Anemia is associated with cognitive impairment in adolescent girls: a cross-sectional survey

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Anemia is associated with cognitive impairment in adolescent girls: A cross-sectional survey

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Anemia is associated with impairment in oxygen transport, affecting an individual's physical and mental wellbeing, and work performance. The aim of this study was to examine the prevalence of anemia and its possible association with serum antibody titers to Hsp27 (as an indicator of cellular stress), cognitive function, measures of emotion, sleep patterns in adolescent girls. A total of 940 adolescent girls were assessed to evaluate neuropsychological function with validated questionnaires. A complete blood count was determined as part of the assessment of hematological parameters. Serum anti-Hsp27 was measured for each subject. Among the total of 940 participants, 99 girls (10.5%) were anemic [hemoglobin <12(g/dl)]. Serum anti-HSP27 was significantly higher in anemic compared to healthy girls ($p<0.05$). There was no significant differences in depression, aggression, insomnia, daytime sleepiness and sleep apnea score between two groups. However, the total cognitive abilities score was significantly lower in the anemic girls (76.8±2.1 versus 85.7±2.5, $p = 0.002$). Logistic regression analysis showed that anemic girls were 1.73 times more likely than non-anemic girls to have cognitive impairment (95% confidence interval [CI] = 1.07-2.78; $P = 0.025$). Anemia was associated with elevated levels of anti-HSP27 and supports the hypothesis that cellular stress may be associated with anemia. Anemia was adversely associated with an assessment of cognitive abilities and was an independent risk factor for cognitive impairment in this group.

Keywords: Heat shock protein; cognitive ability; depression; sleep disorder

1. Introduction

Anemia is a global health problem in which the size and/or number of red blood cells (RBC) or the concentration of hemoglobin (Hb) falls below healthy values. The cut-off values of Hb concentrations have been defined by the World Health Organization (WHO) for various population groups (Khusun, Yip, Schultink, & Dillon, 1999; Organization, 2001).

Anemia has been related to several indicators of poor nutrition, health and wellbeing, of which fatigue and weakness are common features. Anemia is associated with impairment of oxygen delivery to tissues and affects physical and work performance, growth and reproductive function(Organization, 2001).

In women of childbearing age, anemia has several consequences, including having children of low birth weight, perinatal and maternal mortality, and high risk of some diseases in later life (Allen, 2000). Anemia may have a direct influence on brain function. Recent studies indicate that iron deficiency anemia adversely affects behavior by producing cognitive and non-cognitive disturbances (Algarín et al., 2013).

Heat shock protein 27 (HSP27) is a highly conserved small protein and intracellular molecular chaperone which up-regulated in cells in response to the exposure to stressful stimuli (Zilaee, Ferns, & Ghayour-Mobarhan, 2014). HSP27 has been proposed as a biomarker of inflammation, since immune response to HSPs may be involved in the progression of atherosclerosis, lupus erythematous, multiple sclerosis, inflammatory bowel syndrome, and possibly mental disorders (Abdi et al., 2012; Kim, 2003; Singh & Newman, 2011). Serum antibodies against HSP27 (anti-HSP27) may be raised in subjects with inflammatory conditions (Zilaee et al., 2014). In the last decade, there has been an increasing interest in the association between Hsp27 and cardiovascular disease (Ghayour-Mobarhan, Rahsepar, Tavallaie, Rahsepar, & Ferns, 2009). In thalassemia, the increased levels of pro-oxidative stress and higher risk of infectious complications may trigger HSP27 expression

(Saber et al., 2013). It has also been reported that Hsp27 release may stimulate an autoimmune response leading to Hsp27 antibodies that may be involved in cardiovascular disease (Ferns, Shams, & Shafi, 2006; Lamb, El-Sankary, & Ferns, 2003).

In the present study, we assessed serum anti-Hsp27 antibody titers in anemic adolescent girls to determine potential therapeutic target of these indices in the treatment of cardio-metabolic burdens in these patients. We have recently found that adolescent girls with dysmenorrhea experience more depressive mood, aggressive behavior and sleep disorders than those who do not(Afsane Bahrami, Sadeghnia, Avan, Mirmousavi, Moslem, Eslami, Heshmati, Bahrami-Taghanaki, et al., 2017). We also wished to investigate the association between anemia and neuropsychological parameters including mood and emotional function, cognitive abilities and sleep disorders between anemic and non-anemic adolescent girls to detect significant relationships and clinically important factors.

2. **Material and methods**

2.1 .Study population

This cross-sectional study was conducted on subjects from the cities of Mashhad and Sabzevar, in northeastern Iran, from January to April 2015, as previously described (A. Bahrami et al., 2018; Afsane Bahrami, Sadeghnia, Avan, Mirmousavi, Moslem, Eslami, Heshmati, Bahrami-Taghanaki, et al., 2017). A cluster randomization sampling method was used to recruit adolescent girls from schools in the two cities. Written consent was obtained from subjects and their parents. Exclusion criteria included: any auto-immune disease, cancer, metabolic bone disease, hepatic or renal failure, cardiovascular disorders, malabsorption, thyroid, parathyroid or adrenal disease. After applying these criteria, 940 participants aged 12-18 years old remained, and completed this study. The Ethics Committee of the Mashhad University of Medical Sciences approved the study.

2.2. Laboratory measurements

Anthropometric parameters were obtained in health centers, by trained paramedical staff. Body weight was measured while subjects were in over-night fasting state and without shoes and with minimal clothing. After assessment of height, body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

A complete blood count (CBC) was determined as part of the assessment of hematological parameter performed using the SysmexK-800. A Hb concentrations <12 g/dL was used to define anemia, which is the threshold value of the World Health Organization's gender-based definition in women(Organization, 2001).

An in-house ELISA assay was used to quantify serum anti-Hsp27 levels as described previously(Sahebkar et al., 2011). Briefly, serum was diluted with 2% goat serum in PBS was added to microplates which were coated with recombinant human HSP27. After washing, diluted peroxidase conjugated-goat antihuman IgG in PBS, was added to each well. After incubation and washing, TMB substrate was added per well and incubated in the dark room. The reaction was terminated by HCl addition. The optical density at 450 nm was determined with 620 nm reference wavelength.

2.3. Neuropsychological status

2.3.1. Cognitive abilities

Cognitive abilities magnitude was evaluated using the Cognitive Abilities Questionnaire (CAQ)(Nejati, 2013; Piryaei, Khademi Ashkzari, Nejati, Arshadi, & Talkhabi, 2017). The CAQ composed of 30 items, each of which is scored on a 5-point Likert scale (1–5) and then the minimum and maximum total scores for questionnaire are 30 to 150, respectively. The CAQ judged seven distinctive abilities: memory (6 items), inhibitory control and selective attention (6 items), decision making (5 items), planning (3 items), sustained attention (3 items), social cognition (3 items), and cognitive flexibility (4 items). Higher scores in each tasks show better abilities. Total and subscale scores of CAQ for each subject were recorded.

2.3.2. Emotional function

Depression status: The Beck Depression Inventory (BDII) is a standard questionnaire with 21- self-report items which assess depression status. Item scored from 0 to 3 and total score obtain 0 to 63. Regarding to the total score, participants categorized as : score of 0-13 were considered (Minimal or No depression), 14-19 (mild depression), 20-28 (moderate depression) and 29-63 (severe depression)(Beck et al., 1996).

Aggression status: The self-report 29-questions with 5-point Likert scale (1-5), the Buss-Perry Aggression Questionnaire (BPAQ) was used to investigate the aggression level of the participants (Motevalian et al., 2011). By increasing BPAQ score, aggression level is enhanced.

2.3.3. Sleep pattern

Insomnia: The Insomnia Severity Index (ISI) is a reliable questionnaire which quantifies insomnia intensity. The instrument consist of 7 items concerning on sleep disorder severity, satisfaction of sleep and anxiety related to the sleep problems which rated based on a 5-point score (0–4) and provide a final score ranged between 0 and 28. Subjects classified based on acquired scores as follows: no clinically significant insomnia (score: 0–7), mild insomnia or (score: 8–14), moderate insomnia (score: 15–21) and severe insomnia (score: 22–28) (Yazdi et al., 2012). Higher scores positively associated with increased insomnia degree.

Sleepiness: The Epworth Sleepiness Scale (ESS) is a reliable questionnaire which assesses the degree of daytime sleepiness. It is an 8-item with 4-point Likert scale (0-3) that judged the frequent probability to fall asleep in routine conditions of daily living. Total scores are wide range from a value of 0 to 24. Degree of daytime sleepiness is interpret according to

subsequent criteria: normal (score<10), mild to moderate (score:10–16), severe (16 <score) (Haghighi et al., 2013).

Sleep apnea: the STOP-Bang sleep apnea questionnaire was recruited for further assessment of sleep complaints(Sadeghniiat-Haghighi et al., 2015). But, STOP-Bang sleep apnea questionnaire is a scoring model includes 8 questions with Yes/No answers (score: 1/0, respectively) which provide total scores range from 0 to 8 which classified as; low risk (score<2), intermediate risk (score: 3-4), and high risk (score>5).

It should be mentioned that the Persian version of all the questionnaires were used their validity and reliability were previously confirmed.

2.4. Statistical method

The Kolmogorov-Smirnov test was applied to assess the normal distribution of variables. Categorical variables were expressed as number (percentage) and quantitative variables as mean±SD or median (interquartile range [IQR]), as appropriate. For normally distributed variables, an independent sample t-test and for non-normal variable Mann-Whitney test was performed. Chi-square tests were applied to compare quantitative variables. To determine the relationship between Hb and other variables, Spearman's correlation coefficients were calculated. Subjects divided into two groups (those with or without cognitive impairment) according to the median score (75.0) of CAQ to perform logistic regression analysis and evaluate risk of cognitive impairment. A P <0.05 was used for statistical significance. All statistical analyses were performed using the Statistical Package for Social Sciences version 17 (SPSS Inc., Chicago, Illinois, USA).

3. Results

Of the 940 girls in the population sample, 99(10.5%) were anemic. The clinical characteristics of the subjects are summarized in Table 1. The anemic girls had a significantly higher diastolic blood pressure and anti-HSP27 than healthy subjects (p<0.05)(Table 1). The

socioeconomic status of participants in relation to anemia are shown in table 2. Pubertal status, education year as well as father's education and occupation were significantly different between the groups ($P<0.05$).

As shown in table 3, the anemic girls had a higher score for depression and aggression, but the difference was not statistically significant. According to the cognitive abilities score, non-anemic girls achieved significantly better scores than their anemic peers (85.7±2.5 versus 76.8±2.1, p= 0.002). The anemic girls had significantly lower scores for memory, inhibitory control and selective attention, decision-making and planning (Table 3).

There was a significant positive correlation between Hb and total cognitive abilities score and subscales including memory, inhibitory control and selective attention, decision making and planning (Table 4).

Using logistic regression analyses, the group with a normal Hb, served as a reference group. Logistic regression analysis show that anemic girls were more likely than non-anemic girls to develop cognitive impairment (OR=1.73, 95% confidence interval [CI] = 1.07-2.78; P = 0.025) (Table 5).

4. Discussion

Our results suggest that anemia is associated with an enhanced cellular stress, as assessed by higher serum anti-HSP27 levels. We found significantly higher levels of serum anti-Hsp27, as an indicator of cellular stress such as inflammation, and oxidative stress in anemic subjects compared with healthy subjects. Most previous reports have studied Hsp-65, -70, and -90, there has been recent interest in exploring the potential role of the smaller Hsps (sHsps), such as Hsp-27, in different clinicopathological conditions(Okuno, Adachi, Kozawa, Shimizu, & Yasuda, 2016). We have previously demonstrated that anti-HSP27 titers were higher in patients with beta-thalassemia major(Saber et al., 2013), but data about this issue for comparison is scarce. The erythrocytes represent an important component of the

antioxidant capacity of blood, containing particular intracellular enzymes such as superoxide dismutase, catalase, and also the glutathione system. Oxidation of RBC membrane may lead to impairment of red cell membrane integrity and premature erythrocyte fragility and lysis (Jilani & Iqbal, 2011).

Anemic adolescent girls were found to have lower cognitive abilities scores compared to non-anemic girls, with significantly lower scores in memory, inhibitory control and selective attention, decision making and planning abilities subscales. The results reported in several previous studies, in different age groups, are consistent with our findings. Carter *et al* found that infants with iron deficiency anemia (IDA) and those with Hb level ≤105 g/L had poorer recognition memory than infants without IDA (Carter et al., 2010). Algarin *et al* have reported that iron deficiency anemia in infancy is associated with poorer inhibitory control and also slower reaction times. They conclude that IDA has a long lasting effect on myelination and prefrontal striatal circuits(Algarín et al., 2013).

In Mexican children aged 6–8 years, Hb concentration was reported to be positively associated with the results of Number Sequencing performance and Peabody Picture Vocabulary Test (Kordas et al., 2004). In another study, the motor performance in different athletic events and cognitive tests was significantly poorer in anemic children than non-anemic counterparts(Gowri & Sargunam, 2005).

A study performed on rural primary school children in India showed significant difference between the intelligence quotient (IQ) scores of anemic and non-anemic subjects in sub-test digit span. In the arithmetic test, anemic children obtained lower scores for attention and concentration(Agarwal, Upadhyay, Agarwal, Singh, & Tripathi, 1989). Moreover, several studies on hemodialysis patients showed that anemia may be associated with impaired cognitive function and that patients with chronic renal failure commonly suffer from confusion, loss of concentration, impaired memory, and low mental alertness (Pickett,

Theberge, Brown, Schweitzer, & Nissenson, 1999). Acute is ovolemic anemia was reported to result in slowing of data-processing ability and degradation of memory in humans(Nissenson, 1992). Furthermore, correction of renal anemia with erythropoietin improves cognitive ability in hemodialysis patients (Moreno, Sanz-Guajardo, Lopez-Gomez, Jofre, & Valderrabano, 2000). An improvement in psychological symptoms appears to be mediated by an enhancement of oxygen transport to the brain.

In this present, large cross- sectional study, in adolescent girls from Mashhad and Sabzevar, Iran the prevalence of anemia was 10.5%. This is lower than for some other countries. In India, 56% of girls were reported to be anemic and 7.1% suffer from severe anemia (Jawarkar, Lokare, Kizhatil, & Jawarkar, 2015). These differences in the prevalence of anemia between studies may be because of the difference in the study area. Unfortunately the prevalence of anemia in developing countries is high because of dietary factors, poverty, some specific diseases and undesirable health access.

According to our results, BMI was similar in the anemic and non-anemic groups which is consistent with Rodriguez-Zuniga *et al* reported no association between anemia and overweight or obesity in children (Rodríguez-Zúñiga, 2014).

While the total scores for depression, aggression, insomnia, day-time sleepiness and sleep apnea was similar between two groups, Chen-Edinboro *et al*. reported that anemic subjects with iron deficiency had more insomnia compared to non-anemic in elderly population (Chen-Edinboro et al., 2017).

Our study has several strengths. It was a large population-based study which compared anthropometrics and neuropsychological parameters in adolescent girls. A limitation of our study, because of the cross-sectional design of the study, the causal effects of anemia cannot be inferred. Furthermore, we did not classify the type of anemia by assessing the iron profile, and blood film. Also, we could not evaluate the status of other pertaining micronutrients such

as the B-vitamins, vitamins C and D which have all been plausible to be associated with Hb level.

Conclusion

In summary, we found that anemia is associated with elevated levels of anti-HSP27 which support the hypothesis as a cellular stress possibly happens in anemia. Also, as anemic girls have significantly reduced levels of cognitive abilities, precise screening and treatment of anemia in adolescent girls is recommended.

Ethics

Ethics Committee Approval: The approval of the Ethic Committee of the Mashad University of Medical Sciences was obtained about this study (941524); Informed Consent: It was taken.

Authorship Contributions

Concept: Majid Ghayour-Mobarhan

Design: Afsane Bahrami; Najmeh Seifi

Data Collection or Processing: Zahra Khorasanch, Gordon A. Ferns

Analysis or Interpretation: Maryam Tayefi

Literature Search: Seyedeh Belin Tavakoly Sany; Hamidreza Bahrami-Taghanaki

Writing: Gordon A. Ferns, Amir Avan

Conflict of interest: The authors of this paper have no conflicts of interest.

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Table 1: Demographic, clinical and laboratory characteristics and biochemical values of anemic and non- anemic girls

Variable	**Anemic** Hemoglobin <12(g/dl)	**Non-anemic** Hemoglobin >12(g/dl)	***P value***
Number (%)	99(10.5%)	841(89.5%)	
Age(year)	14.9±1.55	14.4±1.5	**0.002**
Body mass index(kg/ m^2)	21.2±3.7	21.18±4.48	0.96
Systolic blood pressure (mmHg)	98.2±11.9	95.0±14.7	0.06
Diastolic blood pressure (mmHg)	64.9±12.1	60.8±13.8	**0.011**
Physical activity (MET-h/week)	45.1±2.5	45.4±3.7	0.43
Age of menses (year)	12.5±1.1	12.5±1.2	0.89
Hemoglobin (g/dl)	10.96±1.24	14.34±2.01	**<0.001**
Hematocrit (%)	37.06±4.49	44.19±5.71	**<0.001**
Anti-HSP27(OD)	0.30(0.19-0.31)	0.21(0.11-0.33)	**0.049**

Data presented as mean±SD or median (interquartile range).
By using independent sample t-test (parametric variables) or Mann-Whitney test (non-parametric variables)

Table 2. Demographic and socio-economic features of subjects in association with anemia.				
Variables		**Anemic (n=99)**	**Non-anemic (n=841)**	**P value**
Pubertal	*Yes*	93(94.0%)	720(86.5%)	**0.049**
	No	6(6.1%)	121(13.5%)	
Education (year)	*6-8*	45(45.8%)	500(59.4%)	**0.025**
	8-10	32(32.5%)	235(27.9%)	
	10-12	22(21.7%)	106(12.6%)	
Family members	*2-4*	48(48.5%)	407(48.4%)	0.08
	5-7	43(43.4%)	405(48.2%)	
	>8	8(8.1%)	29(3.4%)	
Exclusive room	*Yes*	56(56.6)	450(53.5%)	0.59
	No	43(43.4%)	391(46.5%)	
Type of house	*Apartment*	33(33.3%)	299(35.6%)	0.68
	Independent home	66(66.7%)	542(64.4%)	
Parent death	*Yes*	2(2.4%)	34(4.1%)	0.46
	No	97(97.6%)	806(95.9%)	
Parent divorce	*Yes*	7(7.3%)	35(4.2%)	0.20
	No	92(92.7%)	806(95.8%)	
Roommate	*Father & mother*	91(91.5%)	776(92.3%)	0.63
	Father	1(1.2%)	6(0.7%)	
	Mother	5(5.0%)	50(6.0%)	
	Other family	2(2.4%)	8(1.0%)	
Father's Occupation	*Worker*	22(21.8%)	293(34.9)	**<0.001**
	Employee	22(21.8%)	140(16.7%)	
	Tradesmen market	23(23.2%)	158(18.8%)	
	Spiritual	5(5.0)	3(0.4%)	
	Other	25(25.6%)	240(28.6%)	
	Deceased	2(2.4%)	5(0.6%)	
Mother's occupation	*Worker*	0(0)	5(0.6%)	0.51
	Employee	7(7.3%)	30(3.6%)	
	Housewife	85(85.5%)	735(87.4%)	
	Other	7(7.3%)	69(8.2%)	
	Deceased	0(0)	3(0.3%)	
Father's education (year)	*0-9*	38(38.6%)	334(39.7)	**0.012**
	10-12	33(33.7%)	378(44.9%)	
	>13	27(27.7%)	132(15.7%)	
Mother's education (year)	*0-9*	2(2.4%)	38(4.5%)	0.78
	10-12	77(77.5%)	632(75.2%)	
	>13	19(19.2%)	171(20.3%)	
Data presented as number (percent). P value obtained from chi-square test.				

Table 3: Score at cognitive abilities, emotional function and sleep pattern tests in anemic and non-anemic groups			
	Anemic Mean ± SD	**Non anemic** Mean ± SD	**P value***
Test of cognitive abilities*			
Memory	11.3±5.7	15.7±8.2	**<0.001**
Inhibitory control and selective attention	15.3±5.5	16.7±6.2	**0.050**
Decision making	12.5±4.8	14.2±5.6	**0.010**
Planning	7.4±3.1	8.6±3.6	**0.003**
Sustain attention	8.5±3.1	8.6±3.2	0.943
Social cognition	10.6±3.1	10.0±3.5	0.112
Cognitive flexibility	10.9±2.8	11.4±3.5	0.211
Total cognitive ability task	76.8±2.1	85.7±2.5	**0.002**
Tests of emotional function			
Depressive mood**	12.0±10.8	10.7±9.1	0.251
Aggressive behavior(BPAQ)**	79.7±19.6	77.5±20.2	0.353
Tests of sleep pattern			
Insomnia (ISI) **	5.2±6.5	3.9±5.6	0.062
Daytime sleepiness (ESS) **	6.7±3.9	6.6±4.2	0.955
Sleep apnea (STOP-BANG) **	0.6±0.5	0.6±0.5	0.910
* independent sample T-test * Higher scores represent better status. **Lower scores represent better status.			

Table 4. Correlation coefficient and β-coefficients between hemoglobin level and cognitive ability tasks		
Task	**r**	**P value**
Memory	0.198	**<0.001**
Inhibitory control and selective attention	0.127	**0.001**
Decision making	0.118	**0.002**
Planning	0.147	**<0.001**
Sustain attention	0.031	0.407
Social cognition	-0.009	0.801
Cognitive flexibility	0.062	0.096
Total cognitive ability task	0.157	**<0.001**

Table 5. Logistic regression analysis of hemoglobin as a predictor of cognitive impairment			
	OR	95 % CI	P value
Hemoglobin <12(g/dl)	1.73	1.07-2.78	0.025
Hemoglobin >12(g/dl)	ref	-	-
Odds ratios with 95% confidence intervals (95% CI) obtained from binary logistic regression tests adjusted for potential confounders (age, diastolic blood pressure, and menstruation)			