

# Effect of Anaemia on Cognitive Ability in Female Dental Undergraduate Students: A Cross-sectional Study

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## ABSTRACT

**Introduction:** Anaemia not only impairs physical growth but also affects behavioral and cognitive abilities. Past studies have proved that increased haemoglobin levels result in improved Central Nervous System (CNS) functions. However, there is a significant lacuna between cognition and anaemia among young adults, especially females.

**Aim:** To assess the cognitive ability of female undergraduate dental students with and without anaemia.

**Materials and Methods:** This cross-sectional study included 100 young female dental students, aged between 18 and 23 years, who presented in the Department of Physiology, Vivekanandha Dental College for Women, Tiruchengode, Tamil Nadu, India, from February to July 2019. Haemoglobin levels were analysed using the colourimetric cyanmethemoglobin method with Drabkin's solution, and based on this, the students were divided into anaemic and non anaemic groups. Cognitive status was assessed using the Digit Symbol Substitution Test

(DSST), letter cancellation test, and Stroop test. The Student's t-test and Pearson's correlation were used to examine the association between haemoglobin levels and cognition.

**Results:** The mean age in the anaemic group and non anaemic group was 19.95±1.80 years and 19.64±1.60 years, and the Body Mass Index (BMI) was 19.54±1.62 kg/m<sup>2</sup> and 19.32±1.43 kg/m<sup>2</sup>, respectively. Haemoglobin levels were significantly lower in the anaemic subjects at 10.408 gm/dL compared to the non anaemic subjects (p-value<0.001). A statistically significant difference was observed between the two groups on DSST scores (21.10±2.99 versus 32.60±14.69), letter cancellation test (25.80±4.63 versus 35.80±4.56), and Stroop test (132.10±8.15 versus 123.56±10.52).

**Conclusion:** The present study reported a significant association between anaemia and cognitive function. Therefore, early screening and prompt treatment of anaemia are crucial for improving both physical as well as mental health.

**Keywords:** Cognition status, Haemoglobin, Malnutrition, Mental health

## INTRODUCTION

Anaemia is a frequently encountered global health concern in both developing, as well as, developed countries [1]. According to National, regional and global estimates of anaemia in 2019, the prevalence of anaemia was 30% of the world population in the women of reproductive age [2]. Anaemia is a sign of poor health and reduced nutrition. Due to the increased demand for nutrition and poor eating patterns, adolescents (undergraduates) are the most vulnerable category of people to develop anaemia [3,4]. Anaemia in adolescents not only leads to impairment of physical growth but also affects behavioural and mental functions, such as lack of concentration, increased mood changes, irritability, and impairment of academic performance among students, mainly due to a reduction in energy level, endurance, and activity [5,6]. It has been found that anaemia is linked with reduced cognition, as shown by the effect of anaemia on age-related cognitive decline, and it is also an independent risk factor for poor cognition. Anaemia has also been linked with a 41-61% increased risk of dementia, especially in the elderly [7,8]. Reduced haemoglobin levels in anaemia cause tissue hypoxia and reduced oxygen delivery to the brain, producing brain ischaemia, which is the most crucial factor in developing cognitive dysfunction and dementia [9-12]. It has also been found that increased haemoglobin results in improved CNS functions [13]. Past studies have concentrated on cognition and anaemia in middle-aged adults or the geriatric population [14,15]. However, there exists a significant gap in understanding the relationship between cognition and anaemia among young adults, especially females. Therefore, this present study was designed to determine the effect of anaemia on cognitive function in undergraduate dental students.

## MATERIALS AND METHODS

This cross-sectional study was selected for an Indian Council of Medical Research (ICMR) Student Short Term Project (STS) with the reference ID (2019-03648) in 2019. The study was conducted for six months from February to July 2019 in the Department of Physiology at Vivekanandha Dental College for Women in Tiruchengode, Tamil Nadu, India. The study was approved by the Institutional Ethical Committee (IEC No. VDCW/IEC/155/2019), and written informed consent was obtained from all the study subjects.

**Inclusion criteria:** All young female dental students, aged between 18-23 years, who were willing to participate, were included in the study.

**Exclusion criteria:** Subjects who had iron supplementation within one month, smokers, alcoholics, subjects with cardiovascular and respiratory diseases and those on medication that affects cognitive performance, subjects with neural disease, hearing impairment, and visual impairment were excluded from the study.

**Sample size:** The sample size was calculated by using the formula:

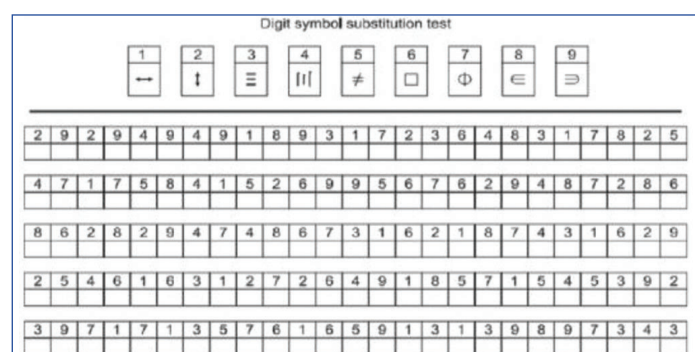
$$N = z^2 * p * (1-p) / e^2$$

According to a previous study, the prevalence rate of anaemia in college-going female students was 72.12% [16]. Using  $z=1.96$  (95% confidence interval),  $e=10\%$  (margin of error), and  $p=0.7212$  (proportion),  $N = 1.96 * 1.96 * 0.7212 (1-0.7212) / 0.1 * 0.1$ ;  $N=77.27$ . Present study included 100 participants randomly.

**Data collection:** General information such as age and family history of diabetes mellitus, hypertension, and cardiovascular disorders were collected. Then, anthropometric measurements

such as height and weight were recorded to calculate the BMI. BMI was calculated using Quetelet's index, i.e., weight (kg)/ (height in meters)<sup>2</sup> [17]. This was followed by drawing 2 mL of venous blood from the antecubital vein, collected in Ethylene Diamine Tetra Acetic Acid (EDTA) vacutainers for analysing the haemoglobin. The blood haemoglobin was analysed using the colourimetric cyanmethaemoglobin method with Drabkin's solution, which contains ferric cyanide, potassium cyanide, and potassium dihydrogen phosphate. This solution oxidises haemoglobin to methaemoglobin and then to cyanmethaemoglobin. The haemoglobin levels were estimated using a spectrophotometer at 530 nm [18]. As per WHO classification, haemoglobin levels <12 g/dL in adult females were considered as indicative of anaemia [19]. Based on this classification, the subjects were divided into anaemic (50 individuals) and non anaemic groups (50 individuals). After this division, the cognitive status was assessed between the two groups by the following tests:

**1. DSST:** This is a standard neuropsychological test used to assess cognitive functioning. Clear instructions were given to the participants before commencing the test. It encloses the key consisting of numbers 1 to 9, each paired with unique symbols like (&, +, >). A series of random numbers from 1 to 9 are repeatedly shown below the key. Participants were asked to fill in the corresponding symbol for each number within 90 seconds. The correct number-symbol pairs inserted by the participants in 90 seconds were considered as test scores [Table/Fig-1] [20,21].



**[Table/Fig-1]:** Showed the DSST symbol coding sheet: Figure reprinted from Patel T and Kurdi MS [21]

**2. Letter cancellation test:** In this test, a worksheet consisting of the English alphabet, jumbled and printed in black on a white sheet of paper arranged in 22 rows and 14 columns was used. Participants were instructed to cancel as many of the six target letters randomly, horizontally, or vertically in one minute. The net score was calculated by subtracting the wrong cancelled letters from the total number of cancelled letters [22].

**3. Stroop test (NIMHANS version):** In the Stroop test, block letter words are printed in a sheet in 16 rows and 11 columns. Each word is a colour name such as red, blue, green, and yellow. The printed colour sometimes matches with the colour represented in the word. The subject had to read the stimulus word in each column as fast as possible, and the time was noted in seconds as the reading time. Then the subject has to name the colour in which the word has been printed in all the columns, and the time was noted in seconds as the naming time. The reading time was subtracted from the naming time to get the Stroop effect score [23].

## STATISTICAL ANALYSIS

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software version 20.0 Quantitative variables were demonstrated as mean±standard deviation. Statistical analysis has been done using the Student's t-test. The association between the factors was analysed using Pearson correlation.

## RESULTS

Hundred subjects were divided into two groups: Group 1 contained 50 participants who were anaemic, and Group 2 had 50 participants who were non anaemic.

In this study, there was no statistical difference between the age and BMI of the anaemic and non anaemic individuals, while haemoglobin showed statistically significant value [Table/Fig-2].

The DSST, letter cancellation test, and Stroop test was found to be statistically significant (p-value<0.0001) [Table/Fig-3].

Demographic profile	Group 1 N=50	Group 2 N=50	t-value	p-value
Age (years)	19.95±1.80	19.64±1.60	0.926	0.357
BMI (kg/m <sup>2</sup> )	19.54±1.62	19.32±1.43	0.707	0.482
Haemoglobin in gm/dL	10.408±0.84	12.718±0.65	15.415	<0.001***

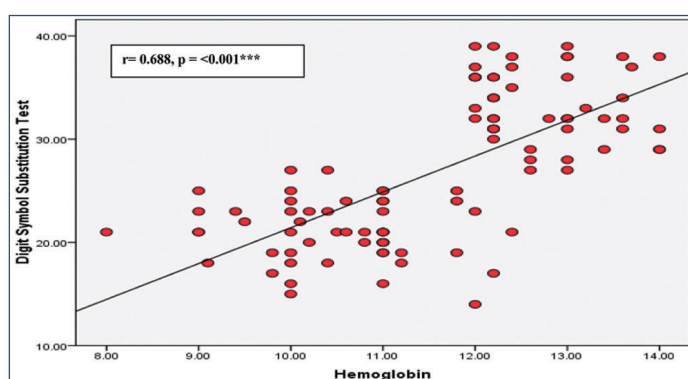
**[Table/Fig-2]:** Comparison of demographic status and haemoglobin level between group 1 and 2 subjects.  
Student t-test. \*p <0.05- \*\*\*p <0.001- statistically significant

Cognitive test	Group 1 Mean±SD N=50	Group 1 Mean±SD N=50	t-value	p-value
Digital Symbol Substitution Test (DSST) score	21.10±2.99	32.60 ±14.69	14.695	<0.0001***
Letter cancellation test score	25.80±4.63	35.80±4.56	10.86	<0.0001***
Stroop test score	132.10±8.15	123.56±10.52	-4.535	<0.000***

**[Table/Fig-3]:** Comparison of cognitive status between group 1 and 2 by student's t-test.  
\*p<0.05- \*\*\*p<0.001- statistically significant

In this study, a significant positive correlation was found between DSST score and haemoglobin concentration (r-value 0.688, p-value <0.001). As the haemoglobin increased, the DSST scoring increased. This showed that anaemic subjects substituted the symbol corresponding to the digit less than the non anaemic subjects [Table/Fig-4].

A significant positive correlation was found between the level of haemoglobin and letter cancellation test scores (r-value 0.642, p-value <0.001). As the haemoglobin increased, the letter cancellation test scores increased. This showed that non anaemic subjects cancelled the letter faster than the anaemic subjects [Table/Fig-5].

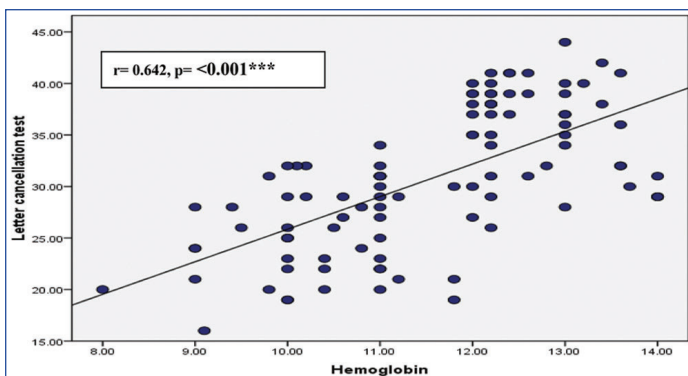


**[Table/Fig-4]:** Correlation between haemoglobin concentrations with Digit symbol substitution test (DSST) scores by Pearson's correlation test.

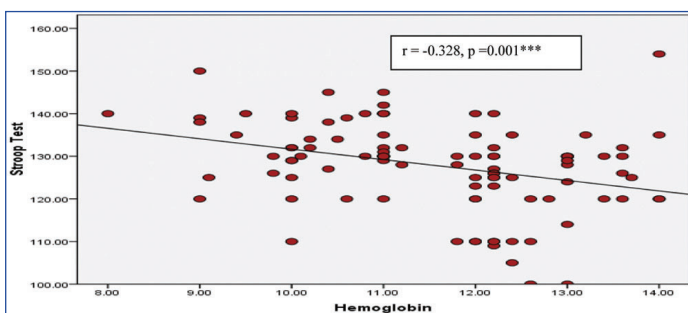
A significant negative correlation was found between the level of haemoglobin and Stroop test readings. When the haemoglobin level increased, the test readings decreased (r value -0.328, p-value 0.001). This showed that the non anaemic subjects were able to do the test faster than the anaemic subjects [Table/Fig-6].

## DISCUSSION

The prevalence of anaemia in the present study was 50%, which was found to be very high, with the mean haemoglobin of 10.40 g/dL. Anaemia is a frequently encountered global health concern



**[Table/Fig-5]:** Correlation between haemoglobin concentrations with Letter cancellation test, by Pearson's correlation test.



**[Table/Fig-6]:** Correlation between haemoglobin concentrations with Stroop Test (ST) By Pearson's correlation test

in both developing, and developed countries. Iron deficiency anaemia, in particular, not only impairs physical growth but also impairs behavioural and cognitive abilities. Anaemia is more frequently observed among pregnant and non pregnant women, as well as elderly women [1,24]. Menstrual blood loss and the inhibitory action of oestrogen increase the vulnerability to anaemia in females [25].

The present study reported an association between cognitive function and haemoglobin levels among the age group 18-23 years in female subjects, showing declined cognitive function in anaemic individuals. More S et al., reported that reduced haemoglobin was associated with decreased verbal learning, attention, concentration, and memory [26]. Terekeci HM et al., found that anaemic individuals had reduced Mini-Mental State Examination scores compared to non anaemic ones and concluded that anaemia may weaken cognitive functions in the elderly [27]. Another study reported that reduced haemoglobin concentrations were linked with worse performance on semantic memory and perceptual speed [28]. Iron is an essential constituent of the main enzymes that take part in vital reactions, neurotransmitter synthesis and removal, and myelin formation. A study done by Chandra MD et al., showed that depletion of peripheral iron causes a major impact on the brain and induces cognitive decline [29]. It is also evident that decreased concentrations of haemoglobin or anaemia can cause chronic brain hypoxia and reduced aerobic capacity, thereby increasing the risk of dementia or cognitive decline [30].

In the present study, the cognitive domains of anaemic individuals were reduced compared to non anaemic subjects, and it showed statistical significance. Black MM reported that anaemic children underperformed compared to normal children in psychomotor skills [31]. A similar study in children also found that a significant decrease in anaemic children showed impaired cognitive skills [32]. It has been found that anaemia is linked with reduced cognition, as shown by the effect of anaemia on age-related cognitive decline, and it is also an independent risk factor for poor cognition. Anaemia has also been linked with a 41-61% increased risk of dementia, especially in the elderly [5,6]. Reduced haemoglobin levels cause tissue hypoxia and reduced oxygen delivery to the

brain, leading to cognitive impairment [7]. Processes of cognitive function like letter cancellation, Stroop test, and DSST are mainly dependent on brain oxygenation [33]. It has also been found that a low level of haemoglobin delays the planning time and central decision-making [34,35].

### Limitation(s)

One of the limitations of present study was the use of data from a homogenous group. In the future, authors will try to incorporate this study in a heterogeneous population with a larger sample size.

### CONCLUSION(S)

The present study reported a significant association between anaemia and cognitive function. The prevalence of anaemia in the study was 50%, indicating a very high prevalence. As haemoglobin levels increase, cognitive function also improves. Therefore, early screening and prompt treatment of anaemia is most important to improve both physical, as well as, mental health. Future studies should analyse the biological mechanisms between anaemia and cognitive impairment and also carry out the randomised controlled trials to improve haemoglobin levels and assess the effectiveness of cognition.

**Author's contribution:** SQ and THG proposed the research plan, design and drafted the study with intellectual content, TGP acquired the data, MVM and ID did analysis of the data with clinical suggestion.

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