Journal of Oral Biology and Craniofacial Research 2012 May–August
Volume 2, Number 2; pp. 77–82

Original Article

Measurement error of 3D cranial landmarks of an ontogenetic sample using Computed Tomography

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ABSTRACT

Background/Aim: Computed Tomography (CT) is a powerful tool in craniofacial research that focuses on morphological variation. In this field, an ontogenetic approach has been taken to study the developmental sources of variation and to understand the basis of morphological evolution. This work aimed to determine measurement error (ME) in cranial CT in diverse developmental stages and to characterize how this error relates to different types of landmarks.

Material and methods: We used a sample of fifteen skulls ranging from 0 to 31 years. Two observers placed landmarks in each image three times. Measurement error was assessed before and after Generalized Procrustes Analysis.

Results: The results indicated that ME is larger in neurocranial structures, which are described mainly by type III landmarks and semilandmarks. In addition, adult and infant specimens showed the same level of ME. These results are specially relevant in the context of craniofacial growth research.

Conclusion: CT images have become a frequent evidence to study cranial variation. Evaluation of ME gives insight into the potential source of error in interpreting results. Neural structures present higher ME which is mainly associated to landmark localization. However, this error is irrespective of age. If landmarks are correctly selected, they can be analyzed with the same level of reliability in adults and subadults.

Keywords: *Craniofacial growth, Morphometry, Intra-observer error*

INTRODUCTION

Computed Tomography (CT) has been used in different areas of craniofacial research such as medicine, odontology and physical anthropology. It has been applied in surgical planning[1–3] and to compare pathological and normal conditions.[4,5] Also, it is a useful tool in fossil preparation, as it gives the opportunity to access unobserved structures and reconstruct fragmentary fossils.[6]

Craniofacial landmark-based studies are increasingly based on CT. Landmarks are discrete anatomical points that are homologues in all specimens. The configuration of landmarks can be analyzed by geometric morphometrics (GM), a methodological approach that focuses on the geometry of structures of interest. To capture this geometric information, after collecting landmark coordinates in the space, some mathematical procedures are applied to eliminate information about scale, position and orientation.[7] As a result, shape and size can be taken as two independent aspects of morphological variation.

Three types of landmarks can be recognized[8]: type I are those localized in biological structures that are easy to identify repetitively, such as the intersection of sutures. Landmarks type II are observed considering geometry, for example they are points of local maxima or minima curvature and finally, landmarks type III include extremal points,

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Received: 16.4.2012; Accepted: 25.5.2012

http://dx.doi.org/10.1016/j.jobcr.2012.05.005

for instance endpoints of a breadth. The concept of semilandmark was introduced to assess shape variation in structures such as curves and surfaces where landmarks are rare.[9] They are usually distributed between landmarks using an algorithm.

An unavoidable stage in any morphometric research is the measurement error (ME) estimation, which is defined as the deviation of the result of a measurement from the true value of the measured variable.[10] To date, ME in CT has been assessed in mandibles,[11] skulls of adult individuals[12] and in the vault of a paediatric sample.[13] In subadults the registration of landmarks can be problematic because of underdevelopment of some structures. As craniofacial morphology changes along postnatal life, it would be expected that ME vary. For instance, vault bones are separated by fontanelles in infants and by sutures after 3 years old all of which would produce variation mainly in landmarks type I. Additionally, some landmarks are located on sites of muscular attachments or they are sexually dimorphic, thus they are easily measured among adults, but not among subadults. This work aimed to determine ME in cranial CT in diverse developmental stages and to characterize how this error relates to different types of landmarks.

MATERIALS AND METHODS

We randomly selected fifteen CT cranial images from a data set constructed at FLENI (Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia, Buenos Aires, Argentine), which belonged to non-pathological humans, 0 to 31 years old, of both sexes and described neurocranial structures (vault and basicranium) and the upper face[14](Table 1).

The skulls were scanned with a General Electric Light Speed RT 16, using two different scan protocols: one with less exposure to X-rays (Protocol 1) for individuals from 0 to 15 years old because they have thinner bones and another one for 16 to 31 years old (Protocol 2). Protocol 1 followed axial mode scan, 150 mA1 of current, 120 kVp2 of accelerating voltage, and a gantry/detector tilt positioned in 0.0° that produced 275 axial 512 × 512 pixel CT images with a voxel size equal to 0.449 × 0.449 × 0.625 mm. Protocol 2 consisted in axial mode scan, 200 mA, 120 kVp, gantry/detector tilt position at 0.0° which gave 275 axial 512 × 512 pixel CT images with a voxel size equal to 0.449 × 0.449 × 0.625 mm.

We used the trial version of Aviso 6.0 software (Visualization Science Group) to examine CT images, create reconstructions and collect landmark data. From CT slice, a 3D superficial reconstruction was created using a chosen density threshold that corresponded to the Hounsfield unit scale (Spoor et al, 2000). Surface extraction thresholds, which needed to be stipulated to produce a reconstruction, were determined empirically. A threshold of 1150 Hounsfield units was chosen to show the maximum amount of bony tissue with the least amount of distortion. The 3D reconstructions were used to identify landmark localization.

Table 1 Sample composition.

Identification	*Age (years)*	*Sex*	*Threshold*
01M-006	0.58	Male	1150
01M-010	1.17	Male	1150
01M-011	1.42	Male	1150
03M-013	2.58	Male	1150
06M-020	6	Male	1150
07M-025	7.08	Male	1150
09M-035	9.17	Male	1150
10F-036	10	Female	1150
10F-037	10.25	Female	1150
10M-041	10.42	Male	1150
25F-106	25.17	Female	1150
28F-133	28.42	Female	1150
29F-137	29.17	Female	1150
31F-146	30.58	Female	1150
31F-147	31	Female	1150

Three dimensions (x-, y- and z-) of 51 landmarks and 17 semilandmarks were registered in fifteen specimens. Two of us (JBA –observer 1- and MA –observer 2-) registered them three times (Fig. 1). Observer 1 has previously registered the same landmarks and semilandmarks in dried skulls using Microscribe.

First, the dispersion of repeated measures was analyzed from the raw coordinates in order to observe landmark placement. CT specimens held in a constant orientation in each measurement as the x-, y- and z- axes, are generated when the study is carried out and the frame of reference is the same in all the measurement events.[15] This approach was an extension of the research design introduced by Corner et al,[16] where several observations were done on a specimen that was kept in a constant orientation, using a digitizer in the same position to avoid any displacement of the measured object or the equipment. Raw coordinates could be compared without the need of any transformation, because the landmarks had the same position in space in all the measurement events. This method provided an estimation of individual landmark position and it gave the opportunity to identify problematic morphometric points where larger deviations were found.

Second, superimposed landmark configurations were analyzed to evaluate whether repeated measures fell within

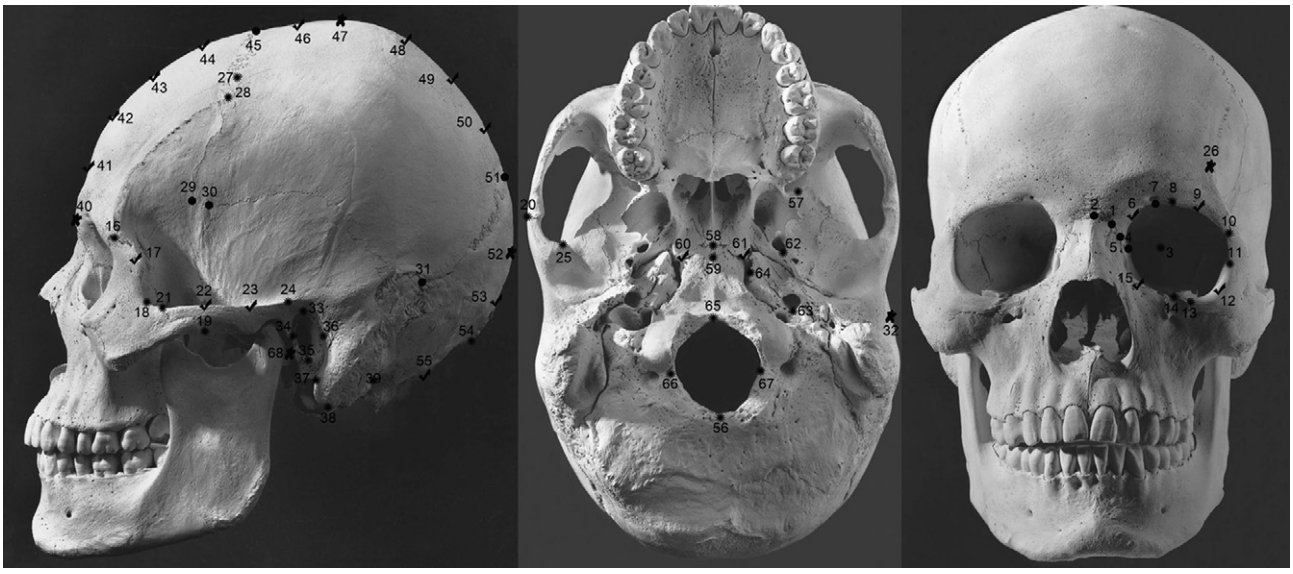

Fig. 1 Landmarks location. Landmarks type I (circle), landmarks type II (pointed circle), landmarks type III (asterisk) and semi-landmarks (tick).

some established limits of variation. For this purpose, the raw coordinates were transformed to adjusted landmarks using the standard procedure known as Generalized Procrustes Analysis where individuals were rotated, translated and scaled to keep only shape information. Once these shape coordinates were obtained, we compared trials by means of Analysis of the Variance for Repeated Measures (ANOVARM).[17] By means of ANOVARM, it was possible to detect differences when measurements were correlated because they were performed on the same object or specimen. In addition, a Principal Component Analysis (PCA) was carried out to assess the magnitude of error of precision relative to the differences in shape.[18–20] This analysis reduces variation and generates axes (principal components) that

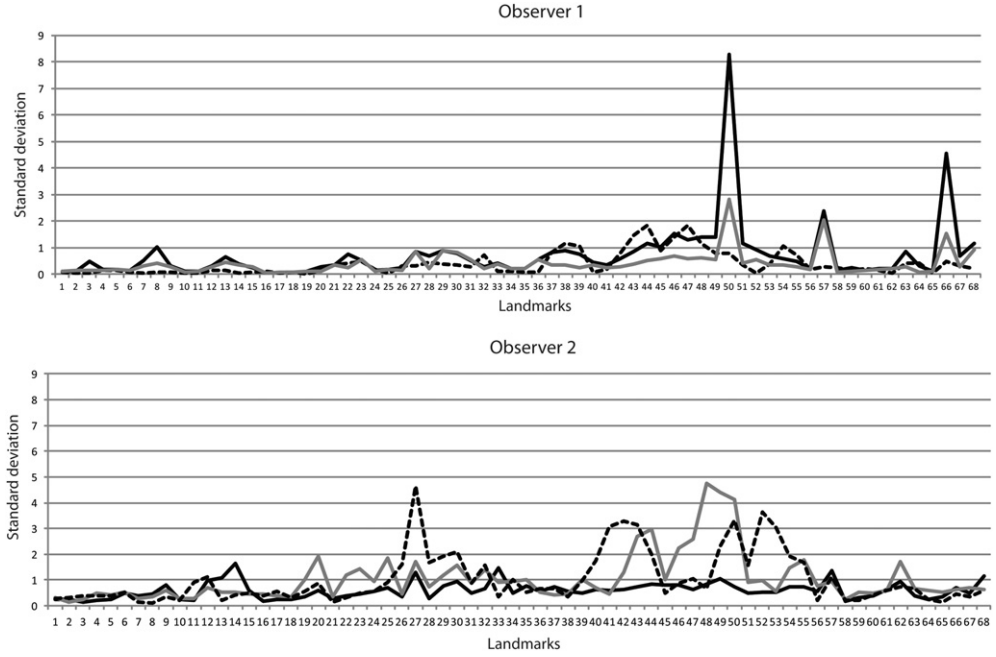

Fig. 2 Standard deviation in x-(solid black), y-(grey) and z-(dotted black) axes.

Table 2 ANOVARM of adjusted landmarks.

Landmarks	*x*		*y*		*z*	
	F	p	F	p	F	p
Observer 1						
1	4.68	0.0176			3.86	0.0331
5			3.83	0.0339		
6					5.57	0.092
9					4.62	0.0184
12					5.98	0.0069
13					5.61	0.009
15			4.46	0.0208		
22					6.05	0.0066
24	3.70	0.0375	3.73	0.0365		
26					5.34	0.0108
32	4.67	0.0177			5.20	0.012
45	5.94	0.0071				
52					3.66	0.0386
65	4.50	0.0202				
66	3.79	0.0349				
67	4.22	0.025				
Observer 2						
12	3.69	0.030				
13					3.75	0.030
17	3.64	0.030			3.75	0.030
18					7.08	0.003
23						
24			8.33	0.001		
25			4.11	0.020		
27	8.35	0.001	4.79	0.010	5.44	0.010
30	4.17	0.020				
33			6.85	0.003		
37			6.47	0.004		
38			3.47	0.040		
39	4.76	0.010			4.92	0.010
41						
46			6.07	0.006		
52					5.37	0.010
53					5.81	0.007
59			9.61	0.0007		
60					3.49	0.040
61			3.77	0.030	3.75	0.030
66			3.74	0.030		
67			4.52	0.010		

explain an important amount of variation in a little number of variables. In a graphical representation of the distribution of specimens along the two axes that explain most of variation, the position of each specimen in each trial can be observed. So, if repeated measurements on the same individual are similar, they must be in a near or in the same position along the axes described by the principal components.

RESULTS

Neurocranial structures showed higher deviations along trials than facial landmarks; these ones were placed with a high level of correspondence (Fig. 2). This pattern was specially clear for observer 1. Both observers produced the largest deviations in neurocranial landmarks and

semilandmarks. Levels of deviation in the x-, y- and z- axes were similar to observer 1. In contrast, observer 2 showed different levels of deviation in the axes, except for the x-axe where deviations were always remarkably low.

Through ANOVARM we assessed error differences between the three axes of each landmark in each trial (Table 2). Error was greater on semilandmarks along three axes. Observer 1 had less ME than observer 2, but although both observers had error on neurocranial and facial landmarks, landmarks of largest ME were not coincident between both observers.

According to the PCA, which helps to visualize error differences between different ontogenetic stages, both observers placed landmarks with a consistent level of correspondence (Fig. 3). While along PC1 the distribution of specimens shows ontogenetic changes, the variation that could be related to ME is seen along PC2. In this latter axe, the similar dispersion was found in adults and subadults.

DISCUSSION

In this work, we evaluated different aspects of ME in the context of landmark-based studies. Although in practice ME is tested before carrying out the definitive measurements, it is important to be aware of some points that may lead to inaccuracy in order to improve the research design.

Each methodological approach and statistical analysis allowed us to assess different aspects of ME. Standard deviation and ANOVARM showed in which particular landmarks there was larger error, as well as interobserver differences. On the other hand, PCA illustrated ME in each particular specimen so that problems of accuracy due to developmental changes can be inferred. Therefore, it is essential to choose the right methods to evaluate the aspect of ME of interest.

Our results suggest that ME increases in landmarks type III (Table 2), as was suggested by Ross and Williams[21] and Williams and Richtsmeier.[11] As a consequence, ME is greater in neurocranium than in the face since the former is mainly described by landmarks type III, as well as by semilandmarks. It is because landmark type III and semilandmarks are difficult to visualize and localize. Nevertheless, landmarks type III and semilandmarks are necessary to study areas that would otherwise be unsampled. Landmark-based studies should consider this difficulty to reinforce the training in the localization of these particular points and to review their inclusion if high ME levels remain.

Following Valeri et al[13], it can be inferred that ME depends on the developmental stage of an individual since visibility of a given structure change across ontogeny; i.e. these authors found that landmarks of the cranial bosses have less ME in younger individuals where the bosses are more pronounced. According to our results (Fig. 3), there is not enough evidence to associate ME with development because, despite anatomical changes, both observers could identify the measured structures in all specimens along the whole age range. Research planning, taking into account a rigorous selection of landmarks that are identifiable in the entire sample, is crucial to avoid homology problems during the study.

Differences between observer 1 and 2 may be associated with their previous experiences. According to Valeri et al[21], the experience of the observer influences the number of trials needed and this trend could be described

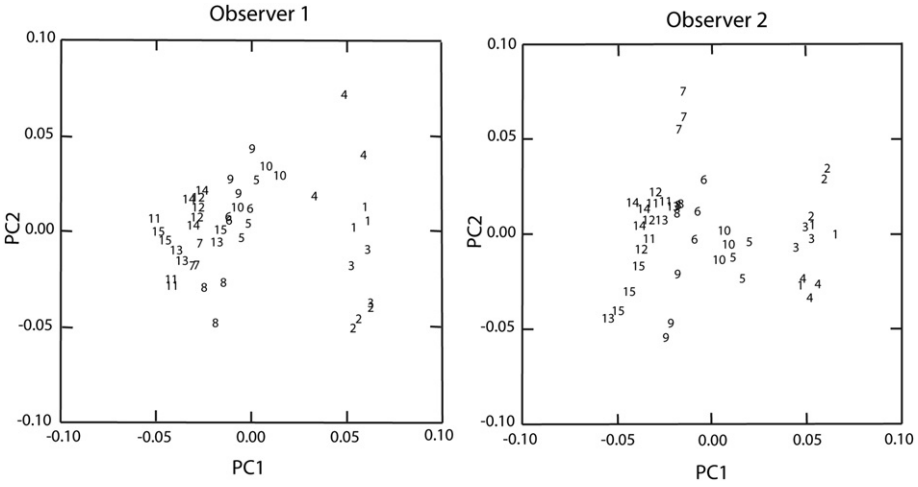

Fig. 3 Principal Component Analyses. Distribution along PC1 and PC2. Each number represents one specimen of the sample as named in Table 1.

as a learning curve where ME is reduced as experience is gained. This fact is not exclusive of landmark-based studies, for example Yeserinac et al[22] showed that measurer's experiences influences substantially precision of linear measurements.

CONCLUSION

CT images have become a frequent evidence to study cranial variation, hence the evaluation of ME gives insight into the potential source of error in interpreting results. Our results are coincident with other studies, suggesting that neural structures present higher ME. This is mainly associated to landmark localization of some particular kind of points. However, this error is irrespective of age of individuals and, if landmarks are correctly selected they can be analyzed with the same level of reliability in adults and subadults. This fact is important in a scientific context where ontogenetic studies are growing in different fields and disciplines that focus on craniofacial morphology.

CONFLICTS OF INTEREST

All authors have none to declare.

ACKNOWLEDGEMENTS

Grant sponsorship: Proyecto de Incentivos N663, Universidad Nacional de La Plata.

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