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# RESEARCH ARTICLE

# 2D:4D ratio and its association with examination scores and cognitive abilities in adolescent students - A cross-sectional study

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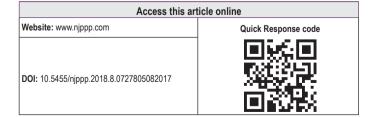
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#### **ABSTRACT**

**Background:** 2D:4D ratio or the ratio of lengths of 2<sup>nd</sup> (index finger) and 4<sup>th</sup> (ring finger), a proxy marker for prenatal testosterone exposure, is known to be sexually dimorphic with males having lower values. Low digit ratio is associated with certain characteristics such as aggression in males and assertiveness in females. Similarly, high digit ratio in males is associated with increased sperm counts, increased risk for heart disease, obesity and metabolic syndrome, depression, and anxiety. Aims and Objectives: The objectives of the present study were to measure 2D:4D ratios of adolescent students and look for associations between 2D:4D ratios and their Higher Secondary School Board examination scores and their cognitive abilities such as attention, memory, spatial ability, and intelligence. Materials and Methods: The present study was conducted on 70 adolescents of 18-19 years age group pursuing their education in the campus of Saveetha University. The 2D:4D ratio was measured from the midpoint of bottom crease where the finger joins the hand to the tip of the finger using digital Vernier calipers. Selected few domains of cognition were evaluated using the tests for free and placement recall, category fluency tests, and visual N-back tests. Correlation between 2D:4D ratio and Higher Secondary Board examination scores and mean cognition test scores were tested using Pearson's correlation test. Comparison of test scores between genders and low scorers versus normal scorers was performed using student t-test. Results: The mean 2D:4D ratio of the study population was  $0.97 \pm 0.034$ . The mean 2D:4D ratio of males  $(0.97 \pm 0.32)$  was not significantly different from that of females (0.98  $\pm$  0.035). There was no significant correlation between higher secondary scores and the 2D:4D ratio. Significant relationships with 2D:4D ratios and domains of cognition tested (free and placement recall, category fluency, and working memory) were not observed. Existing literature also revealed many inconsistencies within the pattern of associations between 2D:4D digit ratio and cognitive abilities. Conclusion: The future studies in a larger population with more and different tests for assessment of cognition would help us in ascertaining the role of 2D:4D ratio as a proxy marker for prenatal androgen exposure.

**KEY WORDS:** Digit Ratio; 2D:4D Ratio; Cognition; Working Memory; Free Recall; Placement Recall; Prenatal Testosterone; Academic Performance



#### INTRODUCTION

A great deal of recent research has focused on the relationship between digit ratios (an index of prenatal androgen exposure) and various facets of health and disease.<sup>[1]</sup> The digit ratio is the ratio of the lengths of different digits or fingers measured from the midpoint of

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bottom crease where the finger joins the hand to the tip of the finger. <sup>[2]</sup> 2D:4D ratio or the ratio of lengths of  $2^{nd}$  (index finger) and  $4^{th}$  (ring finger) is affected by *in utero* exposure to androgens, especially testosterone. A longer index finger will result in a ratio higher than 1, while a longer ring finger will result in a ratio of <1. 2D:4D ratio is negatively related to prenatal testosterone and positively related to prenatal estrogen. <sup>[3]</sup> Normal values of 2D:4D ratios in males and females have been found to be  $0.947 \pm 0.029$  and  $0.965 \pm 0.026$ , respectively. <sup>[4]</sup> Further, Manning et al. have also observed racial and geographic variations. <sup>[5,6]</sup>

A literature search revealed interesting associations between 2D:4D ratio and various physiological, psychological, and performance traits in adulthood.<sup>[7]</sup> Low digit ratio is associated with certain characteristics such as aggression in males and assertiveness in females.<sup>[8]</sup> Similarly, high digit ratio in males is associated with increased sperm counts, increased risk for heart disease, obesity and metabolic syndrome, and depression and anxiety.<sup>[9-11]</sup>

Interestingly, prenatal gonadal hormones have also been implicated as important factors in the development of different cognitive abilities such as memory, spatial skills, numerical, and verbal intelligence.[12] Testosterone promotes the development of brain areas associated with spatial and mathematical skills. Similarly, estrogen also promotes the development of areas associated with verbal ability.[13,14] These findings motivated us to look for associations between 2D:4D ratio and the academic success and learning abilities of the adolescent student population pursuing various disciplines of the study in our University. The objectives of the present study were to measure 2D:4D ratios of adolescent students of various disciplines of education and compare the 2D:4D ratios between male and female participants. Furthermore, the investigators planned to evaluate an association between 2D:4D ratios and their Higher Secondary School Board examination scores and their cognitive abilities such as attention, memory, spatial ability, and intelligence.

# MATERIALS AND METHODS

#### **Study Setting and Population**

This study was conducted on the adolescents of 18-19 years age group pursuing their education in the campus of Saveetha University.

# Sample Size

Based on existing literature, (by allowing alpha error of 5%, beta error of 20% (power 80%), the sample size was estimated to be 58. The authors conducted the study on a sample of 70 to account for absenteeism and non-availability of students on the day scheduled for measurement and tests.<sup>[15,16]</sup>

#### **Sampling Method**

The sampling frame comprised of the list of all eligible students pursuing various courses at our university. Stratified random sampling technique was used, and 70 students were randomly included in the study by computer-generated random numbers. Participants with any history of injury in the second and/or fourth digits and participants with a history of diagnosis of/treatment for any learning disability were excluded from the study.

#### **Procedure**

The study was approved by the Scientific Review Board and the Institutional Ethics Committee (007/04/2014/IEC/SU). The participants signed a written informed consent after reading the information sheet which offered a detailed explanation of the objectives of the present research project and procedures to be performed. Basic demographic details such as name, age, sex, present discipline, and year of the study and board of higher secondary school examination were collected. Further, higher secondary school board examination scores were noted down after verification of their mark sheets.

#### Measurement of 2D:4D Ratio

The 2D:4D ratio was measured from the midpoint of bottom crease where the finger joins the hand to the tip of the finger using digital Vernier calipers. [17,18] The principal investigator was trained to take the digit length measurements. Two sets of measurements were taken in the right hand and tabulated.

# **Cognitive Ability Tests**

Few domains of cognition were evaluated using the following tests.

# Free and placement picture recall

Free recall and picture placement recall tests are the assessments of visual recall (memory) and spatial recall (memory and spatial skill). For 20 s, the participants were asked to reflect on a  $4 \times 4$  grid with 10 PowerPoint Clipart graphics of common objects, located in 10 of the squares (Figure 1). They were asked to remember both the objects



Figure 1: Free and placement recall activity sheet

and their locations. For the free recall task, the participants were instructed to immediately turn the page and write down the names of the objects that they had just viewed (test duration: 2 min). The participants were then asked to turn the page to a  $4 \times 4$  blank grid and write as many names of objects as they could remember, placing the names of objects in words, in the location where they had originally viewed the object (test duration: 2 min). One point was scored for each correct response, for a maximum of 10 points per test. [19]

# Category fluency test

This test evaluates an executive function of behavior, namely, fluency. Participants were asked to list and write as many words as possible from a category (such as wild animals/flowers/fruits) in 60 s. One point was scored for each correct response.

# Visual N-back test

The Visual N back task captures the active part of working memory. It is a memory test where n refers on how many previous stimuli must be remembered. The test used 36 cards which had one black dot of uniform dimension placed anywhere on each card. Each card was individually presented to the participant for 1 s, and he/she was requested to respond whenever the location of the dot repeated itself in the successive cards. The number of hits and errors formed the test score.

Procedures for category fluency and visual N-back tests and their scoring were performed as per available standard charts with age-adjusted cutoff scores in the NIMHANS

**Table 1:** Distribution of participants based on the 15<sup>th</sup> percentile cutoff for tests for cognition

percentific content for tests for cognition					
Assessment	15 <sup>th</sup> percentiles	Population with score <15 <sup>th</sup> percentile n (%)	Population with score >15 <sup>th</sup> percentile n (%)		
Free recall	5/10	7 (9.9)	64 (90.1)		
Placement recall	4/10	8 (11.3)	63 (88.7)		
Category fluency	5/10	7 (9.9)	64 (90.1)		
Visual N back	3/8	4 (5.6)	67 (94.4)		

Neuropsychology battery 2004. [20] For each attribute tested, the quantitative scores were taken first and then interpreted as percentiles. The scores which fell below the 15th percentile were considered to be deficient scores (low scorers) in that particular functional domain and thereby were indicative of cognitive impairment.

# **Statistical Analysis**

Data were double entered into MS Excel and checked for data entry errors. Statistical analysis was performed using SPSS 16.0 and STATA 11.0. Descriptive data were presented as mean  $\pm$  standard deviation for continuous variables and as proportions for qualitative variables. The correlation between 2D:4D ratio and Higher Secondary Board examination scores and mean cognition test scores were tested using Pearson's correlation test. The study population was divided into two groups based on their mean scores of the abovementioned tests as low scorers and normal scorers. Comparison of test scores between genders and low scorers versus normal scorers was performed using student t-test.

#### **RESULTS**

A total of 71 students participated in the study, out of which 27 (38%) were males and 44 (62%) were females. The mean age of the study population was  $18.08 \pm 0.470$  years. The mean age of males and females who participated in the study was  $18.19 \pm 0.396$  years and  $18.02 \pm 0.505$  years, respectively. The mean 2D:4D ratio of the population was  $0.97 \pm 0.034$ . The mean 2D:4D ratio of males  $(0.97 \pm 0.32)$  was not statistically significantly different (t = -1.653, P = 0.103) from that of females  $(0.98 \pm 0.035)$ . There was no statistically significant correlation between higher secondary scores and the 2D:4D ratio (Pearson's coefficient = 0.086, P = 0.475), both overall and among males (Pearson's coefficient = 0.115, P = 0.566) and females (Pearson's coefficient 0.098, P = 0.525).

Based on the scores for the 4 cognition tests, percentiles were calculated, and those scores which fell below the 15<sup>th</sup> percentile in the domains of free recall, placement recall, category fluency test, and visual N-back test were 5/10, 4/10, 5/10, and 3/8, respectively. Participants were thus

Assessment		Mean±SD			P value
	Total	Male	Female		
Free recall	6.66±1.61	6.63±1.39	6.68±1.75	-0.132	0.896
Placement recall	5.54±1.93	5.52±1.89	5.55±1.98	-0.057	0.955
Category fluency	6.61±1.71	6.70±1.68	6.55±1.75	0.376	0.708
Visual N back	5.46±1.66	5.63±1.50	5.36±1.77	0.651	0.517

SD: Standard deviation

classified as low scorers (scores below the 15<sup>th</sup> percentile) and normal scorers (scores above 15<sup>th</sup> percentile). Table 1 shows the distribution of the study population based on the 15<sup>th</sup> percentile cutoff in the four tests for cognition.

There was no statistically significant difference between males and females in the mean scores for the 4 cognition tests performed (Table 2).

Table 3 shows the comparison of proportions of male and female low scorers (below the 15<sup>th</sup> percentile cutoff) in the 4 tests for cognition. Males had performed better in all the tests, and this was indicated by the lower proportion of low scorers among males when compared to females. In the visual N-back test, there were no males who scored below the 15<sup>th</sup> percentile. However, the differences were not statistically significant.

Mean 2D:4D ratios of normal scorers (above the 15<sup>th</sup> percentile cutoff) and low scorers (below the 15<sup>th</sup> percentile cutoff) in cognition tests were compared and found not to be statistically significantly different (Table 4). The mean 2D:4D ratios of all the participants were correlated with their average test scores in 4 tests of cognition, but there were no statistically significant trends observed (Table 5).

**Table 3:** Comparison of proportions of male and female low scorers (below the 15<sup>th</sup> percentile cutoff) in tests for cognition

Assessment	Males	Females	Chi-square	P value
Free recall	2 (7.4)	5 (11.4)	0.295	0.701
Placement recall	2 (7.4)	6 (13.6)	0.649	0.701
Category fluency	2 (7.4)	5 (11.4)	0.295	0.701
Visual N back	0*	4 (9.1)	2.601	0.290

<sup>\*</sup>There were no males with scores <3 in visual N-back assessment. P<0.05 was considered to be statistically significant

#### DISCUSSION

Development and organization of the brain are influenced by the same prenatal androgens that determine 2D:4D. Thus, differences in prenatal testosterone exposure may play a role in between and within sex differences in cognitive abilities. Information regarding the differences in the functioning and performance of brain systems of individuals, particularly cognition, is of scientific and practical importance among adolescent students. This motivated us to assess the cognitive abilities of the adolescents of our university and compare it with their 2D:4D ratios. The present study compared the 2D:4D between male and female adolescents and correlated it with examination scores and competencies in domains of cognition such as fluency, working memory, recall memory, and spatial skills. The mean 2D:4D ratio of the study population was  $0.97 \pm 0.034$ . The mean 2D:4D ratio of males  $(0.97 \pm 0.32)$  was not statistically significantly different from that of females (0.98  $\pm$  0.035). There was no statistically significant correlation between higher secondary scores and the 2D:4D ratio. Significant relationships with 2D:4D ratios and domains of cognition tested (free and placement recall, category fluency, and working memory) were not observed.

The mean 2D:4D ratio of the study population was  $0.97 \pm 0.034$ . The ratios tabulated in the participants of the present study were similar with those reported by Bailey and Hurd. However, the mean 2D:4D ratio of males  $(0.97 \pm 0.32)$  was not significantly different from that of females  $(0.98 \pm 0.035)$ . Lack of gender differences in the present study was in concurrence with the observations of Bull et al. in children. Non-significant differences in 2D:4D ratios between sexes have also been reported in adults. Digit ratio is sexually dimorphic. Various studies have reported that males tend to show lower 2D:4D values when compared to females. AD:4D ratio has been negatively related to prenatal testosterone and

**Table 4:** Comparison of mean 2D:4D ratios of normal scorers (above the 15<sup>th</sup> percentile cutoff) and low scorers (below the 15<sup>th</sup> percentile cutoff) in tests for cognition

Test of cognition	Mean 2D:4D ratio	SD	t-statistic	P value
Free recall test				
Normal scorers	0.97	0.035	-0.649	0.518
Low score	0.98	0.028		
Placement recall test				
Normal scorers	0.97	0.034	-1.041	0.301
Low	0.99	0.031		
Category fluency test				
Normal	0.97	0.034	0.628	0.532
Low	0.97	0.038		
Visual N-back test				
Normal	0.97	0.035	0.314	0.755
Low	0.97	0.018		

P<0.05 was considered to be statistically significant. SD: Standard deviation

**Table 5:** Correlation between 2D:4D ratios and mean scores of cognition tests

Test of	Correlation coefficient (P value)			
cognition	All participants	Males	Females	
Free recall	0.078 (0.518)	0.007 (0.974)	0.095 (0.541)	
Placement recall	0.124 (0.301)	0.154 (0.443)	0.089 (0.564)	
Category fluency	-0.075 (0.532)	-0.119 (0.555)	-0.077 (0.621)	
Visual N-back	-0.038 (0.755)	*	-0.096 (0.536)	

<sup>\*</sup>There were no males with scores<3 in visual N back assessment. P<0.05 was considered to be statistically significant

positively related to prenatal estradiol levels.<sup>[23]</sup> Importantly, no correlation has been demonstrated between the adult sex hormone levels and digit ratios.<sup>[24]</sup> Further, at molecular level studies, the Hox genes that coordinate the development of gonads are also shown to play a role in the development of fingers. [25] Digit ratio may be thus considered as a proxy marker for prenatal androgen exposure. In the present study, there was no significant correlation between higher secondary scores and the 2D 4D ratios (both overall and among gender wise). Romano et al. have reported correlations between 2D:4D ratios and degree examination marks in males but not in females.<sup>[7]</sup> Furthermore, significant correlation between 2D:4D ratios and scores of standard assessment tests in children in the UK has been reported by Brosnan. [14] However, results similar to the present study were observed in adults by Burton et al. [26] Free recall and picture placement recall scores were not statistically significant difference between males and females. The proportion of low scorers of females was higher than males, though not statistically significant. Furthermore, the scores did not show any significant correlation with the 2D:4D ratios. The results of the present study were concurrent with few other reports. [26,27] On the contrary. females (higher 2D:4D ratios) outscored males on picture free recall and picture placement in the study by Mary et al. [19] The abovementioned study also used the same tests as the present study to assess free and placement recall. Interestingly, lower 2D:4D ratios (masculine ratios) correlated with the better spatial recall in the studies conducted by Bull and Benson and Peters et al.[3,12] However, most of the previous studies that reported a significant correlation with lower 2D:4D ratios in this domain have used mental rotation performance tasks for the evaluation of spatial skills.[12] The lack of significant correlations in the present study could be attributed to the difference in methodology and smaller sample size. Fluency and working memory come under the group of executive functions which mediate goal directed behavior. These tests evaluate those aspects of behavior, which are regulated and occur to fulfil the motivational goals of an individual. Verbal fluency refers to the capacity to generate words in a regulated manner. Asking the person to generate words beginning with a particular consonant is known as phonemic fluency while asking the person to generate words belonging to a particular category is known as category fluency test. On the other hand, working memory refers to the capacity to hold and manipulate information for the ongoing processes. One of the major components of working memory is visuospatial working memory. In the present study, category fluency test was performed by asking the participant to list the names of wild animals, and visual N-back test was performed to assess the working memory. Category fluency tests and visual N-back tests did not show any statistically significant differences between males and females. The proportion of low scorers of females was higher than males, though not statistically significant. There were no low scorers at all in visual N-back test among males. Furthermore, the scores did not show any significant correlation with the 2D:4D ratios. In the study by Csathó et al., subjects with lower 2D:4D ratio had significant correlation with visuospatial working memory.<sup>[13]</sup> Similarly, lower 2D:4D ratios were associated with better verbal fluency in females and males with feminine/higher 2D:4D ratios.[26]

In the present study, significant relationships with 2D:4D ratios were not observed during the performance of any of the tests for cognition. The results of the present study were in concurrence with the reports of Bull and Benson and Austin et al.[3,22,23] The strengths of the study include the measurement of 2D:4D ratio in a precise manner using the digital Vernier calipers. However, variations in results obtained in the present study could be attributed to factors such as ethnicity and limitations such as choice of sample, smaller sample size, and choice of tests for assessment of cognition used. Existing reports are predominantly based on the studies conducted in non-Asian populations. The studies in the Indian population in this regard have been initiated recently and are fewer in number. Hence, effective comparisons and conclusions could not be performed and arrived at with the data of the present study.

#### **CONCLUSION**

The present study throws light on the possible associations between the 2D:4D ratios and various domains of cognition in an individual. The mean 2D:4D ratio of males (0.97  $\pm$  0.32) was not statistically significantly different from that of females (0.98  $\pm$  0.035). There was no significant correlation between higher secondary scores and the 2D:4D ratio. Significant relationships with 2D:4D ratios and domains of cognition tested (free and placement recall, category fluency and working memory) were not observed. Existing literature also revealed many inconsistencies within the pattern of associations between 2D:4D digit ratio and cognitive abilities. Nevertheless, the future studies in a larger population with more and different tests for assessment of cognition would help us in ascertaining the role of 2D:4D ratio as a proxy marker for prenatal androgen exposure.

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