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Gender assessment through three-dimensional analysis of maxillary sinuses by means of Cone Beam Computed Tomography

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Abstract. – OBJECTIVE: The availability of a low dose radiation technology such as Cone Beam Computed Tomography (CBCT) in dental practice has increased the number of scans available for forensic purposes. Moreover, specific software allows for three-dimensional (3D) characterization of the maxillary sinuses. This study was performed to determine whether sinus maxillary volumes can be useful to identify gender after validating the use of the Dolphin software as a tool for volumetric estimation of maxillary sinus volumes.

PATIENTS AND METHODS: The validation was performed by four different operators measuring the volume of six phantoms, where the real volume was already known. The maxillary sinus volumes of 52 patients (26 males and 26 females) mean age 24.3 were calculated and compared between genders and sagittal skeletal class subdivision. The measurements for patients and phantoms were based on CBCT scans (ILUMA™) processed by Dolphin 3D software.

RESULTS: No statistical difference was observed between the real volume and the volume measurements performed by the operators. No statistical difference was found in patient's maxillary sinus volumes between gender.

CONCLUSIONS: Based on our results, it is not possible to support the use of maxillary sinuses to discern sexual difference in corpse identification.

Key Words:
Forensic anthropology, Cone beam computed tomography, Three dimensional imaging.

Introduction

Recently, judicial demand for gender identification has increased because of an increase in criminal cases involving young people, irregular immigration and modern crimes. Gender identification is a classic procedure in forensic medicine as sex assessment constitutes an important step in constructing a post-mortem profile. It is also useful in identifying skeletal remains as it is not uncommon to recover only partial remains. Common post-mortem forensic procedures for identification, including general external examination, radiographs and complementary biological methods, are sometimes insufficient. Thus, forensic medicine would benefit from new identification methods that combine simplified procedures, lower costs and especially, maximum accuracy. The primary goal when performing the identification of corpses is to compare ante-mortem and post-mortem records[1,2]. In fact, massive post-mortem changes could complicate identification[3]. According to the literature, the skull, the pelvis and long bones with an epiphysis and a metaphysis in skeletons have been used to

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determine gender. Moreover, it has been reported that maxillary sinuses maintain their anatomical shape and structure intact following fire accidents although the skull and other bones may be badly disfigured[1]. In this respect, the maxillary sinuses have been proposed for corpse identification[4]. The maxillary sinus is a triangular pyramid in the body of the maxilla. It presents three recesses: an alveolar recess pointed inferiorly, bounded by the alveolar process of the maxilla; a zygomatic recess pointed laterally, delimited by the zygomatic bone, and an infraorbital recess pointed superiorly, bounded by the inferior orbital surface of the maxilla. The size of sinuses varies in different skulls, and even on the two sides of the same skull. To measure the internal dimension of the maxillary sinus has proven to be a challenge for researchers[5]. Considering the complex structure of maxillary sinuses, magnetic resonance imaging (MRI) and x-ray computed tomography (CT) are the gold standard methods to depict the true anatomy of the Highmore's antrum. Nevertheless, their use is limited by high dose, cost, or restricted accessibility[6,7]. These drawbacks were overcome with the introduction of cone-beam computed tomography (CBCT)[6,7]. Using CBCT technology, measurements of the maxillary sinus volume and the quantification of craniofacial structures are now available that reduce radiation dose compared with CT scans as well as reduced costs compared with MRI. CBCT data sets allow the possibility of a realistic representation of the head of the patient and has expanded diagnostic possibilities, enabling three-dimensional (3D) simulation of surgical and orthodontic procedures. In addition, since its introduction in 1998, CBCT technology has also been improved in terms of accuracy in identifying the boundaries of soft tissues and empty spaces (air). Concerning these advantages, CBCT technology became the elective tool in Orthodontics and Maxillofacial surgery when three-dimensional analysis was required. It is now easier to get a CBCT full skull report instead CT or MRI of orthodontics patients. Most CT studies only determined the linear metric variables of the maxillary sinus for forensic purposes. Conversely, a valid protocol for 3D volume assessment is still required[8,12]. The main obstacle is still the lack of information on the influence of human error on instrumentation, which could cause measurements to deviate from their actual values[13]. Moreover, only a few studies have tested volumetric analysis techniques with a phantom model[14,15]. For these reasons, validation studies to define the experimental uncertainty are decisive in the estimation of volumetric analysis techniques[13]. The aim of our study is to validate the use of Dolphin Imaging software to analyze CBCT images as a tool for volumetric estimation of maxillary sinus volumes and to test the intra- and inter-examiner reproducibility of this technique. In addition, we determine whether sinus maxillary volumes can be useful as a means to identify the sex of unknown persons.

Materials and Methods

A validation of the method with well-characterized phantoms was conducted to test the experimental procedure in advance.

To evaluate software reliability, we used six known-volume phantoms replicating the geometrically complex anatomy of the maxillary sinuses (Figure 1):

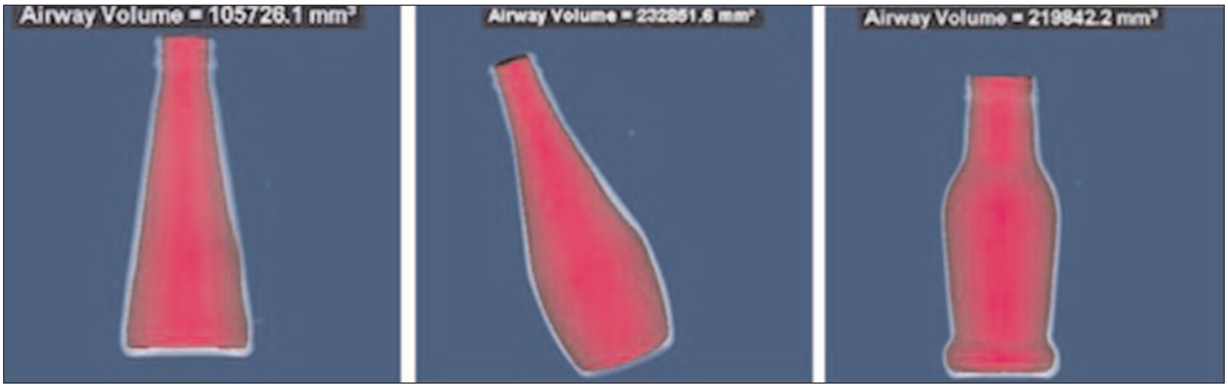

Figure 1. Known-volume phantoms.

- Three empty glass containers dipped inside a cylinder of alginate; (Palgat™ Plus 3M Espe, Germany).
- Three empty glass containers dipped inside a cylinder of alginate and partially filled with alginate (Palgat™ Plus 3M Espe, Germany).

These custom-made phantoms with known volumes were used as the gold standard, and their volume was confirmed by using the water weight equivalent. The volume of the glass containers was calculated by filling them completely with distilled water (1 atm at 20°C). Water weight was determined by using a digital scientific scale (Gibertini TM560, Max 560 g d = 0.01 g). The precision scale was first calibrated by measuring the empty containers. The weight of the distilled water (calculated in grams) was converted in volume (calculated in mm^3) using a specific conversion table. Therefore, we dipped the containers inside a cylinder made of alginate to mimic the soft tissue attenuation of x-rays. Additionally, the 3D scans of 104 maxillary sinuses of a 52 Caucasian adults (mean age 24.3 ± 6.5 years, 26 females and 26 males) was retrospectively examined. All subjects had received a cone beam evaluation of the stomatognatic apparatus for the following reasons: (1) teeth extraction, such as wisdom teeth; (2) orthodontic evaluation of unerupted teeth; (3) the study of cephalometric aspects (lateral and postero-anterior), and (4) a dental implant. The exclusion criteria included history of paranasal sinus surgery and maxillofacial trauma, subjects with upper airway pathology, such as clinical sinusitis, and/or cysts of the maxillary sinus and odontogenic cysts. A cephalometric study was conducted on lateral cephalograms obtained from the volumetric 3D data. Subsequently, patients were divided according to Angle's skeletal classifications. SNA, SNB, and ANB angles and AoBo distance has been used to assign patients to the appropriate skeletal groups: Skeletal class I ($0° < ANB < 4°$); Skeletal class II ($ANB > 4°$), and Skeletal class III ($ANB < 0°$). Patients and phantoms were scanned with ILUMA™ Cone beam volumetric tomography (IMTEC, 3 M Company, Ardmore, OK, USA), with a reconstructed layer thickness of 0.3 mm and a 512 × 512 pixel matrix. The device was operated at 120 kVp and 3-8 mA by using a high frequency generator with a fixed anode and a 0.5 mm focal spot. A single 40-s high-resolution scan was made of each skull. The voxel size was set at 0.25 mm, and the images were exported as DICOM files. Each maxillary sinus was visualized in the proper bone density range (1350-1650 gray scale range) and then graphically isolated prior to 3D and volumetric measurements[15].

Segmentation Procedure

The obtained CT data were analyzed with Dolphin Imaging software (Dolphin Imaging and Management solution, USA). The raw data sets, in DICOM format, were imported in the program 3D Dolphin Imaging. To segment the data correctly, a calibration of the software should be performed following the procedure described below:

1. The type of tissue should be set: Hard (hard tissues), Soft (soft tissue), or Soft + Hard tissues (both), depending on the volume imported and of which part you want to examine.
2. Radiological artifacts were eliminated, as well as any excess in the volumetric reconstruction; thus a precise portion to be examined were delimited.
3. Reorientation of the volume in the three spatial planes was applied, correcting any errors in the positioning of the skull (or phantom) during the CT scan, or simply orienting it in a desired position (see Figure 2).
4. The Sinus/Airway tool function of Dolphin was set as follows (see Figure 2):
   - **Step 1:** "clipping boundary and seed points", where the airway structure has been identified selecting boundary points (seed points) in the coronal, axial and sagittal planes.
   - **Step 2:** "slice airway sensitivity", where the sensitivity of the virtual sensor was applied to discriminate airspace. In this study, the "sensitivity tool" was set to a value of 85/100.
   - **Step 3:** "Update volume", where selecting this option the volume was calculated.

Measurements

All examiners were trained to use the above standardized study procedure and software tools. They performed phantom measurements in a dark room independently from each other and were blind to previous readings. Each measurement cycle was performed three times with an interval of 2 weeks between sessions to minimize personal memory effects. All segmentations were evaluated in random order by (A1) one graduate dental student (A2), one well experienced radiologist (A3), one well experienced oral surgeon (A4) and one associate professor of Orthodontics. Each phantom was evaluated four times by

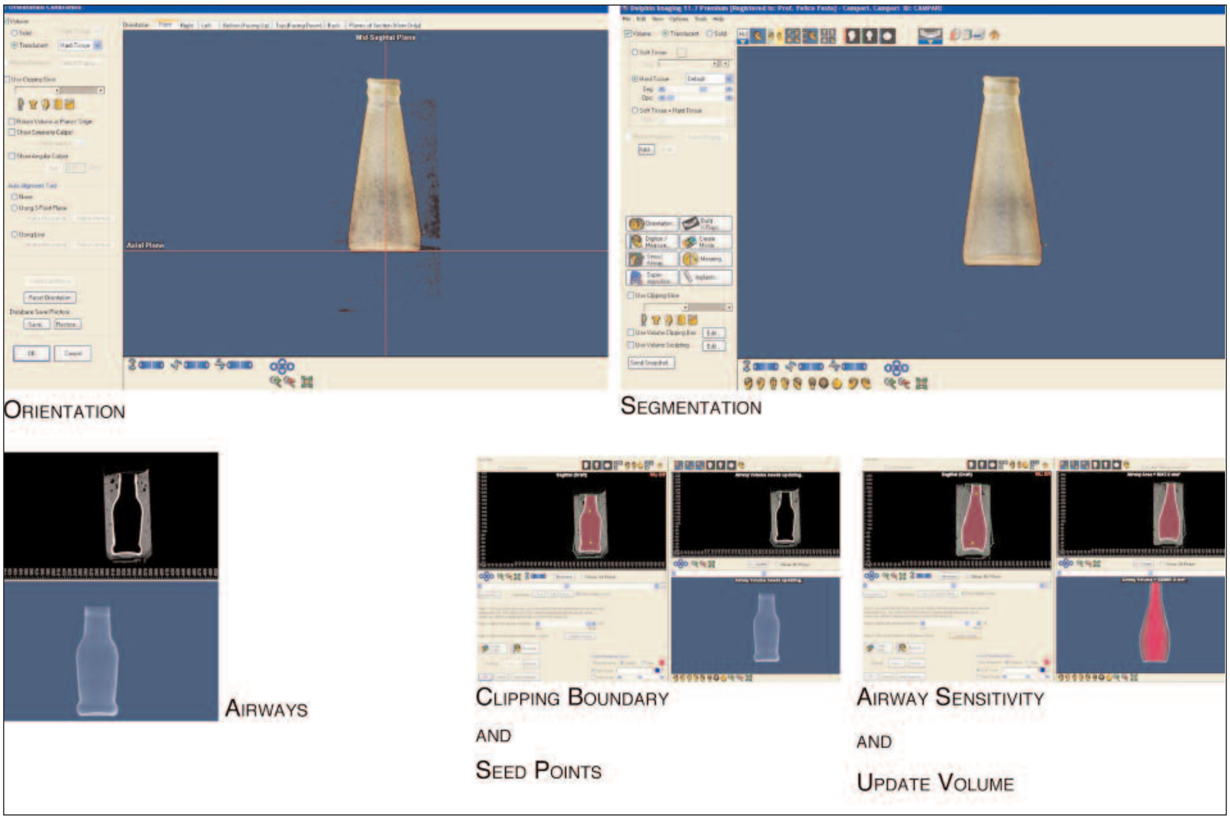

Figure 2. Segmentation procedure.

the single operators. The error of the method of the maxillary sinuses analysis was then calculated. One operator performed 30 double measurements with an interval period of 30 days. Finally, all measurements on maxillary sinus volumes were assessed (see Figure 3).

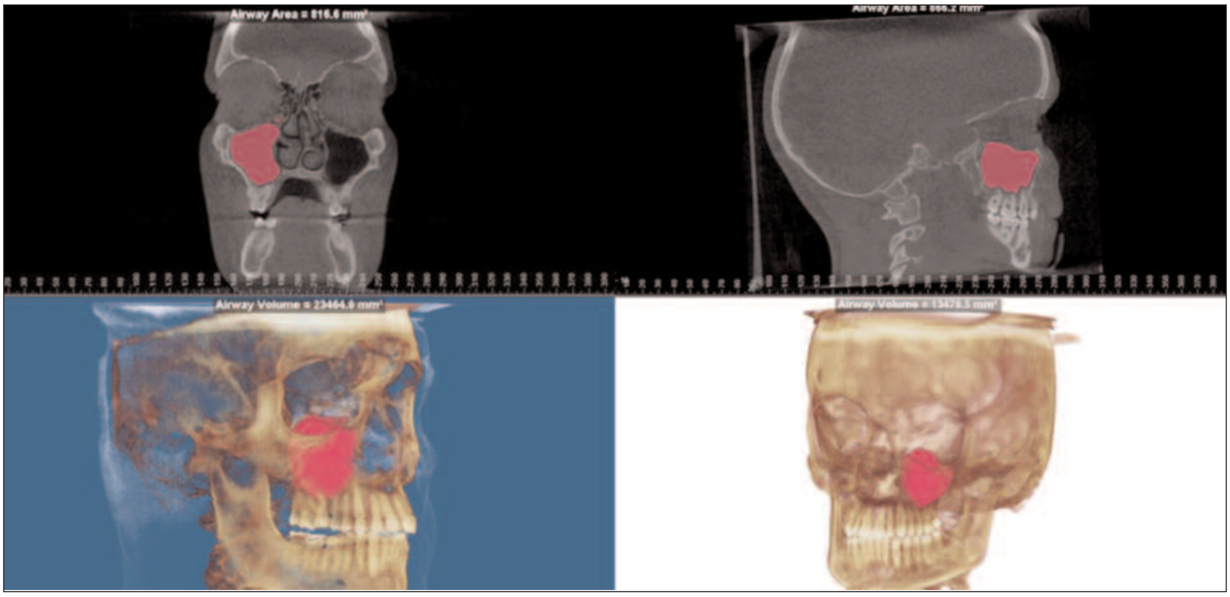

Figure 3. Airway area and volume.

Data Analysis

Data were analyzed on a personal computer using Excel 2000 (Microsoft Corporation, Redmond, WA, USA) and SPSS 14.0 (SPSS Inc., Chicago, IL, USA) on a Microsoft Windows XP platform.

To assess the repeatability of multiple measurements for each Operator, and to compare the measurements among Operators, RM ANOVA was applied[16].

The Correlation Coefficient (Pearson Product-Moment Correlation Coefficient, r) was calculated to describe the strength of the association between the real and Operator measurements. Each pair of data were plotted and a line of equality was plotted. Additionally, the method described by Bland and Altman was applied to assess the agreement between target measurements and those of each Operator[16]. The most straightforward measure of disagreement between the two observations is simply the difference between the real measurements and those of each Operator; the mean difference (d) is a measure of the bias and the standard deviation (s) is a measure of the variation between the two observations. The difference against the mean between the target and Operator measurements was plotted.

To analyze the error of the method, a non-parametric Kolmogorov-Smirnov test (KS test) and a parametric test (*t*-test) were performed. Before running the *t*-test, the normality hypothesis and the equal variance hypothesis were tested. The Student's *t* test was applied to compare right and left sinuses in the skeletal classes' groups.

RM ANOVA was applied, independently from the skeletal groups division, to compare between them: (1) all the sinuses' volumes, (2) all the right sinus volumes, (3) all the left sinuses volumes. A Mann-Whitney Sum rank test was applied to investigate the differences between male and female sinus volumes.

Table I. RM Anova results: comparisons among multiple measurements for each Operator and among Operators.

Real measurements	$p = 0.2$
Operator 1 measurements	$p = 0.3$
Operator 2 measurements	$p = 0.2$
Operator 3 measurements	$p = 0.8$
Operator 4 measurements	$p = 0.08$
Operators 1, 2, 3, 4	$p = 0.1$

Statistical significance ($p = 0.05$) of comparisons among multiple measurements for each Operator and among Operators (RM Anova).

Results

The RM ANOVA results demonstrated the repeatability of the measurement for each Operator, confirming that the variability among observations isn't statistically significant. No statistically significant differences were observed among Operators, confirming the reliability of the method, independently from the Operator (Table I).

The Correlation Coefficient (Pearson Product-Moment Correlation Coefficient, r) demonstrated a strength association between real measurement and that of each Operator (r = 1). The plot of the difference against the mean between the target measurements and the measurements of each Operator is shown in Figures 4 to 7.

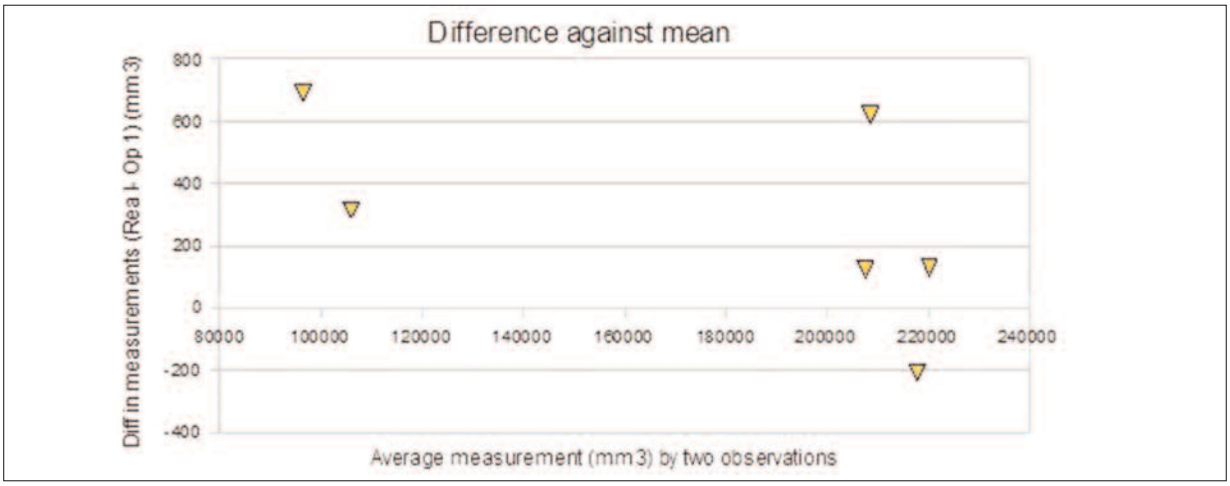

Figure 4. Plot of the difference against the mean between the target measurements and the measurements of Operator 1.

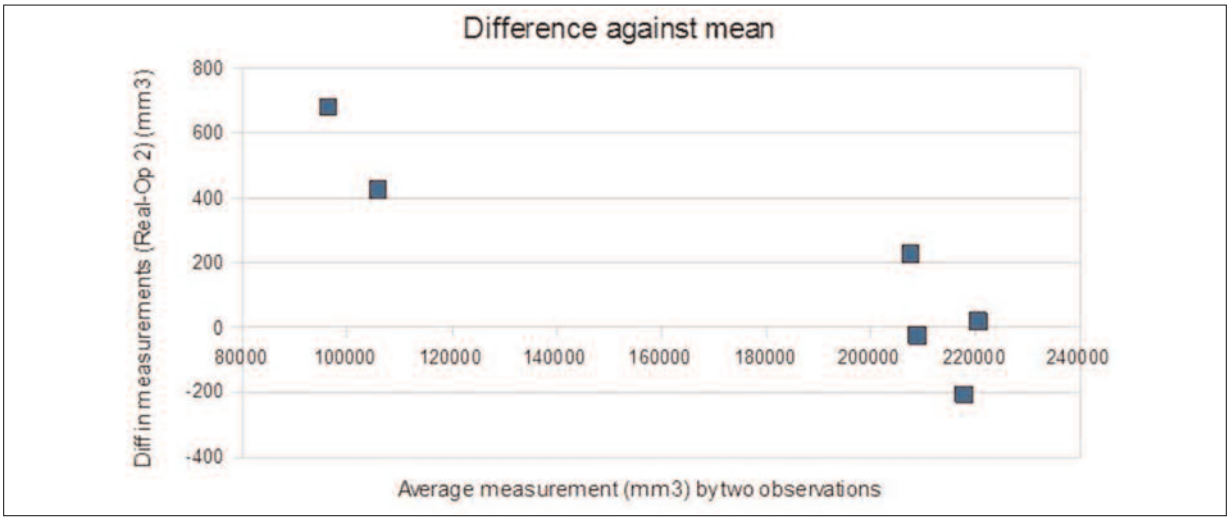

Figure 5. Plot of the difference against the mean between the target measurements and the measurements of Operator 2.

The mean difference (d) between the real observations and Operator 1 data, 277.56 mm3, and the standard deviation (s), 338.39 mm^3, are small compared with the values of volume observed (which range up to 220332.75 mm^3). The agreements limits are –399.22 mm^3 and 954.34 mm^3. As shown in Figure 4, there is an agreement with a discrepancy only up to 691.31 mm^3.

The Correlation Coefficient between the real measurement and that of Operator 1 (r = 1) confirms a direct relationship between variables.

The mean difference (d) between the real observations and Operator 2 data, –193.38 mm^3, and the standard deviation (s), 344.67 mm^3, are small compared with the values of volume observed (which range up to 220332.75 mm^3). The agreements limits are –495.21 mm^3 and 882.72 mm^3. As shown in Figure 5, there is an agreement with discrepancies only up to 678.5 mm^3.

The Correlation Coefficient between the real measurement and that of Operator 2 (r = 1) confirms a direct relationship between variables.

The mean difference (d) between the real observations and Operator 3 data, 163.81 mm3 and standard deviation (s), 261.51 mm^3, are relatively small compared with the values of volumes observed (which range up to 220332.75 mm^3). The agreements limits are –359.21 mm^3 and 686.83 mm^3. As shown in Figure 6, there is agreement with discrepancies only up to 586.75 mm^3. The

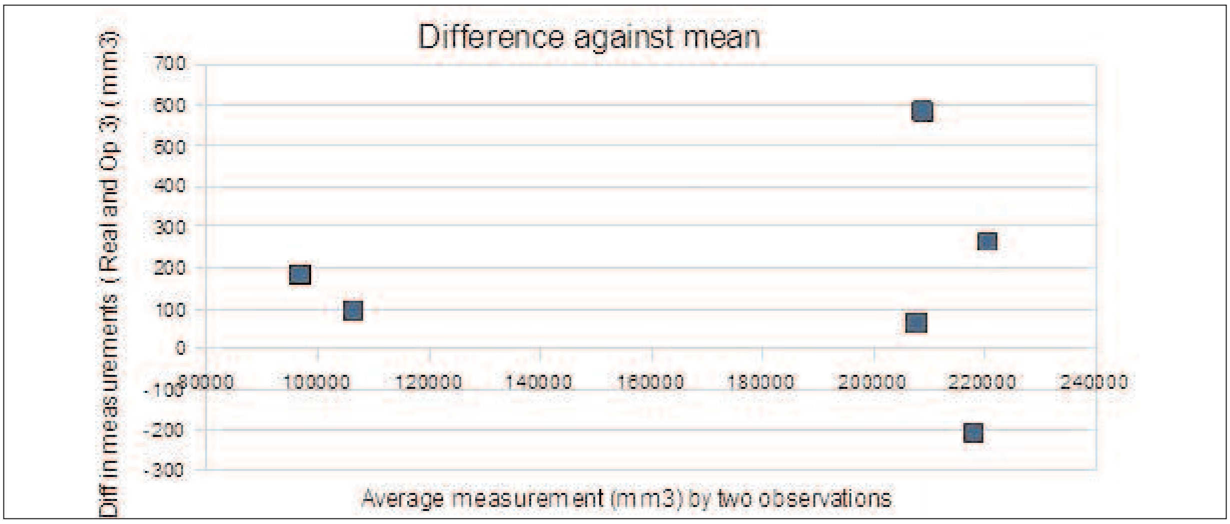

Figure 6. Plot of the difference against the mean between the target measurements and the measurements of Operator 3.

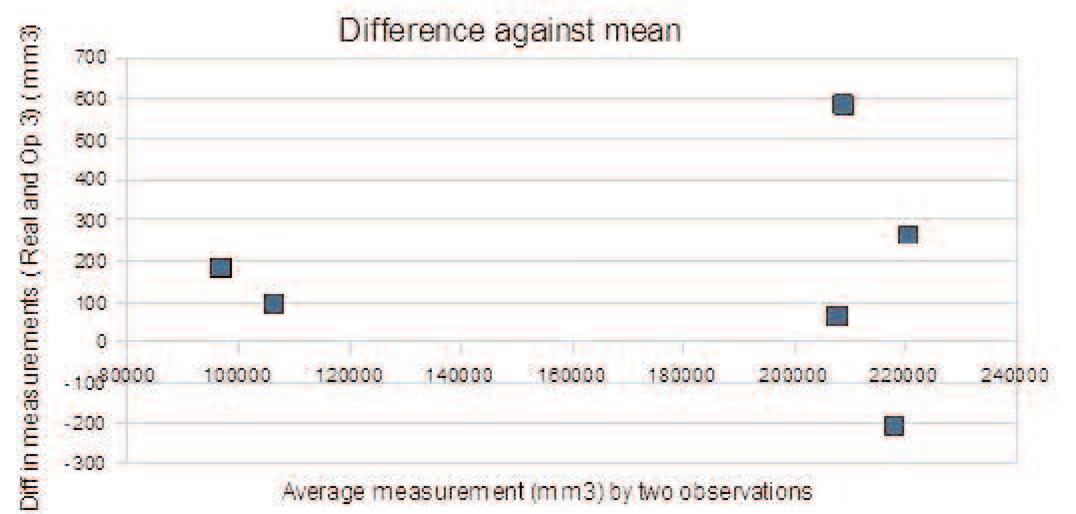

Figure 7. Plot of the difference against the mean between the target measurements and the measurements of Operator 4.

Correlation Coefficient between the real measurement and that of Operator 3 (r = 1) confirm that a direct relationship between variables.

The mean difference (d) between the real observations and the Operator 4 data, 108.04 mm3 and the standard deviation (s), 224.44 mm^3, are small compared with the values of volume observed (which range up to 220332.75 mm^3). The agreements limits are –340.84 mm^3 and 552.92 mm^3. As shown in Figure 7, there is a discrete agreement with discrepancies only up to 474 mm^3.

The Correlation Coefficient between the real measurement and that of Operator 4 (r = 1) confirm a direct relationship between variables.

The KS and *t*-tests show that the differences between the two sets of measurements are not statistically significant. According to the KS test, the two sets of measures follow the same distribution. According to the Shapiro-Wilk test the two sets of measurements can be considered as normally distributed. According to the F test, the hypothesis of equal variance in the two sets of measurements is accepted, so in the following *t*-test the Welch approximation to the degrees of freedom is not necessary. According to the *t*-test on paired samples, the null hypothesis that the two sets of measures have the same mean is accepted (Table II). Comparing right and left sinus volumes for each Skeletal Class, no statistical differences were observed, with $p = 0.3$ (First Class), $p = 0.7$ (Second Class), and $p = 0.8$ (Third Class). Comparing all sinuses' volumes without any subdivision for Skeletal Class, no statistical difference was observed ($p = 0.2$). Similarly, no statistical difference was observed when right and left sinus volumes were taken into account (Table III). Comparing the volumes of all male and female subjects, without any subdivision for Skeletal Class, no statistical difference was observed ($p = 0.1$).

Discussion

The first stage of the current study assessed repeatability and accuracy of 3D measurements delivered by Dolphin software. The RM ANOVA

Table II. Difference test on two sets of measures of sinus volumes.

Error of the method	SW	KS	F test	*t*-test
Volume I	W = 0.9594 *p* value = 0.2188	D = 0.0857 *p* value = 0.997	F = 1.0385 *p* value = 0.9129	*t* = -0.2709 *p* value = 0.7881
Volume II	W = 0.9608 *p* value = 0.2418			

SW Shapiro-Wilk test on normality; (KS) Kolmogorov-Smirnov test; (F test) F test to compare two variances; (T test) T test on equal means.

Table III. *t*-test between right and left sinuses volumes (mm^3) for each Skeletal Class; ANOVA results for maxillary sinuses without any subdivision for skeletal Class.

	I Class right vs left	II Class right vs left	III Class right vs left
t test $p = 0.05$	0.3	0.7	0.8
	I-II-III Class	**I-II-III Class right**	**I-II-III Class left**
ANOVA $p = 0.05$	0.2	0.3	0.1

results (Table I) demonstrate the repeatability of the measurements for each operator, confirming that the variability of the measurements between the various observations is not statistically significant. In addition, no statistically significant difference was observed between the data of four operators, confirming the reliability of the measurement method, independently of the observer (Table I). The correlation coefficient (r) showed a strong association between the real and observed measurements of each operator (r = 1), demonstrating the reliability of the software to identify and measure the volume of different phantoms. For the second part of the study, the reliability of the operator who performed the measurements was tested, assessing the correlation between two different measurements performed at an interval of 30 days. The statistical analysis showed the existence of a strong correlation between the first and second set of measurements. Regarding the correlation between the volumes of 104 maxillary sinus and the three skeletal classes, the *t*-test (Table III) indicated a lack of correlation between right and left maxillary sinus size, in each skeletal class, taken individually. The ANOVA test (Table III) shows:

1. The absence of a default size of the maxillary sinuses, in different skeletal classes, and inside each of them, and
2. The absence of correlation between the volumetric dimensions of the paranasal maxillary spaces and the three different skeletal types.

The "Sinus/Airway" volume measurement tool was then proved to be a valid and reliable instrument in the measurement of the upper airway. The setting of the sensitivity (slice airway sensitivity), the identification and design of boundaries of the sample examined (clipping boundary) are fundamental to allow a reliable measurement (with a relative error < 0.4%). According to Alves et al[15], there is no established protocol for the threshold that should be used when airway volume is measured with Dolphin 3D software. Although manual thresholding is more time consuming and might generate errors if not correctly applied, it has been shown to be more reproducible when compared with the automatic technique. Indeed, El Palomo[17], making a comparison between three automatic procedures and a manual segmentation technique, stated that the latter was the method with the greatest accuracy and allows the greatest operator control. In attempting to establish a standardized method for using the maxillary sinus, our results show no difference in maxillary sinus volumes between male and female subjects. These data reject the hypothesis that maxillary sinus morphology is crucial to determine gender. Conversely, Uthman et al[8] concluded that reconstructed CT images can be used for sexing. Amin et al[18] detected in a CT study that size of the left maxillary sinus are a useful feature in gender determination although only related to Egyptians population. These previous studies focused upon taking linear measurements of the sinus using a 2D analysis, but were considered suitable for 3D characterization. The reproducibility of linear measurements is questionable when a convex shape is chosen to point out a landmark used for measurements[19,20]. On the contrary, volumetric analysis of maxillary sinuses is independent from bias regarding point identification. Our study is conducted using CBCT datasets. Currently, the technical features of CBCT scans as well as the reduced cost compared with CT scans increased their use in hospitals and dentistry. This will enhance the availability of CBCT scans in cases requiring personal identification. The independence of maxillary sinuses sizes between male and female subjects has been elucidated by the current study, which seems to refute the possibility of performing gender identification using maxillary sinuses as suggested in previous research. Besides, taking into account different sagittal skeletal patterns as Angle's classification the independence between maxillary sinus dimensions is unchanged. This shows how the maxilla and jaw positions are independent with respect to the maxillary sinus size.

Conclusions

In conclusion, forensic investigations would benefit from gaining more precise indications via the use of digital radiology, and in particular CBCT, exams and modern software for reconstructing 3D virtual models. The ability to perform 3D measurements appears to be much more accurate compared with linear measurements, and reduces bias on intra-observer reproducibility due to higher accuracy of the image investigated. This approach appears to be a promising direction for further research on all possible anatomical characteristics that may differentiate individual groups, which may prove useful for forensic investigation on human identification.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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