33.9	0.92	12	339.1	±	30.7	0.44
Excess weight	3	332.3	±	16.1		4	353.5	±	36.5	
Sex										
Male	3	330.3	±	20.3	0.90	3	327.5	±	16.7	0.37
Female	13	332.9	±	33.5		13	346.2	±	33.4	
Age (years)										
13 ├┤ 15 y 11 m	9	347.8	±	25.1	0.02	9	331.4	±	20.6	0.10
16 ├┤ 18 y 11 m	7	312.8	±	27.3		7	357.2	±	38.2	
School year										
Elementary school	8	349.5	±	22.2	0.02	10	335.3	±	16.4	0.24
High school	8	315.4	±	29.9		6	354.9	±	46.7	
Retention										
Yes	10	320.3	±	25.4	0.04	7	354.1	±	33.9	0.21
No	6	352.7	±	30.3		9	333.8	±	28.0	
Socioeconomic status[3*]										
B_2 and C_1	9	318.8	±	26.6	0.04	8	335.8	±	33.1	0.33
C_2 and D	7	350.1	±	28.3		5	356.1	±	37.5	

[1]Hb<12g/dL from 12 to 14 years of age and for females. From 15 years of age on, Hb<13g/dL in males (OMS, 2001), [2] Eutrophy: ≥ Z Score -2 and ≤ 1; Excess weight: > Z Score +1, [3] Brazilian Association of Research Enterprises, 2010: B_2 and C_1 groups - R$2,327.00 and R$1,391.00, respectively, C_2 and D groups – R$933.00 and R$618.00, respectively.
[a] - statistically significant values (p≤ 0.05) - t-student test for unpaired data
* 03(three) cases with no record of the information within the group with adequate Hb concentrations.
Legend: n= number of adolescents that fit the category studied; SD = standard deviation

The adolescents with inadequate Hb concentrations and a history of school retention showed a significantly longer latency, when compared to those students with adequate Hb concentrations and who had a history of school retention.

Discussion

It is important to point out to the study's limitations, such as the fact that Hb concentrations were the only proxy for defining the iron-deficiency anemia, without supplementary data from other parameters of iron nutritional status, such as serum ferritin and iron, total iron-binding capacity, transferring saturation percentage, free erythrocyte protoporphyrin, and, above all, transferrin receptors. Other limiting factors were due to the reduced sample size, which may cause interference in the statistical treatment of data, in which the conclusion of statistical inference tests may be negatively affected, and the fact that the case series studied comprised only patients classified as having mild anemia.

The case series with inadequate hemoglobin concentrations showed Hb values very close to normal ones, therefore indicating only mild cases of anemia, which was one of the study's limitations. The lack of association between TO and the inadequate Hb concentrations seen in this study's casuistry was an unexpected finding, given that this skill depends on AA, which is presumably altered in anemic patients. Therefore, one must take into account that the study involved only students with mild cases of anemia, leading to the assumption that Hb concentrations so close to normal would not be sufficient to produce a negative impact on these skills.

This lack of correlation was evidenced even when the TO was assessed in the case series and in the control group analyzed according to the variables that could, in theory, influence the test results, as with the nutritional status and the socioeconomic and demographic characteristics.

Regarding age, a study[22] showed that this variable is not a TO-altering factor, as the development of the auditory system in 9-year-old children is similar to that of adults, and does not change after the age of 11 [22,23].

We expected schooling to have an influence in TO skills, as a steady school learning is considered to facilitate test comprehension[8], as seen in studies conducted with individuals of other age brackets[11,24], which showed a better performance in this skill. This effect may be due to stimuli provided by day-to-day activities such as listening to music, gaming, and others, rather than by formal school education.

The lack of influence of school retention in the FPT behavior was also reported in another study[22], which investigated students aged 11-13 from a private school of Belo Horizonte, Brazil, and also found no association between the test and formal education.

The lack of association between socioeconomic status and this type of skill may be attributed to the fact that most of the adolescents who participated in the study belonged to very similar socioeconomic groups, with only one belonging to the B_2 group (a higher socioeconomic level). It bears stressing that there is a gap in the literature regarding investigations on this association among patients with inadequate hemoglobin concentrations.

The homogeneity in the distribution of the means of latencies in the N_2 and P_3 components was also an unexpected result, considering that AA should be expected to be influenced by the inadequate Hb concentrations, given that iron is necessary for brain function and an essential component of hemoglobin[25], leading to the belief that its decrease would cause changes in the conduction of cortical fibers, negatively affecting neurotransmission and the development of oligodendrocytes, leading to hypomyelination, which could result in a slowing of information processing and have a negative impact on cognitive development[25].

Iron depletion, as well as low ferritin levels, have been described in attention deficit hyperactivity disorders (ADHD)[26], evidencing the prolonged latencies in individuals presenting with this disorder[27]. Consequently, it would be plausible to assess AA with the use of P_{300} to evaluate this skill in patients with iron-deficiency anemia.

Regarding nutritional status, studies have shown an association between obesity and the prolonging of latencies of the N_2 component in adolescents and adults[28]. Despite the lack of association between N_2 and the nutritional status in our casuistry, we may assume that this prolonging is due to a slower cortical response of the component in question, even though additional and deeper investigations are needed in order to strengthen this relationship and establish the likely mechanism of cognitive impairment in cases of obesity[29].

The N_2 is generated by the attention and discrimination of a passive, automatic pre-attentional response elicited by the discrimination of the rare event[14]. The socioeconomic and demographic characteristics seem to influence the latency response in component N_2, and may significantly interfere in the integration between the auditory association area and the cortical and subcortical areas of the nervous system, and may be related to functions such as the ability to establish objectives, control impulses, and make decisions, in addition to organizing and planning actions in order to reach an objective[11].

The values of the component P_3 latencies are expected to be influenced by factors such as sex, age and attention level[12,30]. In this casuistry, the shorter latency in the P_3 component among the older adolescents with better schooling could be attributed to the fact that these socioeconomic and demographic characteristics may cooperate to an adequate auditory attention, even in adolescents with inadequate Hb concentrations.

The decrease in the prolonging of latency in component P_3 among adolescents with inadequate Hb concentrations and a higher socioeconomic level may be explained by factors that may benefit these individuals, such as being exposed to a greater diversity of stimuli.

The smaller latency values in the P_3 component among students with a history of school retention and with inadequate Hb concentrations were another unexpected finding, considering that the presence of anemia may reduce attention, cause fatigue, decrease the learning ability, all of which could lead to school retention[2,3].

It is known that the lack of AA compromises school performance and learning, which is why it is important to encourage it during school years, with the purpose of improving the development of oral and written language and learning.

Conclusion

Temporal ordering and auditory attention did not show an association with Hb concentrations. This lack of association persisted even when the analysis model was adjusted for the nutritional status and the socioeconomic and demographic characteristics.

Investigations with a larger number of cases and more varying degrees of inadequate Hb concentrations, including moderate and severe cases, are recommended to better assess this association.

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