

Gender Dimorphism of Skeletal Measurements and Dental Angle Classification in Orthodontic Patients

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ABSTRACT

Aim: The present study aimed to evaluate gender dimorphism of the skeletal and dental angles and measurements among different types of malocclusions in the Population of Jizan, Kingdom of Saudi Arabia (KSA).

Materials and methods: Cephalometric data were retrieved from the archived records of 272 dental patients, comprising 134 males and 138 females. Skeletal and dental malocclusions in both the sub-groups were digitally studied.

Statistical analysis: The measurement reliability was assessed using the intra-class correlation coefficient (ICC). Non-parametric Mann-Whitney *U* tests were performed to evaluate the difference in skeletal and dental parameters between genders. The Chi-square test was done to determine the difference in malocclusion patterns amongst the gender.

Results: Statistical significance was noted in dental malocclusion (p -value = 0.003) and facial height, with females having a steeper mandible plan than males. The Sella-nasion-Nasion-Vertical line angles were reported high in females with no statistical significance. Linear cephalometric values were measured higher in males than females, whereas angular values and facial height parameters were lower in females than male.

Conclusion: Within the limitation of this study, it could be concluded that there is a prominent difference measured in facial profiles of males and females with higher facial height in males.

Clinical significance and limitations: These clinical outcomes will help orthodontists to analyze the accurate base angles and points to draft proper treatment plans considering the variation in the profile of males and females.

Keywords: Cephalometric, Facial profiles, Gender dimorphism, Orthodontics, Sella-Nasion angle.

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INTRODUCTION

As an orthodontist, one needs to appreciate the transformation in facial growth patterns from the embryonic stage through adolescence until maturity. These changes help to measure and evaluate facial growth amongst the gender. While evaluating facial growth patterns, an important step is to study the number of skeletal discrepancies and the degree of dental compensation to deliver accurate treatment.¹ In clinics, 2D measuring techniques such as lateral cephalometric are most used for interpretations.^{1,2} While quantifying the cephalometric analysis, the foremost step is establishing a reference plane to evaluate the facial growth. These reference planes signify the stable anatomical landmark in the cranium, which is helpful for an orthodontist in diagnosis, treatment planning, and the assessment of treatment progress.

Numerous reference and orientation planes are introduced in orthodontics to evaluate and deliver proper treatment plans.^{2,3} Moreover, a valid cephalometric reference plane should be reliable and reproducible with low inter-examiner variability.³ These reference lines should be as close as possible to the actual horizontal (HOR) through the cranium when the face is angulated in the normal position.³ Amongst all the reference lines, the best-known and used in clinical practice is Frankfort horizontal (FH) (porion to orbitale) plane and the anterior cranium base represented sella-nasion (SN) plane.⁴

The SN plane is one of the most reliable references, as it structurally signifies the anterior cranial base and is stable.¹ The SN plane is formed by the line that joins the midpoint of sella turcica with nasion. In the cephalometric study conducted by Bjork, it was established that there might be variation in the cephalometric analysis if the growth and the slope of the cranial base show the

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differential movement of the position of the mandible with SN as a reference point alone or in conjunction with FH plane.⁵ Moore⁶ published correction factors that establish the relationship between the FH and SN planes in individuals with low and high inclinations in their anterior cranial base. Incisivo V and Silvestri A⁷ mentioned that the reliability of orthodontic planes in cephalometric analysis requires more than one reference plane or angle to acquire a proper diagnosis. Alternatively, perpendicular lines from FH and SN planes can be used as a point of reference.

Numerous studies indicate sexual dimorphism in the craniofacial hard tissues, which provide the facial support structure, with regards to their size and shape.^{8,9} The consistent and prevalent analysis reveals that the male skull exhibits a bigger stature than the female skull. Sexual dimorphism is strongly associated with size, and various studies have been practiced in developing a highly predictable formula employing cephalometric analyses.^{8–10} In dental literature, data are scarce regarding the gender dimorphism

of skeletal measurement and angle classification. Further, previously published studies have not considered subjects according to skeletal and dental discrepancies variations.^{2,8,10} Most of the studies evaluated dental angles regarding the type of malocclusion.¹⁰⁻¹²

Studies have evaluated different classes of malocclusion without referencing gender dimorphism, such as the study by Bacon et al.⁸ on patients with dental and skeletal malocclusion. Research by Tsang et al.⁹ revealed a steeper slope of the anterior cranial base in patients with anterior open bite. Alves et al.² compared the inclination between two reference points in a different class of malocclusion, while Shimizu et al.¹⁰ researched reference points among Class I malocclusion patients, and Wu et al.¹¹ reviewed different vertical growth patterns among patients with Class II malocclusion. Alajlan et al.¹² evaluated different malocclusion and treatment needs in children of northern KSA. Other studies performed by Huh et al.,¹³ Kattan et al.,¹⁴ and Hung¹⁵ evaluated gender stratification without dental or skeletal malocclusion, while Shimizu et al.¹⁰ compared SN-FH angles in Class I malocclusion among Japanese and Spanish female patients. Furthermore, most of the studies on dental and skeletal malocclusion with reference to SN angle and gender dimorphism were done on the non-Saudi Population, for instance, Tsang et al.⁹ and Wu et al.¹¹ on the dental malocclusion and gender dimorphism in Chinese population. The prevalence of malocclusion amongst genders in the Brazilian population was studied by Alves et al.² Huh et al.¹³ studied the prevalence of malocclusion and gender dimorphism in the Korean population. Shimizu et al.¹⁰ compared the Japanese and Spanish females for SN angle with Class I malocclusion. A study on the Nepalese population on types of malocclusions and gender dimorphism was done by Giri et al.¹⁶ The South Indian population on dental and skeletal malocclusion amongst gender was studied by Reddy et al.¹⁷

Even though there are studies published on Saudi Arabian cephalometric norms and analysis, none focus on the cephalometric analysis of different types of dental and skeletal malocclusion and vertical and angular measurement with emphasis on gender dimorphism.^{12,18-22} Moreover, for evaluating orthodontic treatment measures it is important to note the significant point of reference on lateral cephalogram among both genders. Hence, the present study aimed to evaluate gender dimorphism of the skeletal measurement and dental angle among the skeletal and dental malocclusion as well as vertical discrepancies in the southern province (Jazan) of the Saudi Arabian population. The main objective of this study is to evaluate the reliability of cephalometric reference parameters to determine the difference in skeletal and dental growth between the genders.

MATERIALS AND METHODS

This was a retrospective observational cross-sectional study approved by the Ethical Committee at the College of Dentistry, Jazan University. The archive of the Department of Orthodontics was screened for lateral cephalometric radiographs, demographic data, and clinical data of all patients attending the department between the years 2011 and 2017. The inclusion criteria were as follows: (A) Age >17 years old; (B) no hereditary disorders, syndromes, or major surgeries affecting the craniofacial complex; and (C) no previous or current orthodontic treatment. The participants with mixed dentition, major orthognathic surgery, and facial deformities were excluded. It was ensured that all the included patients had signed a consent form before registration, which includes the

patient's approval to use his/her data for research purposes. During imaging, the FH line was made parallel to the floor, and the patients were instructed to bite in the maximum intercuspation to avoid any vertical discrepancy.

The G power software (Universität Düsseldorf, Düsseldorf, Germany) was employed to determine the sample size. A power of 90% and an alpha value of 0.05 were considered in the calculation. The calculation was based on previous studies conducted by Modi et al.²³ in which the position of the mandibular incisors to the NB line was 31.45 ± 7.69 mm for the hyper-divergent face and 26.15 ± 6.62 mm for the hypo-divergent pattern was quantified, and another study conducted by Freudenthaler et al.²⁴ in which the inclination of the mandibular incisors to MP line was $94 \pm 10^\circ$ for Class II patients. As per power analysis, 51 patients for each facial pattern and 15 patients in each skeletal class were anticipated for the study.

The final sample consisted of 272 subjects (134 male and 138 female subjects) between the age of 17 and above with complete permanent dentition and no prior orofacial malformation and surgery history. All patients were then categorized based on the linear and angular radiographic measurements as follows: (A) Skeletal classification, based on ANB angle and Ab diff. NV, into Class I, Class II, and Class III; (B) vertical classification, based on MMP angle and S-Go/N-Me, into normodivergent, hypodivergent, and hyperdivergent; and (C) dental classification, based on Angle's classification of malocclusion achieved the clinical data of the patients, into Class I, Class II, and Class III.

The radiographic linear and angular measurements were performed by one examiner (Washington Examiner). Details about the included parameters are shown in Table 1 and Figure 1 respectively. For reliability of the measurements, 100 cephalometric radiographs were randomly selected and measured twice with a 2-weeks interval in between. The intraclass correlation coefficient (ICC) test was used for the reliability test. The collected data was input into a master sheet, double-checked, and transferred to the analysis software and was again evaluated after 15 days to reduce intra-observer validity.

STATISTICAL ANALYSIS

The Statistical Package for the Social Sciences (SPSS) software V25 for Windows (Armonk, NY: IBM Corp.) was used for analysis. The results were descriptively reported as means and standard deviations (for continuous variables) or frequencies and percentages (for categorical variables). The data were subjected to the normality test which revealed the non-normal distribution of the data (Shapiro-Wilk's test; $p < 0.05$). Accordingly, non-parametric tests were used for the differences between the males and females in linear and angular measurements. In addition, the Chi-square test was used for the differences in the distribution of the different classes among both genders. A p -value of < 0.05 was considered significant.

RESULTS

The ICC test for the reliability of the measurements revealed perfect agreement between both measurements for the selected parameters, ranging from 0.996 to 0.999 (Table 2). A total of 267 cephalometric radiographs belong to 132 males (49.4%) and 135 females (50.6%). In total, more participants were found with skeletal Class I (49.1%), normodivergent vertical relation (49.1%), and dental class I (73.0%). Regarding the differences between genders, more

Table 1: The linear and angular radiographic parameters used in the study

<i>Anterio-posterior skeletal parameters</i>	
A-NV	The linear distance measured between point A and nasion vertical line, measuring the anteroposterior position of the maxilla relative to the nasion vertical line.
B-NV	The linear distance measured between point B and nasion vertical line, determining the anteroposterior position of the mandible relative to the nasion vertical line.
Ab diff (NV)	The linear differences between A-NV and B-NV, determining the anteroposterior jaw relation.
SNA	The angle between three point landmarks S, N and A points, determining the anteroposterior position of the maxilla relative to the cranial base.
SNB	The angle between three point landmarks S, N and B points, determining the anteroposterior position of the mandible relative to the cranial base.
ANB	The angle between three point landmarks A, N and B points, determining the anteroposterior jaw relation.
<i>Vertical skeletal parameters</i>	
MP/SN	The angle between sella-nasion (SN) and Go-Me (MP), determining the vertical position of the mandibular base relative to the cranial base.
MP/PP	The angle between sella-nasion (SN) and Go-Me (MP), determining the vertical position of the mandibular base relative to the ANS-PNS (PP) line.
AFH (N-Me)	Face height; Linear measurement from nasion (N) to Menton (Me).
PFH (S-Go)	Posterior face height; Linear measurement from Sella (S) to Gonion (Go).
UAFH (N-ANS)	Upper Anterior Facial Height; Linear measurement from nasion (N) to the most anterior point on the maxilla at the nasal base (ANS).
LAFH (ANS-Me)	Lower anterior facial height; Linear measurement from the most anterior point on the maxilla at the nasal base (ANS) to the lowest point on the symphysis of the mandible (Me).
FH ratio SGo/Nme	Jarabak's ratio; The ratio between posterior and anterior face height. (S-Go/N-Me).
F prop LAFH/Nme	The ratio between lower anterior facial height and anterior face height
<i>Dental parameters</i>	
U1-NA	The linear distance between Nasion-point A line and the most protruded point in the maxillary incisors.
L1-NB	The linear distance between Nasion-point B line and the most protruded point in the mandibular incisors.
U1/PP	The angle between the long axis of the most protruded maxillary incisor and the ANS-PNS (PP) line.
L1/MP	The angle between the long axis of the most protruded mandibular incisor and the Go-Me (MP) line.

female participants presented with skeletal class I and II, while more male participants presented with class III, with no significant difference ($p = 0.281$). Hypodivergent was more prevalent in male participants than in females, while normodivergent and hyperdivergent were more prevalent in females than in males, with no significant difference ($p = 0.158$). However, the difference between genders in dental classification was significant ($p = 0.003$), with class II in females more prevalent and class III in males. More details are presented in [Table 3](#).

[Table 4](#) shows the angular measurements of the anterior-posterior jaw relationship regarding the cranial base (S-N) and nasion vertical line (N-V). Although the female participants showed higher values than males, no significant differences were found between both genders concerning all the measured parameters.

Female participants showed higher values than males of the mandible plan relative to the cranial base (S-N) (34.6 ± 4.1 compared to 34.0 ± 5.0) and palatal plane to the cranial base (S-N) (26.9 ± 4.3 compared to 26.1 ± 5.3) but with no significant differences ($p = 0.351$ and $p = 0.425$, respectively). However, there were significant differences ($p < 0.001$) between male and female participants in relation to all facial height parameters, with higher values for males. The highest mean difference was for anterior facial height (mean difference = 9.8; 95% CI = 8.5–11.1), followed by posterior facial height (mean difference = 9.8; 95% CI = 8.5–11.1), then lower

anterior facial height (mean difference = 6.8; 95% CI = 5.6–7.9), and upper facial height (mean difference = 3.2; 95% CI = 2.5–3.9). More details are presented in [Table 5](#).

Concerning the dental parameters, there was a significant difference between both genders for the inclination of the lower incisor relative to the mandibular plane ($p = 0.042$), with higher values in females. In comparison, the intra-upper and lower incisor angle was significantly ($p = 0.003$) higher in male than in female participants. However, there were no significant differences between males and females regarding the inclination of the upper central ($p = 0.158$) and the ratio of the upper central to the palatal plane ($p = 0.357$). More details are shown in [Table 6](#). Overall, the result establishes gender dimorphism in the facial planes, height, and dental malocclusion amongst the participants.

DISCUSSION

This study analyzed the difference in cephalometric measurement of different skeletal and dental measurements among the gender, with the aim of evaluating the difference in growth patterns and measuring the reference point. All the subjects presented were above 17 years to present final observational changes in facial growth patterns. Cephalometric reference points are important in classifying and describing skeletal and dental complexes and

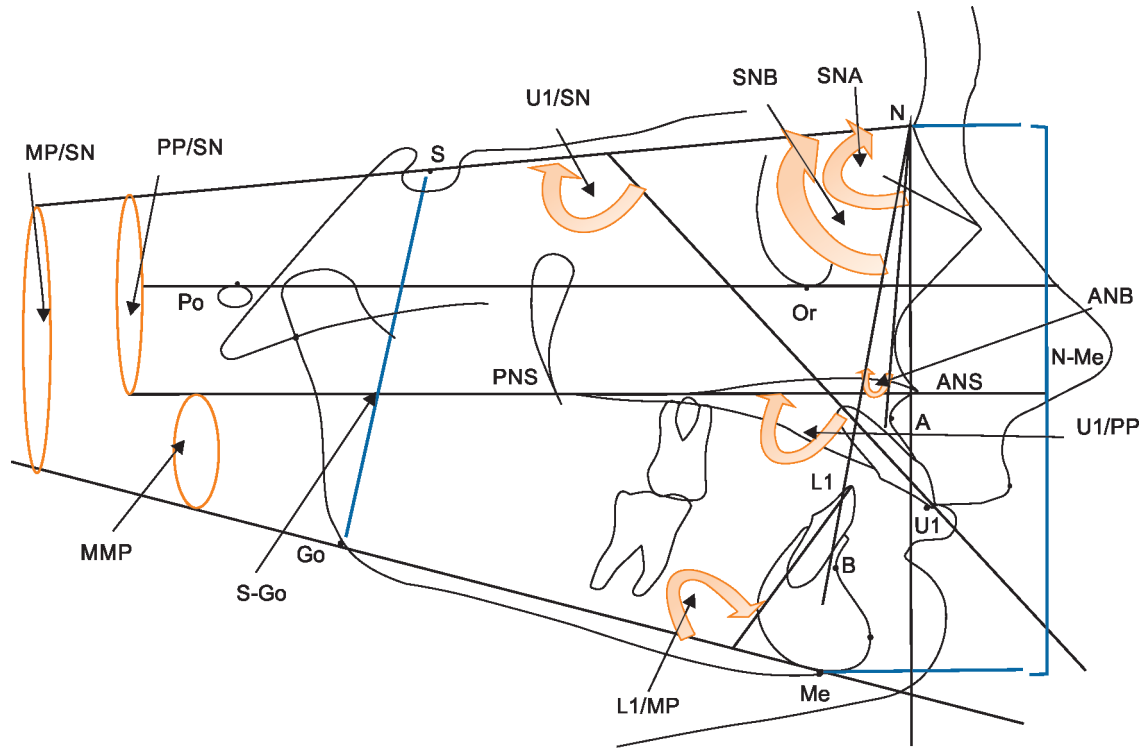


Fig. 1: Different skeletal parameters measured

Skeletal and dentoalveolar landmarks and measurements: (A) Maxillary base: (1) SNA, (2) A-NV, (3) PP-SN, (4) PNS (PP); (B) Mandibular base: (1) SNB, (2) B-NV, (3) MP/SN, (4) MMP, Dentoalveolar measurements: (A) Maxillary incisors: 1.U1-NA, 2. U1/SN, 3.U1/PP; (B) Mandibular incisors: (1) L1-NB, (2) L1/MP, (3) Inter-incisor angles (U1/L1). Porion (Po), Orbitale (Or) line (Frankfort horizontal plane)

Table 2: Intraclass correlation coefficient (ICC) test for reliability

Parameter	ICC
AFH (N-Me)	0.997
PFH (S-Go)	0.996
SNA	0.997
SNB	0.997
ANB	0.999
U1-NA	0.999
L1-NB	0.997

vertical relations. Previous studies have demonstrated that the growth of cranial base angle is relatively stable after the age of 15 years. Hence, cranial base angles can be used as a reference point in cephalometric studies on the facial growth pattern. A comprehensive longitudinal study by Dhopatkar et al.²⁵ and Leighton confirmed cranial base angle stability among genders, with variations in an individual profile.

The SN line lies closest and in the center of the anterior cranial base, further dividing it into anterior and posterior parts. The maxilla is attached to the anterior and the mandible to the posterior of the SN line. With the relationship of the maxilla and mandible to the SN plane, it could be assumed that any variation in the SN line angle may alter the growth of the skeleton. This further causes different skeletal and dental malformations.

Literature suggests that the angle measured between the FH-SN plane is around 7° independent of variable and variation in the

Table 3: Characterizations of the study sample in relation to skeletal class, vertical relation, and dental class for the whole sample and by gender

	Total	Gender		p*
		Male	Female	
Skeletal class				
Class I	131 (49.1)	64 (48.9)	67 (51.1)	0.281
Class II	83 (31.1)	37 (44.6)	46 (55.4)	
Class III	53 (19.9)	31 (58.5)	22 (41.5)	
Vertical relation				
Hypodivergent	54 (20.2)	33 (61.1)	21 (38.9)	0.158
Normodivergent	131 (49.1)	61 (46.6)	70 (53.4)	
Hyperdivergent	82 (30.7)	38 (46.3)	44 (53.7)	
Dental class				
Class I	195 (73.0)	96 (49.2)	99 (50.8)	0.003
Class II	51 (19.1)	19 (37.3)	32 (62.7)	
Class III	21 (7.9)	17 (81.0)	4 (19.0)	

*Chi-squared test

genders also irrespective of the dental and skeletal malocclusion.^{13,16} Conversely, the results of other studies measured varied SN plane angles as a reference.^{2,26} The sample studied in most investigations was non-Saudi descent.^{2,4,8,10,13,15-17,24} The present retrospective cross-sectional study evaluated gender dimorphism of specific parameters related to different angular and linear measurements in dental and skeletal malocclusion, and the subject was drawn from the Jizan population.

Table 4: Anterior-posterior jaw relationships for the whole sample and the mean differences by gender

	Total	Gender		Mean difference	95% CI	p*
		Male	Female			
A-NV	-0.8 ± 3.3	-1.2 ± 3.7	-0.5 ± 3.0	-0.6	(-1.4 to 0.2)	0.133
B-NV	-5.4 ± 5.1	-5.7 ± 5.4	-5.2 ± 4.7	-0.5	(-1.7 to 0.8)	0.616
Ab diff. (NV)	4.6 ± 3.8	4.5 ± 4.1	4.7 ± 3.6	-0.2	(-1.1 to 0.7)	0.750
SNA	82.1 ± 3.2	81.8 ± 3.4	82.4 ± 3.1	-0.6	(-1.4 to 0.2)	0.170
SNB	79.8 ± 2.9	79.6 ± 3.0	79.9 ± 2.7	-0.3	(-1.0 to 0.4)	0.498
ANB	2.4 ± 2.6	2.2 ± 2.8	2.6 ± 2.4	-0.5	(-1.1 to 0.1)	0.375

*Mann-Whitney *U* test**Table 5:** Mandibular plane relations with palatal plane and cranial base and the different facial height measurements for the whole sample and the mean differences by gender

	Total	Gender		Mean difference	95% CI	p*
		Male	Female			
MP/SN	34.3 ± 4.6	34.0 ± 5.0	34.6 ± 4.1	-0.6	(-1.7 to 0.5)	0.351
MP/PP	26.5 ± 4.8	26.1 ± 5.3	26.9 ± 4.3	-0.8	(-2.0 to 0.4)	0.425
AFH (N-Me)	125.2 ± 7.4	130.2 ± 5.9	120.3 ± 5.2	9.9	(8.5-11.2)	0.000
PFH (S-Go)	79.3 ± 7.2	84.3 ± 5.5	74.5 ± 5.0	9.8	(8.5-11.1)	0.000
UAFH (N-ANS)	55.4 ± 3.3	57.0 ± 3.2	53.8 ± 2.7	3.2	(2.5-3.9)	0.000
LAFH (ANS-Me)	69.7 ± 5.9	73.1 ± 5.1	66.3 ± 4.6	6.8	(5.6-7.9)	0.000
FH ratio SGo/Nme	63.1 ± 4.3	64.6 ± 4.3	61.6 ± 3.8	3.1	(2.1-4.1)	0.000
F prop LAFH/Nme	55.4 ± 2.3	56.0 ± 2.2	54.9 ± 2.3	1.1	(0.6-1.6)	0.000

*Mann-Whitney *U* test**Table 6:** Dental parameters for the whole sample and the mean differences by gender

	Total	Gender		Mean difference	95% CI	p*
		Male	Female			
U1-NA	5.8 ± 3.3	6.1 ± 3.4	5.5 ± 3.2	0.6	(-0.2 to 1.4)	0.158
L1-NB	6.8 ± 2.6	7.1 ± 2.9	6.6 ± 2.3	0.5	(-0.2 to 1.1)	0.193
U1/PP	113.1 ± 8.1	112.7 ± 8.2	113.6 ± 8.0	-0.8	(-2.8 to 1.1)	0.357
L1/MP	96.3 ± 7.4	95.3 ± 7.2	97.2 ± 7.5	-1.9	(-3.7 to -0.1)	0.042
U1/L1	124.8 ± 11.7	126.4 ± 11.1	123.1 ± 12.0	3.3	(0.5-6.1)	0.003

*Mann-Whitney *U* test

While studying facial parameters amongst the genders, it is assumed that females exhibit higher angles and measurements in all skeletal and dental malocclusion. The result of the current study exhibits statistical significance in dental malocclusion, and sexual dimorphism was present in dental Classes I, II, and III. This result was in accordance with the study by Alajlan et al.¹² on school children in Hail, KSA, where significant relation was found in dental Class I. However, this study does not focus on gender dimorphism. Similarly, in the study by Reddy et al.,¹⁷ gender dimorphism was noted with Class I malocclusion with predominance in females.

The Mann-Whitney *U* test suggests that Saudi Arabian males and females have notable differences in various cephalometric measured parameters, with higher facial height in males. This could be interpreted as males having longer anterior facial features than their females counterparts. The result of this study was not in accordance with the research done by Reddy et al.¹⁷ on the Indian population, Giri et al.¹⁶ on the Nepalese population, and Huh et al.¹³ on the Korean population. They reported females having

higher facial angulation and height than males. In accordance, the study by Huh et al.¹³ on growing children suggested that the plane angles (SN-FH) were higher in boys, but the difference between plane angles (Ab diff. NV) was higher in girls. Also, the authors have observed a gender dimorphism in some of the angular measurements. The present study measures similar variation, with the difference being adult samples in current research.

Another objective of the present study was to evaluate the vertical and skeletal relationship, namely Class I, Class II, and Class III, with facial profiles of hypo-divergent, normo-divergent, and hyper-divergent. The finding of this study was in accordance with Reddy et al.¹⁷ and Alves et al.,² who found females with greater skeletal Class I and II relationship compared to Class III. However, the Chi-square test result suggests that the difference between the different classes of skeletal malocclusion is not statistically significant. In vertical relation, it was noted that females exhibit more of a normo and hyper divergent profile than males. Similarly, Memon et al.²⁷ observed more female hyper divergent and acute angle profiles

than males. However, statistical significance was noted by Memon et al.²⁷ which contrasts with the result of this study.

The Mann-Whitney *U* test result suggests increased mandibular plane angle (mandibular plane = 97.2 ± 7.5 and palatal plane = 113.6 ± 8.0) in females. However, upper, and lower incisor plane angles were measured higher in males with no statistical significance in the parameters. These findings align with the mandible and palatal plane-related measurement in the study by Alroudhan et al.²⁰ with no statistical significance.

From this discussion, it is hard to substantiate the standardization of angular difference and measurement for both males and females of the Saudi Arabian population. The result of the current study supports this inference in various respects. Firstly, there is a wide range of mean differences in dental and skeletal malocclusions and angulations between males and females. Secondly, the average mean value obtained from the overall Population showed steeper angulation and increased facial height in males. Overall, the calculated values are mostly higher in males than females.

This fact reveals the importance of considering the reference plane while evaluating cephalometric radiographs in gender evaluation. Hence, while succeeding with lateral cephalometric in orthodontics, it is better to evaluate reference mainly SN plane angles. This will eventually help clinicians to make the necessary correction based on the mean value of this plane between men and women of a mentioned population as well as in different malocclusion.

CONCLUSION

This retrospective cross-sectional study on gender dimorphism emphasizes the use of accurate and valid cranial base reference as a fundamental auxiliary element of the aesthetic measurement that interests Orthodontists and Maxillofacial and Cosmetic Surgeons. The result of this study concluded that there is an angle variation (SN and NV) between both genders with the average value greater in females than males. A significant association was found in relation to facial height among the genders, with male participants with greater facial height than female. Statistical significance was found in dental malocclusion amongst the genders, with Class III prominent in males and Class I and II malocclusions in females.

CLINICAL SIGNIFICANCE AND LIMITATIONS

This study is a cross-sectional study with retrospective data, and the available sample size is small, which is appropriate for calculating the power of the study. Due to the limited availability of the subjects, particularly in dental Class III malocclusion, the sample size of this group was undersized when comparing gender dimorphism. Despite the group variation, statistical significance could not be recorded from all the measured data. Hence, this study partially rejects the null hypothesis with statistical significance measured in some parameters of the study. Therefore, future studies should focus on larger samples with different growth patterns to validate and support the findings. Moreover, Orthodontists should evaluate the facial growth patterns among the gender with the help of reference points to provide a substantiated treatment.

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HUMAN RIGHTS STATEMENT AND INFORMED CONSENT

All procedures followed were in accordance with the ethical standards of the responsible committee for human experimentation and with the Helsinki Declaration of 1964 and later versions.

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