Soft tissue facial profile of adult Saudis

Lateral cephalometric analysis

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ABSTRACT

الأهداف: تأسيس مقاييس النسيج الرخوي في منطقة الرأس عند السعوديين البالغين الإناث والذكور، وعمل مقارنة بين المقاييس السعودية والمعايير الأمريكية الأوربية وما إذا كان هناك فروقاً واضحة فيما بينها.

الطريقة: أجريت هذه الدراسة في كلية طب الأسنان التابعة لجامعة الملك سعود، الرياض، المملكة العربية السعودية، وتضمنت سجلات المرضى خلال الفترة من يونيو 2009م إلى يونيو 2010م. شملت الدراسة 61 عينة من صور الأشعة الجانبية للرأس (العدد: 31 ذكراً، و30 أنثى، معدل العمر: 23 عاماً). وقد قمنا بتحليل 13 عينة من قياسات الرأس إلكترونيًا وذلك باستخدام الإصدار العاشر من دلفين وطبقًا لتحليل ليجان وبرستون، وأستخدم البرنامج الإحصائي (SPSS) من أجل تقييم القيم الاحتمالية، والفروق الإحصائية بين المعايير.

النتائج: أشارت نتائج الدراسة إلى إصابة السعوديين البالغين بالتقوس الوجهي الجانبي والذي يرتبط بالتراجع الخلفي للفك السفلي وبزاوية منفرجة أكثر في منطقة ما تحت الذقن، وزيادة في تقدم الشفتين، وزيادة التلم الذقني الشفوي، ونقص في نسبة المسافة بين الشفة والذقن، وزيادة في انكشاف الثنايا العلوية عند مقارنة هذه العينة مع العينة الأمريكية الأوربية. كما أظهرت الدراسة إصابة السعوديات بالزاوية المنفرجة بين الأنف والشفة، ونقص في نسبة الطول العامودي عند مقارنتهن مع عينات الإناث في أمريكا وأوروبا. وعند عمل المقارنة بين نتائج الإناث والذكور تبين إصابة السعوديات بنقص في نسبة الطول العامودي، وزيادة في انفراج الزاوية بين الأنف والشفة، ونقص في المسافة بين الشفتين مقارنة مع الذكور المشاركين في الدراسة.

خاممة: أثبتت الدراسة وجود الفروق الملحوظة في تركيبة الوجه بين عينة المشاركين في المعايير السعودية، والمعايير الأمريكية الأوربية من جهة، وبين الجنسين في عينة المشاركين السعودية من جهة أخرى، وهذا من شأنه أن يساعد على تشخيص وعلاج حالات تقويم الأسنان لدى السعوديين البالغين.

Objectives: To develop cephalometric measurements of soft tissue facial profile for a sample of adult Saudi

males and females and compare it with European-Americans' norms for any significant differences.

Methods: Sixty-one lateral cephalometric radiographs (31 males and 30 females; mean age 23 years) were selected from the archives of cephalometric radiograph files at College of Dentistry, King Saud University, Riyadh, Kingdom of Saudi Arabia between June 2009 and June 2010. Thirteen cephalometric parameters were analyzed electronically using the Dolphin[®] version 10 software according to Legan and Burstone analysis. Descriptive statistics and *p*-values were calculated for the group comparisons using SPSS program. The statistical significance was determined at the 0.05 level of confidence.

Results: Adult Saudis generally had increased facial convexity associated with retruded mandible, more obtuse lower face-throat angle, increased bimaxillary lip protrusion, greater mentolabial sulcus, decreased vertical lip-chin ratio, and increased maxillary incisor exposure than European-Americans. Saudi females had more obtuse nasolabial angle and decreased lower vertical height-depth ratio than European-American females. Comparisons between the males and females indicated that Saudi females had a reduced lower vertical height-depth ratio, smaller lower lip distance more obtuse nasolabial angle and decreased interlabial gap than males.

Conclusion: The significant differences in facial structures of Saudis, European-Americans and between the genders should be of a great help for diagnosis of orthodontic and orthognathic surgical cases in Saudi adults.

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Tarmonious facial aesthetics have long been Π recognized as the most important goal of orthodontic treatment. Knowledge of the facial skeleton and its overlaying soft tissue in determining facial harmony is essential. It was assumed that the soft tissue profile configuration was primarily related to the underlying skeletal configuration.¹⁻⁶ Several investigators have noted that soft tissue behaves independently from the underlying skeleton because the soft tissue covering the teeth and the skeletal face is highly variable in its thickness.^{1,2} Much research has displayed that soft tissues are a major factor in determining a patient's final facial profile.³⁻⁶ The successful treatment planning for patients who require orthognathic surgery should include both hard and soft tissue cephalometric analysis. Detailed cephalometric analyses for orthognathic surgery have been reported by Burstone et al⁶ for hard tissues and by Legan and Burstone² for soft tissues with norms derived from the European-Americans. These analyses have been extensively used for research and for the diagnosis and treatment planning of orthognathic surgical cases because they are based largely upon rectilinear measurements. It has been well established that differences in the dentofacial relationships of various ethnic groups have been observed by many investigators and a single standard of facial esthetics is not appropriate for application as a reference for the diagnosis and treatment to diverse races.⁷⁻⁹ Therefore, the researchers have compared cephalometric characteristics of different races with European-Americans with an intention to establish race-specific cephalometric values.7-12 Currently, an increasing number of Saudis are looking for orthognathic surgery. Therefore, it has become important to develop the cephalometric standards of this ethnic group to be treated according to their own characteristics. Several cephalometric studies that have been carried out among the Saudi population, but were limited to hard tissue analysis.¹³⁻¹⁷ Haider and AlBarakati¹⁸ conducted a cephalometric study on 56 Saudi subjects (30 males, 26 females) with a variety of soft tissue measurements from several analyses; they found significant differences in most of soft tissue parameters when comparing with other ethnic groups. In Middle Eastern countries, some studies^{10,12} were undertaken to investigate soft tissue cephalometric measurements, demonstrated the ethnic differences were of significant difference compared to original published norms and suggesting the need for separate cephalometric standards for each ethnic group. The hard tissue standards for orthognathic surgical analysis have already been investigated for Saudi subjects by Al-Barakati and Baidas.¹⁶ Therefore, the aim of the current study is to develop cephalometric measurements of soft tissue facial profile for a sample of adult Saudi males and females that can provide as a holistic guideline in

diagnosis and treatment planning for cases requiring orthodontics and orthognathic surgery and to investigate whether any significant differences exist between Saudi males and Saudi females and European-Americans according to Legan and Burstone analysis.²

Methods. The study was carried out on standardized cephalometric radiographs of 61 Saudi subjects (31 males and 30 females) were selected from the archives of cephalometric radiograph files at College of Dentistry, King Saud University between June 2009 and June 2010. The age ranged was from 22-24 years. Ethical approval was obtained from the Research Centre at College of Dentistry (CDRC), Riyadh, Saudi Arabia. The following criteria were met: Saudi national adults, a pleasing and harmonious facial profile, Angle Class I molar and Class I canine, normal overjet (1-3 mm), normal overbite (5-20%), no history of trauma, jaw fracture or any craniofacial malformation and syndromes, and no previous orthodontic treatment.

All lateral cephalometric radiographs were obtained with the teeth in maximum intercuspation, a natural head position and lips were in repose. Each radiograph was scanned into an X-Y coordinate system using Epson[®] perfection 4990 photo scanner (Seiko Epson Corporation, Nagano, Japan). Landmark identification was carried out manually on digital images using a mousedriven cursor and was digitized in a darkened room using specific points required by the software. Thirteen soft tissue measurements were calculated electronically using the Dolphin[®] version 10 software (Dolphin Imaging and Management Solutions, Chatsworth, California, USA) according to Legan and Burstone analysis² (Figures 1-3). Calibration of the digital image to define the actual size of the image in millimeters when seen on the screen was

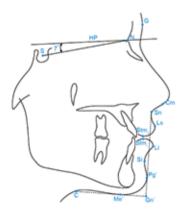


Figure 1 - Cephalometric landmarks. HP - horizontal reference plane, S - sella, N- Nasion, G - glabella, Cm - columella point, Sn - subnasale, Ls - labrale superius, Stms- stomion superius, Stmi - stomion inferius, Li - labrale inferius, Si - mentolabial sulcus, Pg - soft tissue pogonion, Gn'- soft tissue gnathion, Me' - Soft tissue menton, C - Cervical point.

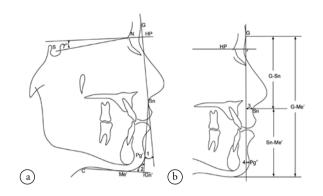
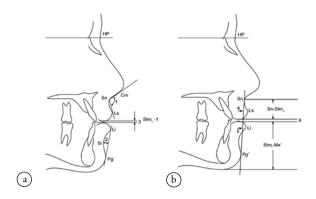


Figure 2 - Facial form a) soft-tissue analysis: Horizontal reference plane (HP), constructed by drawing a line through nasion (N) 7° up from S-N line, 1-facial convexity angle (G-Sn-Pog'), 2- lower face-throat angle (Sn-Gn'-C), lower vertical height-depth ratio, Sn-Gn'/C-Gn'. b) 3- maxillary prognathism (G-Sn), 4mandibular prognathism (G-Pog'), vertical height ratio (G-Sn/Sn-Me').



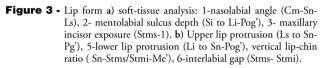


Table 1 - Descriptive statistics and comparison of the soft tissue cephalometric measurements between Saudi and European-American males according to Legan and Burstone analysis²

Variables	Measurements	European- Americans		Saudi		T-value	P-value	Significant
		Mean	SD	Mean	SD			-
Facial form								
Facial convexity angle (dg)	G-Sn-Pg'	12	4	14.85	4.41	2.800	0.006	**
Maxillary prognathism (mm)	G-Sn(HP)	6	3	6.21	4.83	0.212	0.833	NS
Mandibular prognathism (mm)	G-Pg'(HP)	0	4	-2.14	7.70	1.406	0.164	NS
Vertical height ratio	G-Sn/Sn-Me'	1	0	1.00	0.09	0.000	1.000	NS
Lower face-throat angle (dg)	Sn-Gn'-C	100	7	103.89	9.05	1.979	0.052	NS
Lower vertical height-depth ratio	Sn-Gn'/C-Gn'	1.2	0	1.20	0.23	0.077	0.939	NS
Lip position and form								
Nasolabial angle (dg)	Cm-Sn-Ls	102	8	102.85	10.68	0.371	0.712	NS
Upper lip protrusion (mm)	Ls to (Sn-Pg')	3	1	4.24	1.53	3.900	0.000	***
Lower lip protrusion (mm)	Li to (Sn-Pg')	2	1	3.63	2.22	3.805	0.000	***
Mentolabial sulcus (mm)	Si to (Li-Pg')	4	2	4.85	1.17	2.236	0.029	*
Vertical lip-chin ratio	Sn-Stms/Stmi-Me'	0.5	0	0.44	0.06	5.568	0.000	***
Maxillary incisor exposure (mm)	Stms-1	2	2	3.18	2.48	2.179	0.033	*
Interlabial gap (mm)	Stms-Stmi (HP)	2	2	2.69	1.24	1.803	0.076	NS

* $p \le 0.05$, ** $p \le 0.01$, *** $p \le 0.001$. NS - not significant. HP - horizontal reference plane, S - sella, N- Nasion, G - glabella, Cm - columella point, Sn - subnasale, Ls - labrale superius, Stms- stomion superius, Stmi - stomion inferius, Li - labrale inferius, Si - mentolabial sulcus, Pg - soft tissue pogonion, Gn'- soft tissue gnathion, Me' - Soft tissue menton, C - Cervical point.

based on the measurement between 2 points of a known distance on the radiograph. To analyze the difference between double measurements the intra-examiner error and the reliability of the measurements were assessed using Dahlberg's formula.¹⁹ Fifteen cephalometric radiographs from the original sample were selected and redigitized, 2-week interval. The error was found to range from 0.29 to 0.97 for all measurements. Pearson correlation coefficient between respective first and second measurements was all greater than 0.8.

All statistical analyses were performed using SPSS program for Windows (version 12 SPSS Inc., Chicago, IL, USA). Descriptive statistics (mean, standard deviations) were assessed for each measurement in both sexes separately. An independent student's t-test was used to test the gender differences and to compare the mean values of Saudi males and females with European-American mean values originally obtained by Legan and Burstone analysis² at 5% level ($p \le 0.05$).

Results. The results show descriptive statistics of the soft tissue cephalometric measurements for orthognathic analysis in Saudi subjects and compare them to European-Americans norms (Tables 1 & 2).

Table 3 presents the gender differences between theSaudi males and females.

Table 1 displays the results for Saudi and European-American males. The only statistically significant

Table 2 - Descriptive statistics and comparison of the soft tissue cephalometric measurements between Saudi and European-American females according to Legan and Burstone analysis.²

Variables	Measurements	European- Americans		Saudi		T-value	P-value	Sig
		Mean	SD	Mean	SD			
Facial form								
Facial convexity angle (dg)	G-Sn-Pg'	12	4	15.53	4.92	3.210	0.002	**
Maxillary prognathism (mm)	G-Sn(HP)	6	3	6.77	3.63	0.945	0.348	NS
Mandibular prognathism	G-Pg'(HP)	0	4	-0.49	6.62	0.362	0.719	NS
Vertical height ratio	G-Sn/Sn-Me'	1	0	1.02	0.10	1.229	0.223	NS
Lower face-throat angle (dg)	Sn-Gn'-C	100	7	101.12	7.31	0.644	0.522	NS
Lower vertical height-depth ratio	Sn-Gn'/C-Gn'	1.2	0	1.09	0.17	3.659	0.000	***
Lip position and form								
Nasolabial angle (dg)	Cm-Sn-Ls	102	8	109.68	11.40	3.154	0.002	**
Upper Lip protrusion (mm)	Ls to (Sn-Pg')	3	1	3.39	1.61	1.181	0.242	NS
Lower Lip protrusion (mm)	Li to (Sn-Pg')	2	1	2.84	1.91	2.192	0.032	*
Mentolabial sulcus (mm)	Si to (Li-Pg')	4	2	4.32	1.32	0.797	0.428	NS
Vertical lip-chin ratio	Sn-Stms/Stmi-Me'	0.5	0	0.46	0.05	4.558	0.000	***
Maxillary incisor exposure (mm)	Stms-1	2	2	3.37	1.38	3.39	0.001	***
Interlabial gap (mm)	Stms-Stmi (HP)	2	2	1.73	0.58	0.809	0.421	NS

*p≤0.05, **p≤0.01, ***p≤0.001. NS - not significant. HP - horizontal reference plane, S - sella, N- Nasion, G - glabella, Cm - columella point, Sn - subnasale, Ls - labrale superius, Stms- stomion superius, Stmi - stomion inferius, Li - labrale inferius, Si - mentolabial sulcus, Pg - soft tissue pogonion, Gn'- soft tissue gnathion, Me' - Soft tissue menton, C - Cervical point.

Table 3 - Statistical comparison of the soft tissue cephalometric measurements between Saudi males and females according to Legan and Burstone analysis.²

Variables	Measurements	Male		Female		T-value	P-value	Significant
		Mean	SD	Mean	SD			
Facial form								
Facial convexity angle (dg)	G-Sn-Pg'	14.85	4.41	15.53	4.92	0.569	0.571	NS
Maxillary prognathism (mm)	G-Sn(HP)	6.21	4.83	6.77	3.63	0.513	0.610	NS
Mandibular prognathism (mm)	G-Pg'(HP)	-2.14	7.70	-0.49	6.62	0.896	0.374	NS
Vertical height ratio	G-Sn/Sn-Me'	1.00	0.09	1.02	0.10	0.955	0.344	NS
Lower face-throat angle (dg)	Sn-Gn'-C	103.89	9.05	101.12	7.31	1.319	0.192	NS
Lower vertical height-depth ratio	Sn-Gn'/C-Gn'	1.20	0.23	1.09	0.17	2.114	0.039	*
Lip position and form								
Nasolabial angle (dg)	Cm-Sn-Ls	102.85	10.68	109.68	11.40	2.402	0.019	*
Upper lip protrusion (mm)	Ls to (Sn-Pg')	4.24	1.53	3.39	1.61	2.109	0.039	*
Lower lip protrusion (mm)	Li to (Sn-Pg')	3.63	2.22	2.84	1.91	1.495	0.140	NS
Mentolabial sulcus (mm)	Si to (Li-Pg')	4.85	1.17	4.32	1.32	1.666	0.101	NS
Vertical lip-chin ratio	Sn-Stms/Stmi-Me'	0.44	0.06	0.46	0.05	1.763	0.083	NS
Maxillary incisor exposure (mm)	Stms-1	3.18	2.48	3.37	1.38	0.376	0.708	NS
Interlabial gap (mm)	Stms-Stmi (HP)	2.69	1.24	1.73	0.58	3.885	0.000	***

*p≤0.05, ***p≤0.001. NS - not significant. HP - horizontal reference plane, S - sella, N- Nasion, G - glabella, Cm - columella point, Sn - subnasale, Ls labrale superius, Stms- stomion superius, Stmi - stomion inferius, Li - labrale inferius, Si - mentolabial sulcus, Pg - soft tissue pogonion, Gn'- soft tissue gnathion, Me' - Soft tissue menton, C - Cervical point. difference in overall facial form is a greater facial convexity angle in Saudi males indicates a trend toward more convex profile and Class II skeletal relationship which is attributed to a mandibular retrognathism in relation to glabella, but this finding is not statistically significant. For lip position and form, with exception of the variables measuring nasolabial angle and interlabial gap, there are statistically significant differences between the 2 ethnic groups. Saudi males have more protrusive upper and lower lips, deeper mentolabial sulcus, longer lower facial height and larger amount of incisor exposure below the upper lip than European-Americans.

Table 2 demonstrates the differences between Saudi and European-Americans females. No statistically significant differences are observed for the majority of variables with respect to the facial form except 2 variables. Saudis have a greater facial convexity angle and reduced lower vertical height depth ratio values, indicating for a more convex profile, Class II skeletal relationship tendency, shorter neck distance and more reduced chin than European-Americans. The increased Class II pattern in Saudi females is because of retrusive mandible, but the difference is not statistically significant. Regarding the lip position and form (Table 2), Saudi females reveals more obtuse nasolabial angle, more protruded upper and lower lips, longer lower facial height and larger amount of incisor exposure than European-American females. Table 3 illustrates the gender differences of soft tissue variables; no statistically significant differences are noticed except for the 4 variables. These are more obtuse nasiolabial angle, smaller lower vertical height-depth ratio, lower lip protrusion and inter-labial gap distance which indicate that Saudi females have a shorter neck and a more retrusive chin and lower lip than males. On the other hand, Saudi males have longer vertical distance between the upper and lower lips than females.

Discussion. The current study developed and compared cephalometric measurements of soft tissue facial profile of a sample of Saudi adults to European-American's norms using Legan and Burstone analysis.² The sample was limited to young adults with a mean age of 23 years. This correlates well with the age at which patients undergo orthognathic surgical treatment. Other studies included children¹² on the basis that this was the age range at which most patients commenced orthodontic treatment may not be useful for orthognathic surgical cases due to the processes of facial growth and development; therefore, the cephalometric norms for children can be expected to differ from those of adults. Similarly, patients of advanced age may show changes due to the aging process such as loss of vertical dimension between the jaws caused by attrition and loss of teeth.⁷ This is in agreement with previous reports.⁷⁻¹¹ The sample used in the study was 61 radiographs (31 males and 30 females); this size was similar to most other studies^{8,11,14} that have determined cephalometric norms. The criteria on which the sample was selected were based on normal dental and skeletal occlusion which agree and vary with the other studies.⁷⁻¹¹ However, most reports do exclude subjects who undergone orthodontic treatment or facial surgery or had facial trauma.7,9,14,18 With regard to the facial form variables, the Saudi subjects showed closer anteroposterior values when comparing with Legan and Burstone's norms. Both Saudi males and females had a significant increase in profile convexity than the European-Americans as indicated by statistically significant larger facial convexity angle. This suggests a reduced potential for forward mandibular growth rotation and may be explained by reduced chin prominence in Saudis as well as increase in vertical dimensions. However, the finding that the soft tissue facial angle is convex supports our previous finding of similar convexity in hard tissue profile.¹⁶ Saudi females showed shorter neck and reduced chin as indicated by statistically significant smaller lower vertical heightdepth ratio than European-American's females. Also, the lower face-throat angle was more obtuse in Saudi males than that in the European-American norms although it is not statistically significant. In this observation, consideration should be given during treatment. Legan and Burstone suggested, "An appreciation of this angle is critical in planning treatment to correct anteroposterior facial dysphasia. An obtuse lower face-throat angle should warn the clinician not to use procedures that reduce the prominence of the chin". These results were similar with a study conducted for Yemenis in the Middle East by Al-Gunaid et al.¹⁰ This can be a result of a similar ethnic background or adjacent geographic location. A comparison of the mean values of lip form and position indicate that there are significant differences in a number of cephalometric parameters between adult Saudis and European-Americans. Only Saudi females had statistically significantly larger nasolabial angle than those in European-Americans. Nevertheless, maxillary prognathism is normal, this difference apparently could be attributed to an upwardly sloping columella position of nose in Saudi females causing obtuse nasolabial angle. While, the Saudi males showed equal nasolabial angle (102°± 10.68°) to European-Americans; the angle was smaller than those obtained by Yemenis¹⁰ ($106^{\circ} \pm 9.7^{\circ}$). It was also noticed that, the nasolabial angle and the lower face-throat angle were found to have large standard deviations which reveal that these measurements show a great degree of individual variability and indicate that comparisons should be made with the range of normal values rather than with the mean. Similar findings were

observed by Zylinski et al²⁰ in a study conducted for white male Americans. In both sexes, the upper and lower lips were more anteriorly positioned in relation to the line between sub-nasale and soft tissue pogonion than European-Americans, agreeing with the previously reported concept of bilabial protrusion in Saudis.¹⁸ Hashim and AlBarakati¹⁸ determined lips protrusion in Saudi adults using the esthetic plane analysis. In keeping with previous finding¹⁸ and the current finding that indicate adult Saudis have increased lips protrusion relative to the norms of European-Americans, it could be stated the underlying reason for this difference may be due to increased incisor protrusion which indirectly supporting our recent findings of hard tissue study.¹⁶ The mentolabial sulcus depth were significantly greater in Saudi males than in the European-Americans, perhaps this is might be attributed to lower lip protrusion which might compensate for a retruded mandible during lip closure. The vertical lip-chin ratio was statistically reduced in both Saudi males and females indicating increased lower facial height when comparing with European-Americans' norms. This finding may be supported by reduced chin prominence. It is also supporting our previous finding of hard tissue study.¹⁶ Despite the fact that the statistically significant difference of maxillary incisor exposure may be interpreted as vertical maxillary excess. Indeed, indirectly supporting the increased lower facial height. In general, the results of this study agree with some other studies that compared other Middle Eastern populations with European-Americans norms. Al-Gunaid et al¹⁰ found that the Yemenis had increased facial convexity associated with retruded mandible, more obtuse lower face-throat angle, greater mentolabial sulcus, increased maxillary incisor exposure than European-Americans. Al-Azemi et al¹² showed that the Kuwaitis have more facial convexity and more bimaxillary lip protrusion and more obtuse nasolabial angle. The data were separated according to gender to obtain a more specific and useful cephalometric normative values. Of the soft tissue measurements, few parameters showed statistically significant differences between the sexes. Saudi females had a more obtuse nasolabial angle, shorter neck and more reduced chin than male counterparts. This indicates that the males have relatively straighter profile than the females.¹⁸ These findings are consistent with Kalha et al study.¹¹ In contrast to previous research,¹¹ Saudi males had a greater interlabial gap than did the females. This might be due to the gender difference in upper and lower-lips thickness which are more protruded in males than females. The other observed differences between males and females were in linear dimensions, but the difference was not statistically significant. This is to be expected since males have thicker soft tissue structure than females.^{7,11} The significant difference in facial heights between males and females might be of significance in treatment planning because these differences can be indications to increase or decrease face height.¹¹

The results of the current study confirm the differences in facial structures of various ethnic groups and between the sexes. These findings show that the group specific standards should be of useful guide for diagnosis of orthodontic and orthognathic surgical cases in Saudi adults. Although the present study had achieved its aims, However further investigations are needed with several aspects to be considered. These include a large randomly selected sample of both males and females that collected from different regions of Saudi Arabia with different age groups and more strict selection criteria. More variables from different cephalometric analyses are needed to be evaluated to establish Saudi cephalometric norms. Attempts should be made at exploring how the dental professional members, the laity, parents and patients perceive facial esthetics in individuals.

In conclusion, from the results of this study, the followings may be concluded: Soft tissue cephalometric values for adult Saudis males and females have been presented. These findings highlight the racial variations that should be considered during orthodontic and orthognathic diagnosis and treatment planning in adult Saudis. The study revealed that adult Saudis generally had increased facial convexity associated with retruded mandible, more obtuse lower face-throat angle, increased bimaxillary lip protrusion, greater mentolabial sulcus, decreased vertical lip-chin ratio, increased maxillary incisor exposure than European-Americans. The facial structure of Saudi male in general is larger than that of Saudi female.

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Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.