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ASSOCIATION OF HORMONAL FINGERPRINTS WITH MALOCCLUSION AMONG 18-25 YEARS OLD DENTAL STUDENTS – A CROSS-SECTIONAL STUDY

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ABSTRACT

Aim & Objective: The study aims to assess the association of hormonal fingerprints with malocclusion among 18-25 years old dental students. **Introduction:** A 'Hormonal Fingerprint' biomarker is the ratio of the lengths of the second and fourth digits. In dentistry, few research have been conducted to assess the impact of hormonal fingerprints on oral health, prompting us to develop a new approach for predicting malocclusion. **Material & Methods:** A total of 160 dental students were randomly selected from both gender of age group 18-25 years. The hormonal fingerprint (2D:4D) was made by measuring the length ratio of the index and ring finger with the help of digital vernier calliper. Malocclusion was assessed by using the Dental Aesthetic Index (DAI) according to the WHO criteria, 1997. The Kappa value for intra-examiner reliability was 0.98 and 0.95. The data collected were tabulated and statistically analysed using Chi-square and Pearson Correlation test and p-value of < 0.05 was considered statistically significant. **Results:** Out of 160 dental students, 47.5% were males and 52.5% were females in which 82.5% had a low 2D:4D ratio and 17.5% had a high 2D:4D ratio. The results showed lower 2D:4D ratio was associated with normal occlusion. **Conclusion:** These data indicate that testosterone plays a crucial role in mandibular growth. Thus, the 2D:4D ratio, a minimally invasive and repeatable method, can be employed as an early predictor of malocclusion.

INTRODUCTION

According to the World Health Organization, biomarkers are any measurement that reflects an interaction between a biological system and a potential hazard, which can be chemical, physical, or biological, and the measured response can be functional and physiological, biochemical at the cellular level, or a molecular interaction.[1] A number of biological indicators have been identified in dentistry to predict the incidence of oral problems such as dental caries, periodontal disease, and malocclusion (Buzalaf *et al* 2020).[2] One such biomarker is the 'Hormonal Fingerprint', which has received a lot of scientific attention. The study used a new risk marker, the ratio of second and fourth digit lengths (2D:4D), to predict the likelihood of malocclusion (Priyanka *et al* 2016).[3] Although alternate digit ratios (3D:5D) can be employed to study sexual dimorphism and its relationship with other human phenotypic features, the 2D:4D ratio has been shown to be more sensitive and risk-free (Trivers *et al* 2006).[4] Molecular studies have found a relationship between prenatal testosterone and HOX A and HOD A gene expression. HOX A genes are highly conserved in mammals and play an important role in digit and toe development (Zhang *et al* 2013).[5] The first mechanism for sexual dimorphism is that the shared genes Homeobox A and Homeobox D control digit and gonad development. The time of gene regulation yields a digit ratio that remains constant throughout life and represents the amount of androgen produced. The second hypothesis proposes that finger ratio is governed by androgen sensitivity rather than androgen concentration. These sex and individual differences in 2D:4D arise in utero at the beginning of the second trimester and appear to be reasonably stable postnatally. This ratio is beneficial for determining the retrospective impact of prenatal sex hormone on the brain, body, and behavior, as well as predicting an individual's malocclusion and caries risk (Verma *et al* 2013).[6] Testosterone promotes development by acting as a general epigenetic factor and through the Homeobox genes, which operate as intrinsic genetic factors.[7] Sex hormones stimulate bone growth centers directly or indirectly by causing the local production of insulin-like growth factor-1 (IGF-1) or other growth factors. They promote osteoclast

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differentiation and endochondral ossification (Fujita *et al* 2001).[8-10] In an animal experiment, it was discovered that sex hormone secretion levels altered the internal anatomy of the mandibular condyle, which serves as a mandibular growth site and center.[11] As a result, the distinguishing features of mandibular development disharmony may be substantially sex dependent and linked to testosterone differences (Baccetti *et al* 2005).[12] The hormonal fingerprint is consistent, repeatable, and reconcilable for each individual.[13] In dentistry, studies on the effect of hormonal fingerprints on oral health are very sparse, which has led to the proposal that hormonal fingerprints might be useful as a retrospective, indirect marker for malocclusion, and the current study was conducted with the aim of assessing the relationship between 2D:4D ratio and malocclusion might be useful as a retrospective, indirect marker for the prediction of malocclusion in the individual.

MATERIALS AND METHODOLOGY

This Cross - Sectional study was conducted with the aim to assess and compare the association of hormonal fingerprints with malocclusion among 18-25 years old dental students. The study participant consisted of dental students of the institution. The ethical approval for the study was obtained from the Institutional Ethics Review Board.

Sample size: The sample size for the study was estimated to be 158 which was rounded off to 160 using formula

$$\text{Sample size (n)} = \frac{z^2_{1-\alpha/2}\, p(1-p)}{d^2}$$

where;

$z_{1-\alpha/2}$ = Is standard normal variate at 5% type 1 error (P<0.05)

it is 1.96.

p = Expected proportion in population based on previous studies or pilot studies = 64%

d = Absolute error or precision – Has to be decided by researcher=7.5%

Method of data collection: The study was conducted for 2 months in which participants were selected by simple random sampling. The informed consent was taken from the participants after mentioning the objectives of the study.

Inclusion criteria

- Participants aged 18 to 25 years.
- Participants who were classified as ASA Class I by the American Society of Anaesthesiologists.
- Participants whose parents gave consent.

Exclusion criteria

- Participants on hormonal therapy.
- Participants undergoing orthodontic therapy.
- Participants with deformed digits.
- Participants having hand injuries.

Sterilized mouth mirrors and CPI probes were used to examine participants while they were seated in a dental chair in either natural or artificial light. The Dental Aesthetic Index (DAI) components were recorded to determine malocclusion according to WHO standard criteria mentioned in the WHO oral health proforma, 1997.[14]

Anthropometric measurement: The lengths of the index and ring fingers were measured from the ventral surface of the right hand, from the center of the basal crease to the tip of the digit, using a Digital Vernier Calliper with an accuracy level of 0.01 mm. If a participant's hand had more than one crease, the one closest to the hand was taken into account. The digit lengths of the ventral surface of the right hand were measured for each participant, and only right-handed individuals were selected. To find the 2D:4D ratio, divide these values.

A high 2D:4D ratio indicated that the ratio exceeded one, while a low 2D:4D ratio indicated that the ratio was less than one. A 10% re-examination of the samples was performed to assess intra-examiner repeatability of the 2D:4D ratio and DAI. There was almost perfect agreement amongst examinations conducted by the same examiner (**Kappa value = 0.98 and 0.99 respectively**).

Statistical analysis

The data collected was entered in MS Excel Sheet and analyzed using suitable statistical tests like Pearson's correlation coefficient and Chi-square test. The chi-square test was employed to investigate gender differences in 2D:4D ratios and DAI scores. Karl's Pearson Correlation Coefficient (r) was utilized to assess the relationship between the 2D:4D ratio and DAI scores. Statistical analysis was performed using SPSS software (IBM Corp 2013; Version 22.0; Armonk, NY) and P value < 0.05 was selected to be the level of statistical significance.

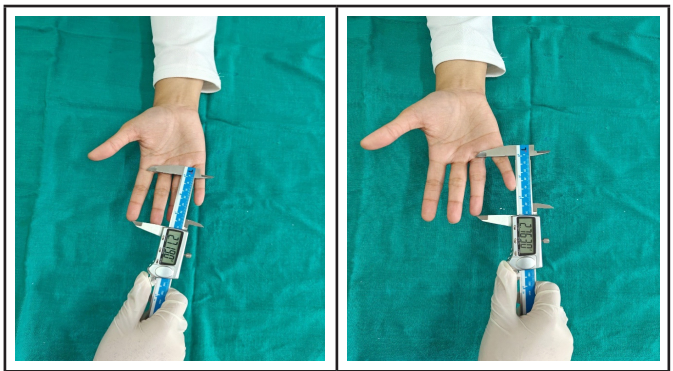

Figure 1. Measurement of 2nd and 4th digit using vernier caliper

Figure 2. Oral examination of study participant

RESULTS

A cross-sectional study was done to examine the relationship between hormonal fingerprints and malocclusion among dental students aged 18 to 25 years. The study population was divided based on the calculated 2D:4D ratio, with 83 (51.87%) having a low ratio (<1) and 77 (48.12%) having a high ratio (≥1). Out of 160 dental students, 47.5% were males and 52.5% were females. Table 1 shows that out of total, 99 (61.87%) participants had DAI Score ≤ 25 suggestive of normal occlusion in which 32 (44.4%) were males and 40 (55.6%) were females. Remaining 61 (38.12%) participants had DAI Score ≥ 26 suggestive of malocclusion in which 44 (50%) were males and 44 (50%) were females. Fig 2 depicts that Out of 72 (45%) participants who had DAI Score ≤ 25 suggestive of normal occlusion in which 25 (15.62%) were males and 27 (16.87%) were females having 2D:4D < 1 whereas 7 (4.3%) were males and 13 (8.12%) were females having 2D:4D ≥ 1.

Remaining 88 (55%) participants who had DAI Score ≥ 26 suggestive of malocclusion in which 20 (12.5%) were males and 11 (6.87%) were females having 2D:4D < 1 whereas 24 (15%) were males and 33 (20.65%) were females having 2D:4D ≥ 1. The Karl Pearson's correlation test revealed a positive correlation between 2D:4D and malocclusion, regardless of gender, implying that the higher the 2D:4D, the higher the DAI score, predicting the probability of malocclusion. However, the tests showed negative correlation of DAI score according to gender.

Table 1. Comparison of 2D:4D ratio (< 1 and ≥ 1) with gender and malocclusion

Gender			Hormonal fingerprints				Total		Chi-Square value	P-value
			<1 2D:4D		≥1 2D:4D		P-value			
			n	%	n	%	n	%		
Male	DAI	≤25	25	15.62	7	4.3	32	20	8.188	0.004**
		≥26	20	12.5	24	15	44	27.5		
Female	DAI	≤25	27	16.87	13	8.12	40	25	15.277	0.000**
		≥26	11	6.87	33	20.65	44	27.5		

*p<0.05 is statistically significant, **p<0.01 is statistically highly significant, NS = not significant, Chi-square test of significance applied.

DISCUSSION

The hormonal fingerprint has been projected as a putative marker for prenatal hormone exposure as well as homeobox (HOX) and androgen receptor gene expression. This suggests that exposure to androgens, such as testosterone, while in the uterus affects the two-digit ratio, and so the 2D:4D ratio can be used as a rough measure of prenatal androgen exposure, with lower 2D:4D ratios indicating higher prenatal androgen exposure.[6,15] In adults, 2D:4D has been found to have a positive correlation with estrogen and a negative correlation with testosterone.[16] While estrogen encourages the growth of the second digit, testosterone enhances the fourth digit's growth during pregnancy.[17] A low 2D:4D (4D longer than 2D) indicates a uterine environment high in testosterone and low in estrogen, and is most often seen in males. Whereas, a high 2D:4D marks a uterine environment low in testosterone and high in estrogen, and is usually found in females.[18] The current study suggests that men (54.2%) have a lower 2D:4D ratio than females (45.8%), which is compatible with the findings of Manning et al, who reported equivalent results, but in contrast to another study in which boys showed higher 2D:4D ratios than girls in another study by Bloom et al.16,19 The lower ratio implies that men may have had higher prenatal testosterone levels but lower prenatal estrogen levels than women. The 2D:4D ratio, which causes the fourth digit to be longer than the second, has been shown to be a reliable diagnostic for prenatal testosterone.[20] Majority of participants (61.87%) had DAI scores of ≤ 25, indicative of normal occlusion consistent to study conducted by Baskaradoss et al where 85% had normal occlusion, 10.4% had score ranging between 26-30, 3% had scores between 31-35 and 1.6% had DAI scores above 35. These findings differ from other Indian studies conducted by Singh et al and Shivkumar et al, where 82% had DAI score ≥ 26 and 80.1% ≤ 25.[21-23] In the current study, a lower 2D:4D ratio was related with normal occlusion (45%), which was similar to studies conducted by Priyanka G et al, Issrani et al, and Garg S et al (50.6%) but different from a study conducted by Premkumar and Gurumurthy. These results suggested that testosterone plays a role in mandibular development.[3,24,25] As established in the current study, these hormones function as epigenetic variables in craniofacial growth and development.

Additionally, substantial correlations were discovered between the 2D:4D ratio and an individual's malocclusion status, supporting the idea that testosterone influences bone formation. The Karl Pearson's correlation test and the Chi-square test revealed that 2D:4D has a strong positive correlation with malocclusion of the individual, implying that the greater the 2D:4D, the higher the prevalence of malocclusion, which is consistent with the study conducted by Priyanka et al.[3] Given that hormones have an epigenetic role in craniofacial growth and development, the idea that hormones, particularly testosterone, affect bone growth is supported by the significant correlations seen between an individual's malocclusion status and the 2D:4D ratio. Further studies can be carried out to assess the relation of hormonal fingerprints with dental caries. To understand that ethnicity and geographical location on individuals have significant consequences on hormonal fingerprints and necessitate exploration to confirm the role with malocclusion. The current study demonstrates that hormones play a role in the prevalence of malocclusion, which may be

used to predict children's malocclusion.

CONCLUSION

Hormonal fingerprints are novel biological indicators that can be used to predict the incidence of malocclusion, as a high 2D:4D ratio is linked to malocclusion. This 2D:4D ratio provides a glimpse into an infant's prenatal life that discloses not just information regarding behavior, sickness risk, IQ, and reproductive ability, but also the likelihood and likelihood of dental cavities and malocclusion. Despite numerous substantial clinical and public health attempts to minimize dental caries over the last several years, it remains the most common microbiological illness, and one of the serious etiologies could be malocclusion. If there were metrics that could anticipate dental caries and malocclusion, the financial burden on a developing country like India would be significantly reduced. The current study confirms the role of hormones in malocclusion prevalence, which influences caries prevalence and could be used as a predictor of malocclusion in a person. Further research is needed in this context to understand the relationships between testosterone and 2D:4D that are involved in bone growth and maturation, with an emphasis on the mandible, which leads to malocclusion.

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