



## Original Research Article

# Prenatal testosterone (2D: 4D Ratio) and its association with learning and memory in medical undergraduates – A cross-sectional study

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## ABSTRACT

**Background & Objectives:** The 2D:4D ratio is a sexually dimorphic trait, negatively related to prenatal testosterone which in turn influences the areas in brain, critical for learning and memory. The objective of the present study was to compare the 2D:4D ratios between sexes, right / left hands and correlate with mean scores of learning and memory.

**Materials and Methods:** The cross-sectional study was conducted in 116 participants (80 males and 36 females) of 18 -19 years of age. Lengths of index and ring fingers were measured using vernier calipers to calculate 2D:4D ratios. Free & Placement recall and Working visual memory (Visual N Back test) were assessed. Verbal learning ability was assessed using Rey's Auditory Verbal Learning Test (AVLT).

**Results:** Mean 2D:4D ratios of right hand ( $0.96 \pm 0.051$ ) were significantly lower than left hand ( $0.98 \pm 0.1$ ;  $p = 0.036$ ). Females had higher ratios than males in both hands. In Free recall (FR) and Placement Recall (PR) tests, average hits and errors scores of females and males were comparable. In Visual N Back test, there were statistically significant correlations between 2D:4D ratios and mean hits & errors scores ( $M > F$ ). The mean hits score in AVLT was lower in males ( $13.9 \pm 1.4$ ) when compared to females ( $14.2 \pm 1.0$ ).

**Interpretation & Conclusions:** The findings of the present study can help us to associate a simple measure like 2D:4D ratio to learning abilities of an individual. Also, 2D:4D ratio, a non invasive measure can be used to evaluate one's cognitive abilities with regard to memory and learning.

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## 1. Introduction

The 2D:4D ratio is the ratio of the length of the second finger to that of the fourth finger. The digit ratio is a sexually dimorphic trait which appears detectable in the fetus as early as 10-40 weeks of gestational age and becomes constant by two years of age.<sup>1</sup> Digit ratio is a putative marker for prenatal testosterone exposure. 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>2</sup> The digit ratios were also reported to vary greatly between the ethnic groups.<sup>3,4</sup>

Prenatal testosterone exposure may influence lateralization of brain, promote development of areas

involved in spatial and mathematical skills and cortical dominance. Meanwhile, prenatal estrogen influences the parts of the brain involving verbal ability. Fetal testosterone has been reported to affect the areas in the brain that are critical for learning and memory - two vital aspects in human life.<sup>5</sup> Studies exploring the association between 2D:4D ratios and various physiological and behavioral measures in the Indian population have been on the rise recently. Further, results were highly variable with regard to the association between digit ratios and cognitive abilities.

## 2. Objectives

The objectives of the present study were

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1. To measure and compare the 2D:4D ratios in adolescent students of 18 -19 years of both sexes.
2. To correlate the 2D:4D ratios and the mean scores of tests of learning and memory in the study participants.

### 3. Materials and Methods

#### 3.1. Ethical considerations

The study was initiated after presenting the proposal and obtaining clearance from both the Institutional Review Board and Institutional Ethics Committee of Saveetha Medical College & Hospital (010/12/2015/IEC/SU). Written informed consent was obtained from all the participants after clarification of all the queries of the participants with the help of a detailed information sheet. The worksheets used in the collection of data from the participants were number coded for protecting the subject's privacy.

#### 3.2. Study setting and population

A total of 116 adolescents (Males =80, Females =36) of 18 -19 years of age, pursuing Engineering at Saveetha University were included in the study. The study participants with the history of injury in the second and/or fourth digits and history of diagnosis of / treatment for any learning disability or behavioral disorders (Attention deficit hyperactivity disorder, dyslexia) or drug intake (sedatives, antidepressants) were excluded from the study.

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

The lengths of the second and fourth digits were measured from the fingertip to the ventral proximal crease of the digit, using the calibrated digital vernier calipers. Two measurements were taken on the both hands by a trained single examiner. Then, the average was calculated. The ratio was calculated by dividing the mean index finger lengths (mm) and mean ring finger lengths (mm) for both the hands.

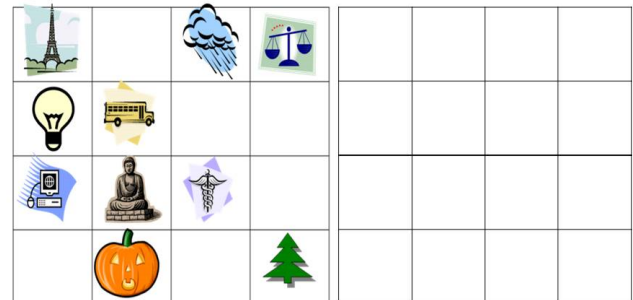
#### 3.4. Assessment of learning and memory

The following tests were performed to evaluate learning and memory of the participants.

##### 3.4.1. Visual learning and memory

Free recall and picture placement recall tests were used for assessments of visual recall (memory) and spatial recall (memory and spatial skill). For 20 seconds, the participants were asked to reflect on a 4x4 grid with 10 PowerPoint Clipart graphics of common objects located in 10 of the squares. (Fig 1) They were asked to remember both the objects and their locations. For the free recall task, the participants were instructed to immediately write down the names of the objects they had just viewed (2 mins duration). The participants were then asked to use a 4 X4 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 (2 mins duration). One point was scored for each correct response, for a maximum of 10 points per test.



**Fig. 1:** Activity sheet for assessment of free and picture placement recall

##### 3.4.2. Verbal learning and memory

It is the capacity to learn and remember verbal material and tested with the learning and memory of word lists and passages. Rey's Auditory Verbal learning Test (AVLT) consists of a list of words designating familiar objects like vehicles, tools, animals and body parts. The NIMHANS battery provides exact details of conduct and scoring of the test.<sup>6</sup> Immediate recall trials, delayed recall trials and recognition trials were conducted to calculate the memory scores of the participants. The test duration was about 20 min.

##### 3.4.3. Visual N Back test

The Visual N back task captures the active part of working memory. It is a memory test which refers on how many previous stimuli must be remembered. The test uses 36 cards which has one black dot of uniform dimension placed anywhere on each card. Each card was individually presented to the participant for one second, 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.

##### 3.4.4. Scoring

All the tests were scored as per available standard charts with age-adjusted cutoff scores in the NIMHANS Neuropsychology battery 2004. For each attribute tested, the quantitative scores were taken first and then interpreted as percentiles. Those scores which fell below the 15th percentile were considered to be deficient scores (Low scorers) in that particular functional domain and thereby, indicative of cognitive impairment.

### 3.5. Data analysis

All data were double entered into MS excel and checked for data entry errors. Statistical analyses were done using SPSS 16.0 and STATA 11.0. Mean±SD of the 2D:4D ratio of the participants was calculated. Comparison of 2D:4D ratios of the right and left hands of the study participants was done using a paired t test, while the difference between sexes was done using an unpaired t test. The correlation between 2D:4D ratio and mean test scores for various aspects of memory were tested using Pearson's correlation test.  $p < 0.05$  was considered to be statistically significant.

### 4. Results

The study participants ( $n=116$ ) comprised of both sexes-36 females and 80 males. The mean age of the study participants was  $20.22 \pm 0.59$  in females and  $20.65 \pm 0.73$  in males.

Table 1 shows the comparison of Mean 2D:4D ratios of the right and left hands of the study participants using a paired t test. There was a statistically significant difference in the ratios of right and left hands ( $p=0.036$ ).

Comparison of 2D:4D ratios between sexes was performed using unpaired t test. The 2D:4D ratios of females were higher than the males in both the right (M:0.964±0.0583; F:0.974±0.0325) and left hands (M:0.989±0.1297; F:0.994±0.0317). However, there was no statistical significance.

In the free recall test for learning, the mean hit scores of males and females were  $8.2 \pm 1.5$  and  $8.2 \pm 1.2$ , respectively. Comparison using unpaired student t test revealed no statistical significance. Likewise, the comparison of placement recall test scores between males ( $7.2 \pm 2.2$ ) and females ( $7.1 \pm 2.3$ ) using unpaired student t test revealed no statistical significance. The mean hit scores of males and females were  $7.1 \pm 2.3$  and  $7.1 \pm 2.2$  respectively.

In the Visual N back test for working memory, the mean hit scores of males and females were  $6.0 \pm 1.9$  and  $5.8 \pm 2.1$  respectively. Comparison using unpaired student t test revealed no statistical significance.

Comparison of auditory verbal learning test scores between males and females using student t test did not show any statistical significance between the mean hit scores of males ( $13.9 \pm 1.4$ ) and females ( $14.2 \pm 1.0$ ) respectively.

In the Visual N Back test scores, 2D:4D ratio showed a statistically significant positive correlation between the mean hits score and a negative correlation with the mean errors score. (Table 2) In the Auditory Verbal learning test, 2D:4D ratio showed a negative correlation with the hits score and positive correlation with the errors. However, there was no statistical significance.

### 5. Discussion

Digit ratio is said to have interesting correlations with key cognition areas like memory, learning, attention, fluency, spatial ability and numerical intelligence. Memory and learning are two vital cognitive aspects of human life. The recent days has seen a rise in the number of studies conducted on digit ratios. Nevertheless, the results of studies on 2D:4D ratio and cognition are highly variable. The variations could be attributed to the small magnitude of the measures involved and the variety of techniques available to evaluate cognition. The present cross sectional study aimed to find the relationship of 2D:4D ratio with learning and memory in adolescent individuals.

In the present study, 2D:4D ratio was measured on 116 participants. There was a statistically significant difference between the 2D:4D ratios of the right and left hands. This result was consistent with the study done by Jeyaseelan Nadankutty et al.<sup>7</sup> Studies by Sarah M Coyne et al. and Allison A Bailey et al. measured only the 2D:4D ratio of the right hand because they observed the right sided ratio to be more sensitive to prenatal testosterone exposure.<sup>2,8</sup> On the other hand Mathangi K et al. and Rebecca Bull et al. reported than an average 2D:4D ratio of both the hands may provide a stable index.<sup>9,10</sup>

2D:4D ratios of females were higher than the males in both the right and left hands. However, there was no statistical significance. This finding was consistent with many of the previous studies.<sup>2,11</sup> The differences were attributed to the lesser prenatal exposure to testosterone in females than males. The prenatal testosterone exposure is related to HOX genes which also has an influence in development of digits. However, few studies have reported no sex differences in 2D:4D ratios.<sup>1,12</sup>

Visual learning and memory is the capacity to learn and remember visual material. The free and placement recall scores revealed that males have better visual and spatial memory. These findings were consistent with the reports of Rebecca Bull et al.<sup>1</sup> However, Mary Poulin et al. reported that females outscored males in both free and placement recall tests.<sup>13</sup> The higher scores in females were attributed to the fetal hormones that affect 2D:4D ratios, which in turn either directly or indirectly influenced the visual recall abilities in females.

Working memory is the capacity to hold and manipulate information for any on-going process. This is needed to integrate the information with long term memory and with other information being processed either serially or in parallel. The working memory evaluation done in the present study with visual n back test showed that males scored better than females. On other hand, Tende J.A et al and Sailesh KS et al revealed that there were no sex differences in working memory.<sup>14,15</sup> Ashley C. Hill et.al suggested that females outscored males in working memory as they use their limbic and prefrontal cortex more while

**Table 1:** Comparison of 2D:4D ratio between right and left hands

2D:4D Ratio	Mean	Std. Deviation	T statistic, p value
Right Hand	0.967508	.0517737	-2.123, 0.036**
Left Hand	0.989454	.1084051	

\*\*p<0.05 was considered to be statistically significant

**Table 2:** Correlation between 2D:4D ratios and scores in all tests of learning and memory

Score	Free Recall		Placement Recall		Visual N Back Test		AVLT	
	Hits	Errors	Hits	Errors	Hits	Errors	Hits	Errors
Spearman's rho	.015	-.055	.039	-.047	0.187*	-.286*	-.062	.093
p value	.870	.560	.682	.619	.044**	.002**	.510	.322

\*\*p<0.05 was considered to be statistically significant

males use more of their parietal regions.<sup>16</sup> The prefrontal cortex has been found to be more active during working memory tasks and it is critical in the maintenance and integration of verbal and spatial memory.

Verbal learning and memory is the capacity to learn and remember verbal material. It is tested with the learning and memory of word lists and passages. Rey's Auditory verbal learning test revealed that there were sex differences with females out performing males. This finding was again attributed to the extensive use of cortical exposure for learning functions by females. Researches also say that females use declarative memory system which is said to be enhanced by estrogen, while males use procedural memory. Declarative memory is a part of long term memory and has extensive storage capacity and longevity. The findings of the present study were also concurrent with Joel H. Kramer et al and Bleecker ML et al.<sup>6,17</sup>

## 6. Conclusion

The findings of this study helped us to associate a simple measure like 2D:4D ratio to learning abilities and memory. 2D:4D ratio is constant since childbirth and it's also a non invasive measure that can help the parents or teachers or the students to evaluate one's cognitive abilities with regard to memory and learning. This will help in incorporating new learning techniques which will result in better education.

## 7. Source of Funding

None.

## 8. Conflicts of Interest

Nil.

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