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Association between Hormonal Fingerprints (2D:4D Ratio) and Anxiety of the Children under Dental Settings – A Cross Sectional Study

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Abstract

The 2D:4D ratio, often referred to as the "hormonal fingerprint," represents the proportional difference between the lengths of the index and ring fingers. This sexually dimorphic physical marker serves as a proxy for investigating the link between prenatal hormone exposure and various behavioral outcomes. Generally, a higher ratio indicates an increased presence of estrogen, whereas a lower ratio is associated with higher levels of testosterone (rather than progesterone) during fetal development.

Aim –To evaluate 2D:4D digit ratio correlates with levels of dental anxiety in pediatric patients undergoing dental treatment.

Materials and Methods - This study evaluated 297 children (ages 6-12) at a pediatric dental clinic. Digital calipers measured 2D:4D ratios, categorizing participants into groups (<1 and ≥1). Behaviour was recorded using Frankl' s scale, with data analysed via SPSS 23.0 using Pearson's correlation and independent t- test to correlate hormonal markers with clinical dental anxiety.

Results - The results showed that The mean age was 7.8 years. No significant correlation was found between 2D:4D ratio and FBRs scores ($r = 0.07$, $p = 0.24$). Mean 2D:4D ratios did not differ significantly between cooperative and uncooperative groups ($p = 0.32$).

Conclusion - The study found no significant association between the 2D:4D digit ratio, considered a hormonal fingerprint, and children's cooperative behavior as measured by FBRs scores. The results suggest that the 2D:4D ratio does not predict behavioral pattern in children.

Introduction

Dental anxiety stands as a premier barrier to effective oral healthcare delivery, particularly among pediatric populations. Extreme fear, apprehension, and resistance during dental visits significantly degrade the quality of clinical treatment outcomes, inflate chairside time, and foster long-term dental avoidance behaviors that persist into adulthood [1]. Managing a child's disruptive behavior in the dental chair requires a nuanced understanding of psychological, environmental, and biological determinants. Despite advanced behavioral management strategies, identifying highly anxious

children prior to treatment remains an ongoing challenge for pediatric clinicians [2].

In recent years, biological and structural markers have gained interest as potential non-invasive metrics to predict stress vulnerability and behavioral temperaments. The second-to-fourth digit ratio (2D:4D)—the relative length of the index finger (2D) to the ring finger (4D)—is widely recognized as a "hormonal fingerprint" reflective of the in-utero balance between prenatal testosterone and estrogen during early fetal development [3]. A lower 2D:4D ratio serves as an established proxy for elevated prenatal testosterone exposure, whereas a higher ratio points to a stronger estrogenic influence [4].

Because prenatal androgens profoundly affect the structural and functional lateralization of the developing brain, including areas governing the hypothalamic-pituitary-adrenal (HPA) axis, variations in the 2D:4D ratio have been linked to distinct neuropsychiatric profiles, physical aggression, pain thresholds, and generalized stress resilience in childhood and adolescence [4, 5].

The primary clinical impetus for investigating the 2D:4D ratio in a pediatric dental context is the need for a rapid, objective, and entirely non-invasive chairside pre-triage tool. Traditional methods of assessing a child's anxiety rely heavily on subjective parental proxy questionnaires or retrospective clinical ratings, which fail to predict a new patient's cooperation before an invasive procedure begins [2]. If a fixed, easily measurable physical marker like the digit ratio were significantly associated with dental anxiety or cooperation boundaries, clinicians could proactively implement targeted behavioral modifications. For instance, children structurally predisposed to low stress tolerance (hypothesized to exhibit lower ratios based on broader psychological literature) could be pre-scheduled for longer appointment slots, designated for advanced pharmacological sedation strategies, or introduced early to desensitization techniques such as "Tell-Show-Do" and virtual reality distraction. This would dramatically optimize clinical workflow, eliminate emergency appointment failures, and mitigate physical and emotional trauma for both the patient and the dental team.

Hence, this study was explicitly executed to bridge the gap between basic endocrine morphology and clinical pediatric dentistry. We hypothesized that individuals with high acute dental anxiety would display altered 2D:4D ratios, establishing whether this anatomical footprint can serve as a viable clinical predictor of cooperative behavior during pediatric dental treatment.

2. Materials and Methods

2.1 Study Design and Ethical Clearance

This cross-sectional clinical study was conducted at the outpatient department of Pediatric and Preventive Dentistry between June and December 2024. Formal ethical clearance was reviewed and approved by the Institutional Review Board (Protocol ID: 2024/IRB-MAY-PEDO-03/APDCH). The study adhered strictly to the guidelines of the Declaration of Helsinki. Prior to participation, the clinical intent, risks, and protocols were detailed to the parents or legal guardians, and signed informed consent was obtained alongside verbal assent from the children.

2.2 Selection Criteria and Sample Size Calculation

To ensure statistical power and minimize confounding systemic factors, sample size calculation was performed prior to enrollment using

G Power software (v3.1.9.7). Assuming a medium effect size ($r = 0.20$), an alpha level of 0.05, and a power of 90%, a minimum sample size of 260 children was determined. To account for potential dropouts or behavioral procedural deviations, a final cohort of 297 children was sequentially selected.

Inclusion Criteria: (a) Chronological age between 6 and 12 years; (b) present with at least one active dental caries lesion requiring routine restorative treatment under local anesthesia; (c) good general health profile with no history of cognitive delays.

Exclusion Criteria: (a) Children presenting with hand injuries, finger amputations, or congenital musculoskeletal deformities affecting digit symmetry (b) severe chronic systemic illnesses or neurodevelopmental disorders (e.g., Autism Spectrum Disorder, ADHD); (c) history of systemic corticosteroid or hormonal medication within the past two months; (d) patients requiring immediate transition from standard local anesthesia to advanced nitrous oxide or general anesthesia due to extreme uncooperativeness, as this would alter post-procedural behavioral grading.

All digit assessments were performed prior to clinical treatment to eliminate evaluator bias regarding behavioral performance. In accordance with established anthropometric protocols, the **right hand** was exclusively selected for measurement, as prenatal dimorphic hormone links are structurally and consistently more pronounced on the right side of the body [4].

Measurements were taken using high-precision digital vernier calipers (Mitutoyo, Japan; resolution 0.01 mm). The lengths of the index finger (2D) and ring finger (4D) were recorded from the most proximal ventral crease to the definitive tip of the digit pads. In cases where multiple proximal creases were observed at the finger base, the crease closest to the palm line was uniformly utilized. The 2D:4D ratio was computed by dividing the 2D value by the 4D value.

To prevent inter-examiner drift and verify measurement reproducibility, an explicit calibration protocol was instituted. A sub-sample of 30 randomly selected children underwent duplicate measurements. Intra-rater reliability was checked by having the primary investigator re-measure the same 30 children 48 hours later. Inter-rater reliability was verified by comparing the primary investigator's data against measurements taken independently by a second blinded co-investigator. Statistical evaluation yielded an Intraclass Correlation Coefficient (ICC) of 0.94 for intra-rater reliability, and 0.91 for inter-rater reliability, demonstrating excellent methodological stability.

All children underwent standard dental restorative treatment involving the administration of local anesthesia (2% lidocaine with 1:100,000 adrenaline). Immediately following the completion of the operative procedure, a secondary independent

evaluator, who remained fully blinded to the child's caliper finger measurements, recorded the patient's anxiety and cooperation using Frankl's Behavior Rating Scale (FBRS).

Definition of Cooperative vs. Uncooperative Cutoffs: The Frankl categorizes pediatric clinical cooperation into four definitive tiers:

- **Rating 1: Definitely Negative (--)**
 - Refuses treatment completely.
 - Exhibits forceful crying, extreme fear, panic, or physical combativeness.
- **Rating 2: Negative (-)**
 - Reluctant to accept treatment and uncooperative.
 - Shows subtle signs of a negative attitude, such as being sullen, silent, or withdrawn.
- **Rating 3: Positive (+)**
 - Accepts treatment and follows directions.
 - Cooperates well, though they may show mild caution or hesitation.
- **Rating 4: Definitely Positive (++)**
 - Establishes excellent rapport with the dental team.
 - Shows interest in the procedures, laughs, and appears relaxed and happy.

To facilitate binary statistical stratification and fulfill analytical clinical utility, the population was split into two explicit clinical categories: **Uncooperative** (incorporating Frankl Scores 1 and 2, indicating clear operational barriers) and **Cooperative** (incorporating Frankl Scores 3 and 4, indicating acceptable or excellent procedural compliance)

2.5 Statistical Analysis

Analytical execution was performed utilizing SPSS v23.0 (IBM Corp., Armonk, NY). A p-value of < 0.05 was recognized as statistically significant. Continuous demographic data were expressed as mean $\pm$ standard deviation (SD). Crucially, data were fully stratified by sex to control for physical dimorphism. Independent-sample t-tests evaluated differences in mean 2D:4D ratios between boys and girls, as well as between cooperative and uncooperative clinical cohorts. Pearson's correlation coefficient (r) evaluated the linear relationship between continuous digit ratios and individual FBRS scores.

3. Results

3.1 Demographic Distribution and Sexual Dimorphism

The study population comprised a final verified sample of 297 children with a mean age of 7.8 ± 2.1 years. Sex distribution analysis showed 154 boys (51.9%) and 143 girls (48.1%). The overall mean

right-hand 2D:4D ratio for the entire sample was 0.96 ± 0.03 .

When stratified by sex, a distinct physical dimorphism was validated: the mean 2D:4D ratio for boys was 0.95 ± 0.02 , which was statistically lower than the mean ratio observed for girls (0.97 ± 0.03), demonstrating high statistical significance ($t = 6.42$, $p < 0.001$). This confirms the structural validity of the digit ratio measurements as a sex-dimorphic biomarker in this study sample.

Table 1: Demographic and Clinical Characteristics Stratified by Sex (N=297)

Parameter / Characteristic	Total Sample (N=297)	Boys (n=154)	Girls (n=143)	p-value (Between Sexes)
Age (Years), Mean $\pm$ SD	7.8 $\pm$ 2.1	7.9 $\pm$ 2.0	7.7 $\pm$ 2.2	0.42
Mean Right-Hand 2D:4D Ratio ($\pm$ SD)	0.96 $\pm$ 0.03	0.95 $\pm$ 0.02	0.97 $\pm$ 0.03	$< 0.001^*$
Mean Frankl Behavior Score ($\pm$ SD)	2.8 $\pm$ 0.9	2.7 $\pm$ 0.8	2.9 $\pm$ 1.0	0.08
Cooperative Behavior Cutoff, n (%)				0.14
Uncooperative (Frankl 1 & 2)	121 (40.7 %)	68 (44.2 %)	53 (37.1 %)	
Cooperative (Frankl 3 & 4)	176 (59.3 %)	86 (55.8 %)	90 (62.9 %)	

*Statistically significant ($p < 0.05$); °Independent t-test; °Chi-square test.

3.2 Correlation Between Digit Ratio and Dental Cooperation

The core objective was evaluating the link between structural hormone markers and clinical behavior. Pearson's correlation analyses demonstrated that the 2D:4D ratio did not significantly correlate with objective FBRS scores across the total sample ($r =$

0.07, $p = 0.24$). Crucially, when continuous data were fully stratified by sex, the correlation remained uniformly weak and statistically non-significant for both boys ($r = 0.08$, $p = 0.32$) and girls ($r = 0.05$, $p = 0.55$).

To test the clinical pre-triage hypothesis, mean right-hand digit ratios were compared between the binary behavioral cutoffs. As summarized in Table 2, there was no statistically significant variance in digit dimensions between patients categorized as clinically cooperative or uncooperative, regardless of sex.

Table 2: Comparison of Mean 2D:4D Ratios Between Cooperative and Uncooperative Groups

Cohort Stratification	Uncooperative (Frankl 1 & 2)	Cooperative (Frankl 3 & 4)	t-value	p-value
Total Sample (N=297)	0.96 ± 0.04	0.95 ± 0.03	1.00	0.32
Boys (n=154)	0.95 ± 0.03	0.95 ± 0.02	0.81	0.42
Girls (n=143)	0.97 ± 0.04	0.97 ± 0.03	0.64	0.52

4. Discussion

Contrary to our primary operational hypothesis, the results of this cross-sectional clinical trial demonstrate that the 2D:4D digit ratio does not correlate with or predict a child's cooperative behavior patterns or situational anxiety during pediatric dental intervention. While our data strongly reaffirmed physical sexual dimorphism—establishing that boys have a significantly lower right-hand mean ratio than girls—this structural biomarker did not translate into a predictable clinical behavioral pattern.

These outcomes directly contrast with foundational psychomorphological investigations that associate lower 2D:4D ratios with heightened stress resilience, lower generalized anxiety traits, and elevated physical pain thresholds in laboratory settings [4]. They do, however, align with emerging studies that report weak, unstable, or completely absent relationships between digit dimensions and acute behavioral metrics under high-stress situational environments [5].

The absence of an association can be scientifically rationalized by analyzing the specific clinical architecture of pediatric dental fear. Generalized stress resilience models are typically validated in tightly controlled, passive laboratory environments. In sharp contrast, a pediatric dental setting introduces a highly dynamic, unpredictable, and

multi-sensory cluster of acute physical and emotional stressors. The invasive nature of an intraoral local anesthetic injection, the high-pitched auditory triggers of a high-speed dental drill, and the claustrophobic sensation of manual clinical manipulation represent profound situational environmental stimuli [1].

These real-time exogenous stressors generate massive, acute activation of the sympathetic nervous system and the HPA axis. This immediate neurological alarm response appears to completely cascade over and override any subtle, foundational neuropsychiatric predispositions linked to prenatal intrauterine hormone exposure [2]. Furthermore, dental anxiety is heavily governed by an array of powerful immediate confounders, including parental anxiety transmission, past traumatic medical or dental experiences, and the child's underlying psychological cognitive maturity [1, 8]. Consequently, structural markers like the 2D:4D ratio are too distal from the acute clinical event to carry predictive weight.

From a practical dental application perspective, these results provide vital guidance for pediatric operators. Because the structural biometric "hormonal fingerprint" carries negligible predictive value under standard operative stress, clinicians cannot utilize digit ratios or matching physical biomarkers as an objective screening mechanism to pre-triage or predict behavioral difficulty. Instead of seeking biometric shortcuts to identify non-compliant patients, clinicians must maintain strict reliance on established, real-time behavioral guidance techniques. Techniques such as active cognitive distraction, structured "Tell-Show-Do" protocols, positive reinforcement, and validated pre-procedural psychological scales (e.g., Facial Image Scales completed by the child in the reception area) remain the gold standards for identifying, managing, and mitigating clinical dental anxiety [2, 6].

Limitations and Future Research Directions:

While this study utilized a robust sample size and strict caliper measurement protocols with validated intra- and inter-examiner calibration, certain limitations are noted. The Frankl Behavior Rating Scale, although universally implemented and highly practical, remains an external behavioral observation metric that captures compliance rather than the precise internal cognitive perception of fear. Future research seeking an objective dental pre-triage biomarker should avoid stable structural metrics and instead focus on active, fluctuating physiological stress indicators, such as salivary cortisol levels, alpha-amylase activity, or real-time heart rate variability (HRV) monitored during treatment [7].

5. Conclusion

This study demonstrates that there is no significant correlation or association between the 2D:4D digit ratio and a child's cooperative behavior or anxiety during dental procedures, across the full sample or when fully stratified by sex. Although the ratio remains a reliable morphological marker of physical sexual dimorphism, it does not possess clinical utility as a biometric tool to predict pediatric dental behavior. Clinicians should prioritize proven psychological and real-time behavioral management strategies over physical biometric pre-screening metrics to effectively handle dental anxiety.

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