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Original research

Bolton's analysis using a photogrammetric method on occlusal photographs

Purpose

The aim of the study is to present a photogrammetric technique using standardized occlusal photographs to perform Bolton's analysis and assess reliability of this new method with plaster study casts.

Materials and Methods

The study was conducted on 16 subjects (8 males, 8 females), aged 18-25 years. Standardized occlusal photographs and plaster study casts were obtained. The occlusal photographs were calibrated in Nemoceph® software. Mesio-distal dimensions of all teeth up to first molars were calculated and Bolton's analysis was performed. Similarly, a digital calliper with 0.1 mm sensitivity was used to measure mesio-distal dimensions of all teeth on plaster study casts to perform Bolton's analysis. 28 parameters were measured on study models and corresponding occlusal photographs. Paired t test and intraclass correlation tests were carried out to test validity and reliability of the photogrammetric method. An intraclass correlation test was calculated for 4 derived parameters to test reliability of Bolton's analysis measurements obtained from occlusal photographs as compared to study models.

Results

All 28 parameters showed a statistically significant and excellent correlation (r>.80) in the Intra Class Correlation test. 4 variables used to calculate Bolton's analysis showed statistically significant correlation (r>.96) in the intraclass correlation test.

Conclusion

Photogrammetry is a reliable tool to measure mesio-distal tooth size. Bolton's analysis from standardized occlusal photographs using the described photogrammetric technique can be used as an effective clinical tool.

Keywords: Photogrammetry, Bolton's analysis, Ophotograph, Nemoceph, Tooth dimensions

Introduction

Effective and practical diagnostic aids that help in seamless and easy acquisition of data are useful in orthodontics. Digitization has been making an impact in the way we practise dentistry and holds a lot of promise in the future. However, when it comes to 3 - dimensional information, particularly in the pre treatment stage, plaster study models remain the most commonly used diagnostic aid.

Digital scanning technologies have been available from the mid 1990's (1) and digital study models were introduced in 1999 by Orthocad[™] (2). Digital study models hold a lot of advantages over plaster study models, obviating the need for physical storage (3), allowing instant accessibility to information, quick referral and virtual treatment planning. Moreover Cone Beam Computed Tomography (CBCT) technology also allow the creation of virtual study models which give 3D visualization of dental crown and root morphology.

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Several studies have been conducted comparing plaster study models with digital study models (4,5). A systematic review concluded that digital models offered a higher degree of validity (2). In spite of evidence showing the diagnostic advantages of digital study models, their widespread clinical use has not permeated in developing countries. This could be attributed to the high cost of scanning technology and dependency on software involved in acquiring digital data. Moreover, both plaster study models and digital study models do not serve as a cost effective or time saving method for acquiring data on tooth dimensions in epidemiological studies.

The technological improvements in digital cameras over the recent years combined with their reduced costs makes digital photography a viable alternative. However, there have been very few studies comparing measurements obtained from occlusal photographs with plaster study casts. The present study describes a photogrammetric method to perform Bolton's analysis on occlusal photographs and assess the reliability of this new method with plaster study casts.

Materials and Methods

The study was approved by Ethics Committee of Army College of Dental Sciences, Secunderabad, India (ACDS/IEC/21/ Jan 2018). The sample size estimation was carried out using GPower software version 3.1.9.2. Considering the effect size to be measured at 55%, power of the study at 80% and error margin at 5%, the total sample size required was 16.

The study was conducted on 16 subjects (8 males, 8 females), aged 18-25 years, with a mean age of 21 years and 5 months with a SD of 1.4.

Inclusion criteria

- All permanent teeth till first molars should be present.
- No restorations or crowns on any teeth.

Exclusion criteria

- Previous history of orthodontic or orthognathic treatment.
- Craniofacial trauma.
- Congenital anomalies.
- Neurologic disturbances

Bolton's analysis on study models

At the outset, upper and lower alginate impressions of the study sample (n=16) were taken and plaster study casts were prepared (Fig 1A). A digital calliper with .01 mm sensitivity was used to measure mesio-distal dimensions of all teeth up to the 1st molars in both arches (Fig 1B). The total arch length (mesio-distal dimensions of all teeth from 1st molar to the contra lateral molar in the same arch) and total anterior arch length (mesio-distal dimensions of all teeth from canine to the contra lateral canine in the same arch) were calculated for both arches. Subsequently, Bolton's analysis was performed using formulae as shown in Fig 2.



Figure 1. A) Plaster study casts were prepared for each subject. *B)* A digital vernier calliper with sensitivity of .01mm was used to measure the mesio-distal tooth measurement of each tooth up to first molars.

$overall ratio = \frac{sum of mandibular 12}{sum of maxillary 12} x 100 \qquad anterior ratio = \frac{sum of mandibular 6}{sum of maxillary 6} x 100$									
If ratio < 91.3%: maxillary TM excess. If ratio > 91.3%: overall mandibular TM excess									
$Overall\ maxillary\ TM\ excess\ =\ sum\ of\ maxillary\ 12\ -\ \frac{sum\ of\ mandibular\ 12}{91.3}\ x\ 100$									
$Overall\ mandibular\ TM\ excess\ =\ sum\ of\ mandibular\ 12-\frac{sum\ of\ maxillary\ 12}{100}\ x\ 91.3$									
If ratio < 77.2%: maxillary anterior TM excess. If ratio > 77.2%: mandibular anterior TM excess									
Anterior maxillary TM excess = sum of maxillary $6 - \frac{sum of mandibular 6}{77.2} \times 100$									
Anterior mandibular TM excess = sum of mandibular $6 - \frac{sum of maxillary 6}{100} \times 77.2$									



Bolton's analysis on standardized occlusal photographs

A digital camera (Canon EOS 600D) mounted with a macro portrait lens (EF 105 mm f/2.8, 1:1 OS, Sigma) was used for obtaining photographic records. A single combination intraoral mirror designed by Ashwin, Pulgaonkar, Chitra(6) and a lip retractor with a 35 mm metal ruler bonded onto its front surface were used to obtain standardized upper and lower occlusal photographs (Figs 3A,3B) of the sample (n-16).

Method of photography: The photographs were taken in a standardized manner to achieve consistent and reproducible images. For maxillary and mandibular photographs, the subjects were seated in a dental chair in a slightly reclined position with the height of the dental chair in its lowest position. All photographs were taken by the same examiner who held the camera in his hand in a stable position and an assistant who retracted the lips and placed the intra oral mirror. For maxillary occlusal photographs, the mirror was kept parallel to the occlusal plane and placed as low as possible until it touched the lower incisors. The entire maxillary arch was reflected in the mirror. For mandibular photographs, the subjects were asked to raise their tongue to the palate and breathe through their nose. The mirror rested on the gingiva distal to the last molars, so as to include all teeth. The mirror was turned upwards with the mouth wide open until it touched the incisal edges of the upper incisors. For both occlusal photographs, the mirror and camera were positioned such that the optical axis was exactly vertical to the mirror image of the occlusal plane of the maxillary or mandibular arch. The distance from mirror to camera is kept constant by

pre selecting the magnification ratio to 1:2. All photographs were taken with the camera settings in manual mode. The shutter speed was set at 1/250th of a second to ensure calibration with the ring flash, aperture at f25 and ISO at 100.

The photographs obtained were uploaded into Nemoceph 10.4.2 (Nemotec Dental Systems, Madrid, Spain) software program for Windows in which the mesio-distal tooth widths of all teeth up to the first molars were calculated. The 35 mm scale in the image was used for the purpose of calibration (Fig 3C). The total arch length and anterior arch length were calculated from the photogrammetric measurements obtained and Bolton's analysis was performed.



Figure 3. A) Modified single combination intraoral mirror which is used to take the occlusal maxillary and mandibular photographs. **B**) 35 mm trimmed metal scale bonded on both the surfaces of cheek retractor. **C**) The scale is used for the purpose of calibrating the images in Nemotec[®] software. Individual mesio-distal dimensions of all teeth up to first molars are measured in the software.

Statistical analysis

28 parameters were measured on study models and corresponding occlusal photographs of the same subjects. The parameters measured included the mesio-distal dimensions of each upper and lower tooth up to the first molars and the upper and lower total arch length and anterior arch length. The variables were paired and subsequently, paired t test and intraclass correlation coefficient (ICC) tests were performed (n=16) (Table 1).

Overall ratio and anterior ratio for the study models and occlusal photographs were calculated for each subject using the measurements from Table 1. The overall and anterior maxillary/mandibular tooth material excess was calculated for study models and photographs of each subject (Table 2).

An intraclass correlation test was calculated for the 4 derived parameters to test reliability of Bolton's analysis obtained from occlusal photographs as compared to study models (n=16) (Table 3).

Results

The mean difference calculated between the 28 variables show that, in general, photographic measurement values are greater than the corresponding study model measurements, except for UR5, LL6 and LR6 (Graph 1). Moreover, all the individual mesio-distal tooth measurements show a difference less than 0.20 mm, except UR4 (-0.22 mm). All the 28 variables showed a statistically significant and excellent correlation in the intraclass correlation coefficient test (r >.75) (Graph 2). The highest correlation was obtained for LL2, LR2 and the lower anterior arch length (r = .97) and the lowest correlation was obtained for LL6 (r = 0.8) (Table 1).



Graph 1. Bar diagram showing mean values of parameters from study model (s) with corresponding parameters from photographs (p) required to calculate Bolton's analysis. Parameters shown are: U6 to 6 = upper total arch length, U3 to 3 = upper anterior arch length, L6 to 6 = lower total arch length, L3 to 3 = lower anterior arch length, ovmme = overall maxillary tooth material excess/mandibular tooth material excess, antmme = anterior maxillary tooth material excess/mandibular tooth material excess.



Graph 2. Scatterplots of 4 parameters measured show excellent correlation. **A)** correlation between overall ratio in study models (s overall ratio) and photograph (p overall ratio); **B)** correlation between anterior ratio in study models (s anterior ratio) and photograph (p anterior ratio); **C)** correlation between overall maxillary/mandibular tooth material excess in study model (sovmme) and photograph (povmme); **D)** correlation between anterior maxillary/mandibular tooth material excess in study model (santmme) and photograph (pantmme).

13 subjects showed an overall ratio greater than 91.3% in study model and photographic Bolton's analysis, indicating an overall mandibular tooth material excess. 14 subjects showed an anterior ratio greater than 77.2%, in study model and photographic Bolton's analysis, thus indicating an overall anterior mandibular tooth material excess (Table 2).

Table 1. Mean, SD* (Standard deviation) and SE** (standard error) of the 28 parameters (n=16)												
Sample No		Study model			Photograph			SM-P	t	Sig	ICC	
	Variable	Mean	SD	SE	Mean	SD	SE	(diff)	value	(2 tailed)	R	Sig†
1.	UR1	08.78	0.43	.10	08.90	0.42	.10	12	- 2.65	.018	.95	+++
2.	UR2	06.70	0.44	.11	06.89	0.39	.09	18	- 3.70	.002	.93	+++
3.	UR3	07.75	0.49	.12	07.85	0.47	.11	10	- 1.84	.085*	.94	+++
4.	UR4	07.16	0.50	.12	07.38	0.41	.10	22	- 3.20	.006	.90	+++
5.	UR5	06.55	0.31	.07	06.51	0.40	.10	.04	.99	.334*	.94	+++
6.	UR6	10.05	0.70	.17	10.15	0.53	.13	09	86	.401*	.87	+++
7.	UL1	08.60	0.51	.12	08.73	0.39	.09	12	- 2.49	.025	.94	+++
8.	UL2	06.65	0.44	.11	06.79	0.41	.10	13	- 2.28	.038	.91	+++
9.	UL3	07.67	0.40	.10	07.76	0.35	.08	09	- 1.99	.065*	.93	+++
10.	UL4	07.15	0.36	.09	07.32	0.37	.09	17	- 4.73	.000	.96	+++
11.	UL5	06.50	0.36	.09	06.54	0.39	.09	04	70	.493*	.89	+++
12.	UL6	10.01	0.46	.11	10.06	0.42	.10	05	52	.609*	.76	++
13.	LL1	05.29	0.28	.07	05.44	0.31	.07	15	- 3.00	.009	.86	+++
14.	LL2	06.05	0.41	.10	06.21	0.36	.09	16	- 4.89	.000	.97	+++
15.	LL3	06.92	0.40	.10	06.97	0.40	.10	04	- 1.07	.302*	.94	+++
16.	LL4	07.11	0.41	.10	07.25	0.42	.10	14	- 2.35	.033	.91	+++
17.	LL5	06.87	0.40	.10	07.03	0.36	.09	16	- 4.11	.001	.95	+++
18.	LL6	11.18	0.69	.17	11.13	0.68	.17	.05	.35	.726*	.80	††
19.	LR1	05.51	0.27	.06	05.59	0.26	.06	07	- 1.38	.188*	.81	+++
20.	LR2	05.99	0.47	.11	06.13	0.42	.10	13	- 3.59	.003	.97	+++
21.	LR3	06.66	0.44	.11	06.76	0.42	.10	10	- 2.68	.017	.96	+++
22.	LR4	07.11	0.37	.09	07.26	0.30	.07	15	- 3.13	.007	.91	+++
23.	LR5	06.93	0.41	.10	07.07	0.40	.10	14	- 2.80	.013	.93	+++
24.	LR6	11.07	0.68	.17	10.91	0.77	.19	.16	1.35	.194*	.87	+++
25.	UTAL	93.63	3.53	.88	95.00	3.04	.76	- 1.36	- 4.02	.001	.95	+++
26.	UAAL	46.18	1.77	.44	46.95	1.55	.38	76	- 5.00	.000	.96	+++
27.	LTAL	86.75	3.69	.92	87.81	3.18	.79	- 1.06	- 2.69	.017	.94	+++
28.	LAAL	36.44	1.69	.42	37.13	1.48	.37	68	- 5.83	.000	.97	+++

UR= Upper right, UL= Upper left, LL= Lower left, LR= Lower right, UTAL= Upper total arch length (sum of UR6 to LL6), UAAL= Upper Anterior arch length (sum of UR3 to UL3), LTAL= Lower total arch length (sum of LL6 to LR6), LAAL= Lower Anterior arch length (sum of LL3 to LR3) SM-P (difference in means between Study model (SM) and photograph(P), Sig= Significance, ICC= Intraclass Correlation coefficient, r= r value, Sig= Singificance † p<.05, †† p<.01, ††† p<.001

The mean difference between the 4 variables derived from Bolton analysis on study models and photographs showed that in general, photographic measurements are greater than corresponding study model measurements. The only exception to this was the mean value of overall ratio. However, all variables showed a mean difference less than 0.20 mm and a statistically significant and excellent correlation in the intraclass correlation (ICC) coefficient test (r >.75) (Table 3).

Discussion

There have been numerous studies comparing Bolton tooth size analysis between digital models and plaster study models, (7,8,9,10) all of which have shown acceptable agreement between the two methods. However, to our knowledge, no study has been conducted comparing Bolton ratio obtained from occlusal photographs to plaster study models. Moreover, only two previous studies have compared measurements obtained from occlusal photographs to plaster study models.

In 1984, Gholston (11) concluded in his study that measurements obtained from intra oral photographs were reliable. However, the Orthoscan camera the author used is no longer in production. In 2011, Normando et al. (12) presented a photogrammetric method where dental arch dimensions and tooth size widths were calculated on standardized occlusal photographs and compared with plaster study models. The authors concluded that the photogrammetric method was a reliable tool for clinical and scientific application to measure tooth size and dental arch widths, except for calculating the mesio-distal width of the upper first molar.

Table 2. Bolton's analysis calculated on study models and photographs ($n=16$)												
Sample no	OR (SM)	OR (P)	AR (SM)	AR (P)	OTM (SM) Excess	OTM (P) Excess	ATM (SM) Excess	ATM (P) Excess				
1.	96.32	96.95	84.7	83.77	4.28	4.95	3.66	3.28				
2.	85.79	84.53	70.74	71.92	6.61*	7.11*	4.22*	3.44*				
3.	94.04	94.16	80.35	81.36	1.98	2.13	1.47	1.97				
4.	97.33	96.51	81.56	79.91	5.52	4.86	1.52	1.26				
5.	90.51	90.34	79.48	77.64	0.84*	1.05*	1.06	0.22				
6.	92.11	92.24	80.25	80.53	0.71	0.85	1.33	1.48				
7.	91.44	92.47	77.39	78.38	0.13	0.69	0.09	0.54				
8.	92.79	92.60	77.52	77.55	1.44	1.22	0.15	0.17				
9.	92.38	92.44	78.48	78.99	1.04	1.10	0.60	0.86				
10.	90.56	89.91	78.28	78.39	0.74*	1.40*	0.51	0.56				
11.	91.36	91.37	77.77	77.39	0.07	0.07	0.28	0.10				
12.	93.62	93.56	79.7	80.53	2.14	2.16	1.14	1.54				
13.	94.26	93.70	78.47	79.25	2.81	2.29	0.59	0.97				
14.	93.80	92.78	80.21	80.79	2.41	2.14	1.41	1.69				
15.	93.19	93.03	76.65	76.94	1.78	1.62	0.34*	0.16*				
16.	93.00	92.81	81.27	82.11	1.63	1.46	1.89	2.27				

OR= Overall ratio, SM= Study model, P= Photograph, AR= Anterior ratio, OTM (SM)= Overall tooth material excess in Study model, OTM (P)= Overall tooth material excess in Photograph, ATM (SM)= Anterior tooth material excess in Study model, ATM (P)= Anterior tooth material excess in Photograph. *Maxillary excess , readings not highlighted denote mandibular excess

Table 3. Mean, SD* (Standard deviation) and SE** (standard error) of the 4 parameters obtained from Bolton's analysis ($n=16$)											
	St	udy model		Р	hotograph	SM-P	ICC				
Variable	Mean	SD	SE	Mean	SD	SE	(diff)	r	Sig		
Overall ratio	92.65	2.60	.65	92.46	2.80	.70	.19	.98	+++		
Anterior ratio	78.92	2.95	.73	79.09	2.68	.67	16	.97	+++		
Overall maxillary/mandibular excess	2.13	1.87	.46	02.19	1.86	.46	06	.98	+++		
Anterior maxillary/mandibular excess	1.26	1.18	.29	01.28	1.05	.26	01	.96	+++		

SM-P (difference in means between Study model (SM) and photograph (P)), Sig= Significance, ICC= Intraclass Correlation coefficient, r= r value, Sig= Sigificance † p<.05, +t p<.01, +t+ p<.001

However, it should be kept in mind that correlations could be influenced negatively if the following precautions are not taken during the photographic procedure. The mirror has to be positioned correctly to ensure parallelism with the camera lens set to the correct magnification ratio of 1:2 and the optical axis of the camera perpendicular to the maxillary or mandibular occlusal plane. Occlusal photographs should be taken consistently and must reproduce the intraoral structures exactly to be of use for measurements. Moreover, if the need arises to crop the images, it must be cropped by maintaining the original ratio of the image so as to negate magnification errors.

In the current study, we evaluated the reliability of Bolton's analysis, which is an application of the photogrammetric method and requires accurate measurement of mesio-distal tooth dimensions from standardized occlusal photographs. The photogrammetric technique which we have used differs from the one used by Normando et al. in two aspects, viz., equipment and software used for calculation. Firstly, we have used a macro lens and a ring flash, which, we believe is essential for capturing standardized occlusal photographs in the correct magnification ratio. Secondly, Nemoceph[®] software, which we have used, allows for calibration of occlusal photographs with the help of the 35 mm scale visible in each photograph. Also, Nemoceph[®] software calculates the distance between any two marked points immediately in millimetres and has the distinct advantage of saving time and effort when compared to other imaging softwares such as Imagetool[®] which give readings in pixels and require calculations and conversions of unit. Moreover, we could not find an Imagetool[®] release supporting Windows 7[®] or higher versions. Nevertheless, further studies must be done comparing the available imaging software to ascertain which software gives the highest accuracy and reliability for calculating photogrammetric readings.

In the current study, even though all variables showed a statistically significant and excellent correlation in the intra-

class correlation coefficient test (r >.75), only 18 out of 28 variables showed a non significant result in the paired t test (Table 1). The parameters that showed a significant difference (p < .05) include the mesio-distal widths of upper and lower first molars, upper second premolars, upper canines and LL3 and LR1. This is in agreement with the findings obtained by Normando et al. who also found that despite the high reliability between the two methods, the paired t test revealed statistical differences in the validity of the two methods. The non significant result in the paired t test of the first molars and upper premolars could be due to the posterior location of the tooth and the difficulty in obtaining standardized images, which could have been influenced by the variations in the angle formed between the mirror and arch. The differences in UR3, LL3 and LR1 could be attributed to the increased occurrence of rotations with respect to these teeth. However, the mean differences between the mesio-distal dimensions of the measured teeth were less than 0.20 mm (except UR4 = -0.22 mm), which is close to the human eye resolution of 0.2mm. (13) Hence, these minor differences are not of clinical significance.

Similarly, the intraclass correlation test of the 4 derived parameters obtained from Bolton's analysis, show excellent correlation between the two methods (r>0.96, for all variables) (Table 3). The mean differences between the measurements obtained for the two methods for the 4 parameters are below 0.2mm, showing that Bolton's analysis measurements from occlusal photographs are clinically useful. This could be used as an advantageous measuring tool in epidemiological and research studies, for assessing Bolton's discrepancy during treatment progress and in conditions were procuring a dental arch impression proves difficult. Moreover, with advances in technology it is expected that digital cameras would further improve their accuracy and be low cost imaging tools for clinicians. Taking standardized occlusal images is not time consuming and negates the need for making study models at various stages of treatment. Measurements can me made directly on occlusal photographs without the need to remove archwires as required prior to making alginate impressions which is a time saver in busy practices. We also believe the photogrammetric method can be used as an effective clinical control for self assessment and to assess changes that occur in the dental arch in between appointments (e.g. assessing midline discrepancies, Bolton discrepancy, changes in arch width due to expansion devices etc).

Conclusion

The present study shows that photogrammetry is a reliable tool to measure mesio-distal tooth size and that quantitative data obtained from photogrammetric measurement of standardized occlusal photographs can provide clinicians with useful and accurate information negating the need for plaster study models. However, taking standardized photographs is a technique sensitive procedure and so the clinician must train himself in taking repeatable photographs with minimal errors. Also there is a need for development of free software that allows for calibration and measurement of distances between two or more points in a photograph so that more clinicians can apply photogrammetry in their clinical practice. Türkçe Öz: Oklüzal Fotoğraflarda Fotogrametrik Bir Yöntem Kullanılarak Bolton Analizi. Amaç: Bu çalışmanın amacı, standardize oklüzal fotoğraflar kullanılarak Bolton Analizi yapılmasını sağlayan yeni bir fotogrametrik tekniği sunmak ve alçı çalışma modelleri ile bu yeni yöntemin güvenilirliğini değerlendirmektir. Gereç ve Yöntem: Bu çalışma yaşları 18-25 arasında olan 16 birey üzerinde (8 erkek, 8 kadın) gerçekleştirilmiştir. Standart oklüzal fotoğraflar ve alçı çalışma modelleri elde edilmiştir. Oklüzal fotoğraflar Nemoceph® yazılımıyla kalibre edilmiştir. 1. büyük azı dişlerine kadar olan tüm dişlerin mezio-distal boyutları hesaplanmış ve Bolton analizi yapılmıştır. Benzer şekilde, alçı çalışma modellerindeki tüm dişlerin mezio-distal boyutları 0,1 mm hassasiyete sahip bir dijital kompas kullanılarak Bolton analizi yapmak için ölçülmüştür. Çalışma modellerinde ve ilgili oklüzal fotoğraflarda 28 parametre ölçülmüştür. Fotogrametrik yöntemin geçerliliğini ve güvenilirliğini test etmek için eleştirilmiş t testi ve sınıf içi korelasyon testleri yapılmıştır. Okluzal fotoğraflardan elde edilen Bolton analiz ölçümlerinin çalışma modellerine kıyasla güvenilirliğini değerlendirmek için 4 parametreye sınıf içi korelasyon testi yapılmıştır. Bulgular: 28 parametrenin tamamı, sınıf içi korelasyon testinde istatistiksel olarak anlamlı ve mükemmel bir korelasyon (r>.80) göstermiştir. Bolton analizini hesaplamak için kullanılan 4 değişken, sınıf içi korelasyon testinde istatistiksel olarak anlamlı korelasyon (r> .96) göstermiştir. Sonuç: Fotogrametri mezio-distal diş boyutunu ölçmek için güvenilir bir araçtır. Standart oklüzal fotoğraflardan fotogrametrik teknik kullanılarak yapılan Bolton analizi etkili bir klinik araç olarak kullanılabilir. Anahtar Kelimeler: Fotogrametri, Bolton analizi, oklüzal fotoğraf, Nemoceph, diş boyutları

Ethics Committee Approval: The study was approved by Ethics Committee of Army College of Dental Sciences, Secunderabad, India (ACDS/IEC/21/Jan 2018).

Informed Consent: The informed consents were provided by the participants.

Peer-review: Externally peer-reviewed.

Author contributions: AP and PC designed the study. AP participated in generating the data for the study. AP participated in gathering the data for the study. AP and PC participated in the analysis of the data. AP wrote the majority of the original draft of the paper. AP and PC participated in writing the paper. All authors approved the final version of this paper.

Conflict of Interest: The authors had no conflict of interest to declare.

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