Characterization of Tympanic Cavity Volume in Newborns using Computerized Tomography Scanning

Caracterización del Volumen de la Cavidad Timpánica en Recién Nacidos usando Tomografía Computadorizada

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SUMMARY: This study reports tympanic cavity (TC) volume in newborns, which was missing in the literature. *Ex vivo* histology and computerized tomography (CT) scans were performed on temporal bone and data were analyzed in part using software developed in house. CT images with a slice thickness of 0.5 mm were obtained from 5 newborn cadavers and analyzed independently by two expert researchers. The border of the TC was delineated manually and measurement of area of interest was calculated on masked images. Then, the area measurements from all sections were added to estimate the total volume. The agreements between the histological and CT findings were then compared for accuracy, repeatability and reliability. The Dice and Jaccard similarity coefficient measures were used as a statistical validation metric to evaluate the assessor's performance in manual volume segmentation. Good assessor agreement was observed with average Dice values above 0.8 indicating that consistent and reliable volume measurements were feasible. The proposed protocol was shown to be accurate in calculating the TC volume, and thus can be used for computer-assisted presurgical planning or for diagnosing structural alterations in TC.

KEY WORDS: Computerized tomography; Tympanic cavity; Tympanic cavity volume; Similarity index.

INTRODUCTION

Tympanic cavity (TC) is an important but highly complicated anatomical structure within the temporal bone of the middle ear. It includes proper and epitympanic recess. The former component is located opposite to tympanic membrane and the later one is positioned above it and covers the upper half of malleus and the greater part of incus (Standring, 2008). Typically, TC volume does not vary between left and right sides or with gender, but increases with age (Kavakli *et al.*, 2004; Kürkçüoglu *et al.*, 2010; Ikui *et al.*, 2000).

Diseases related to middle ear are highly prevalent in pediatric population. An anatomical abnormality, such as perforation of tympanic membrane can cause hearing loss in children. Middle ear volume is interesting because it correlates with otitis. Also, hearing loss is increased by smaller volume caused by a drum perforation or a problem with the ossicular transmission (Mehta *et al.*, 2006). In clinics, visualization of TC and measurement of its volume are used as critical diagnostic markers for assessing the origin of the loss. In addition, such information also aids treatment planning or surgical intervention of such cases. So far, however, there are no reports documenting the TC volume or its expected normative size in newborns. Therefore, this study was initiated to fill this gap relevant to the pediatric anatomy in literature.

In the first part of the study, histological analysis was performed on specimens extracted from 5 pediatric cadavers, and provided a gold standard for the TC volume in newborns.

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Evaluation of temporal bone in clinically relevant fashion in patients would require radiological imaging. Computerized tomography (CT) and magnetic resonance imaging (MRI) are the two common techniques used for reference imaging of temporal bone or its related structures. CT currently offers images with higher resolution than MRI, but its use in pediatric population was avoided since the operation requires ionizing radiation. MRI on the other hand requires patient stability during the scan to eliminate image motion artifacts. For imaging cadaver specimens, CT was prefered over MRI since the latter requires the tissue to be wet.

Based on these considerations, in the second part of the study, CT scans were performed on the cadavers and the TC volumes were measured and compared against the benchmark histological findings. Multiple observers performed the measurements using an image processing software developed in house. The accuracy and reproducibility of the results were tested by characterizing and comparing the inter and intra observer performances. The reliability of the results were evaluated with the help of Dice and Jaccard similarity indices (Somasundaram & Kalaiselvi, 2010, 2011; Kundel & Polansky, 2003).

MATERIAL AND METHOD

The study was performed on 5 newborn cadavers after an approval by the Local Ethics Committee of Erciyes University, Kayseri, Turkey. The cadavers were fixed by carotid artery perfusion and preserved in a 10 % formalin solution for 6 months.

Anthropometric measurements. The body height (cm) and body weight (g) of each newborn cadaver were measured by a trained examiner by following the standardized protocols. Body height was measured to the nearest 0.1 cm with an Iowa Height Board and body weight to the nearest 0.1 kg by using a balance beam metric scale. In addition, head length (HL), head width (HW), head height (HT) and head circumference (HC) were measured using a caliper, as described earlier (Acer *et al*., 2007). Maximum HL (glabella-inion length: HL), maximum head breadth (between right and left eurions: HW), and maximum head height (between the external acoustic meatus and the highest point of the vertex: HH) were recorded. The head circumference (HC) was obtained by placing a flexible measuring tape on the occipital protuberance behind and supraorbital ridge in front (Akhter *et al*., 2012).

CT Scans. The cadavers were placed in a supine position and then went through head scan using a CT scanner

(Toshiba Aquilion One, Medical Systems). Images were acquired sequentially in axial planes parallel to the orbitomeatal line. Multi-slice acquisition and reconstruction parameters were FOV of 18 cm, rotation time of 1 sec, section thickness of 0.5 mm and reconstructed image matrix of 512 x 512, pixel size of 0.12 mm x 0.12 mm while kVP was 120 and mA was 200. The images were stored in Dicom format. Figure 1 shows TC using one of the CT data sets after volume rendering in 3D.

Histology. After the CT scans, temporal bones were dissected from the cadavers and decalcified in 5 % nitric acid in formaldehyde. Each sample was then paraffin embedded by preserving its anatomical orientation same as in cadaver for enabling one-to-one comparison of the final histology sections with the corresponding CT images in axial direction. The blocks were subjected to 20 µm thick axial sectioning at 0.2 mm intervals using a rotary microtome. The resulting slides were stained with hematoxylin-eosin (HE) and examined under a stereomicroscope (Carl Zeiss, Germany) with magnification at 5x. In the presence of a reference ruler, the slides were digitally photographed with high resolution camera (3 mega pixels) and saved in tiff format.
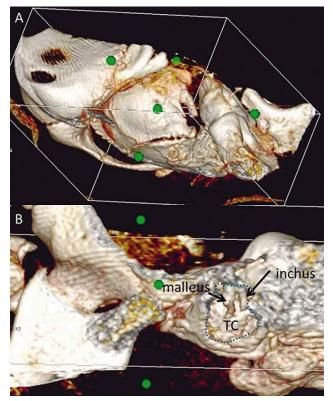

Fig. 1. Visualization of TC with 3D volume rendering of CT images. Top: Slightly superior view of the lateral skull base and Bottom: Lateral view of ossicular chain. Dotted line denotes the boundary of TC.

TC volume estimation. The software ImageJ (http://www.rsb.info.nih.gov/ij/download) was installed in a PC and used for estimating the TC volumes from both the histology and CT data. Region of interests (ROI) covering the inner lining of the TC were manually traced on images as shown in Fig. 2. The total volume of TC was calculated from the selected areas in the consecutive images using the formula:

$$V_{TC} = txPx \sum_{i=1}^{N} A_i$$

Here, the coefficient t represents slice thickness in mm (t= 0.22 mm for the histology data and 0.5 mm for the CT data), P denotes pixel area in mm^2 and Ai represents the number of pixels in the selected region of image i out of total N.

The TC tissue was hard and thus did not shrink or deform during the histological preparation or sectioning. The spatial resolution of the histology images was significantly higher than the CT images. Therefore, the errors made in estimating the volumes using histology data were substantially lower and the volume measurements would be closer to the real values. Thus, the histology based volume estimates were considered to be ground truth reference for benchmark comparison of the CT based estimates.

Accuracy, repeatabilty and reliability of the TC volume measurements. Accomplishing accuracy, repeatability and reliability analysis involved two anatomists, participating as assessors measuring the TC volumes. Each assessor independently and blindly outlined the TC on the CT images. The selected ROIs on the CT images were converted into binary masks and saved in bitmap format. Figure 3 presents the steps involved in masking out TC. The spatial domain of
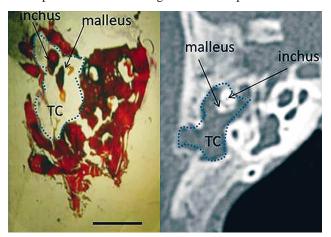

Fig. 2. Representative slides showing TC on histology (H&E stained) and CT slices. Bar represents 500 µm of length. The closed dottet lines denote the manually selected boundaries of TC, from which the area on the images and subsequently the volume of TC were estimated from the consecutive images collectively by taking the slice thickness into account.

the masks collectively represented the total volume of TC in 3D. Next, the TC volume was calculated according to formula described above. Assessors repeated the measurements 3 days later. The results were tabulated for statistical analysis, as discussed below.

The accuracy of the TC volume measurements was determined by statistically comparing the volume readings against the histology-based measurements. The repeatabilty was determined by statistically evaluating the intra and inter assessor variabilities in the volume readings. Accurate and repeatable results necessitate consistency in the selected volumes and its limits are ultimately affected by the degree of agreement or disagreement between the volumes segmented out by the assessors. To explore this further, additional numerical analysis was performed to characterize the similarity of the selected volumes by the assessors, as a predictor of reliability. In the literature, several measures were reported for quantitatively comparing the structural similarities between the two data sets (Somasundaram & Kalaiselvi, 2011). In this study, we employed Dice and Jaccard similarity indices (Dice, 1945; Jaccard, 1912), as they had the capacity to define the quality of spatial overlap between the two volumes. Given two volumetric data sets A and B, the Dice and Jaccard Indices were calculated by

$$Dice = 2 \frac{|A \cap B|}{|A| + |B|}$$

$$Jaccard = \frac{|A \cap B|}{|A \cup B|}$$

Here $\cap$ denotes intersection and $\cup$ denotes union of the volumes and $|\ |$ denotes the volumetric size of the argument. According to the equations above, the indices range between 0 and 1, where 0 represents no spatial match and 1 represents an excellent consistency or overlap between the selected two volumes. The calculations of the indices were carried out using a software developed in house under Matlab platform (version 2012a, MathWorks Inc., Natick, MA, USA).

Statistical Analysis. Overall, there were 10 TCs extracted from 5 cadavers. Thus, histology-based volume data included 10 measurements. Since, two assessors performed the CT-based volume measurements twice, the resulting data were separated into 4 groups, each with 10 measurements. The accuracy and repeatability of the CT-based volume measurements were characterized by calculating inter-class correlation coefficient between the histology-based volume data and the CT-based data in all 4 groups together. The reliability of the results was measured with Dice and Jaccard similarity indices as statistical validation metrics and involved comparing the performances of the assessors in their repeatitive or cross manual segmentations of the TC volume.
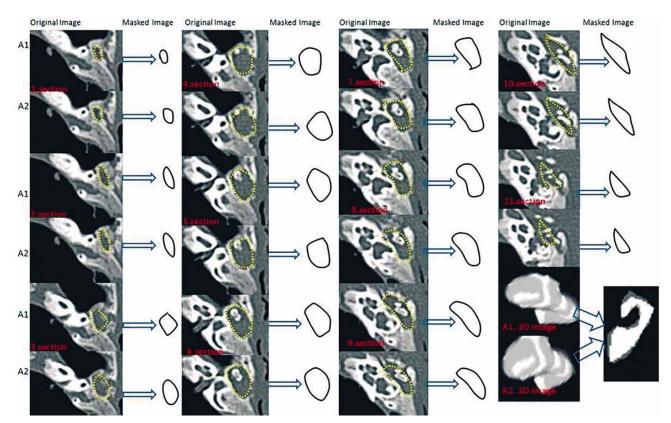
Fig. 3. Image processing steps employed for the analysis of inter and intra assessor performances using Dice and Jaccard similarity measures. A1:Assessor 1, A2: Assessor 2.

RESULTS

The physical parameters measured from the cadaver head specimens were described in Table I. The measurements of TC volumes based on the analysis of histology and CT data were listed in Table II. Spearman's correlation coefficient between the histology-based TC volumes and HC, HL, and HC values were positive and significant (>0.9). TC volumes measured from the right and left sides were not significantly different statistically (P > 0.001).

The TC volume assessed from the CT data second time around by both assessors appeared to be closer to the real values on the average (Fig. 4). This trend suggested assessor's ability in correctly identifying and segmenting out the TC improves with repetition or training on the same subject. However, intra-class correlation analysis performed to test the accuracy of the measurements yielded mixed results (Data in accuracy column of Table III). With repetition, the level of correlation increased for the Assessor 1, but decreased for the Assessor 2.

Table I. The anthropometric parameters of the newborns; body weights, heights and head dimensions.

	Minimum	Maximum	Mean	SD
Body weight (g)	1100	3400	2220	1056.88
Body height (cm)	37	57.8	46.68	8.78
Head circumference (cm, HC)	27	34	31	3.17
Head width (cm, HW)	6.9	8.9	7.78	0.87
Head height (cm, HH)	5.4	10.5	7.78	1.86
Head length (cm, HL)	8.9	13.6	11.22	1.75

Table II. Measurements of TC Volumes based on the histology and CT data. CT based measurements were performed independently and blindly by two assessors (A1 and A2) at 2 different days (M1 and M2) of 3 days apart.

Histology (mm^3)	CT			
	Assessor 1 (A1)		Assessor 2 (A2)	
	Measurement 1 (M1)	Measurement 2 (M2)	Measurement 1 (M1)	Measurement 2 (M2)
380	416	404	347	310
340	440	369	382	355
185	273	177	178	151
155	156	183	149	160
230	302	274	238	255
256	333	268	289	256
228	260	280	216	226
214	254	255	235	222
311	285	283	319	300
319	367	372	342	351

Table III. Observer performances as evaluated by intra-class correlation coefficients computed between the histology and CT based TC volume measurements.

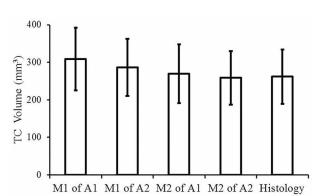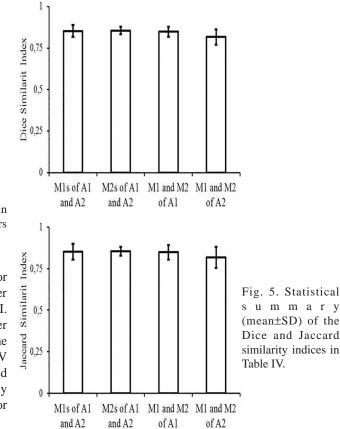|	Accuracy	Repeatability			
	Assessor Performance	Intra-assessor Performance		Inter-assessor Performance	
	Histology	M2 of A1	M2 of A2	M1 of A2	M2 of A2
M1 of A1	0.884	0.901	---	0.868	0.835
M1 of A2	0.939	---	0.923	---	---
M2 of A1	0.957	---	---	0.906	0.966
M2 of A2	0.914	---	---	---	---

Fig. 4. Statistical summary (mean±SD) of the measurements in Table I. The TC volumes assessed second time by both assessors appeared to be closer to the real value on the average.

But, in both times the two measurements were consistent for both assessors, as indicated by the high correlation values under the intra and inter assessor performance columns of Table III. These findings indicated high repeatability and were further supported by the results of the reliability analysis based on the Dice and Jaccard metrics, which were presented in Table IV and Fig. 5. These data suggested that the TC volumes selected by the two assessors at two different time points were highly coincided and thus stable. Therefore, manual segmentation for measuring the TC volume from the CT data was reliable.

Fig. 5. Statistical summary (mean±SD) of the Dice and Jaccard similarity indices in Table IV.

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Table IV. Results of Dice and Jaccard similarity indices computed from the volumes segmented manually by two assessors on 2 different days.

M1s of A1 and A2		M2s of A1 and A2		M1 and M2 of A1		M1 and M2 of A1	
Dice	**Jaccard**	**Dice**	**Jaccard**	**Dice**	**Jaccard**	**Dice**	**Jaccard**
0.86	0.75	0.88	0.77	0.84	0.72	0.86	0.75
0.87	0.78	0.87	0.74	0.83	0.71	0.74	0.59
0.88	0.78	0.88	0.77	0.87	0.77	0.86	0.75
0.77	0.63	0.85	0.74	0.89	0.8	0.74	0.59
0.87	0.77	0.85	0.73	0.87	0.77	0.84	0.72
0.86	0.76	0.83	0.71	0.82	0.7	0.81	0.68
0.83	0.73	0.86	0.74	0.82	0.7	0.83	0.72
0.89	0.76	0.81	0.7	0.87	0.76	0.81	0.7
0.87	0.78	0.87	0.77	0.87	0.78	0.87	0.77
0.82	0.7	0.85	0.77	0.8	0.67	0.81	0.68

DISCUSSION

In the literature, traditional serial histological sectioning has often been used for volumetric sizing, including middle and inner ear (Decraemer *et al.*, 2002; Rau *et al.*, 2013; Wang *et al.*, 2006; Sun *et al.*, 2002). This approach provides the greatest detail but is very time consuming. Being an *ex-vivo* procedure also makes it not applicable for *in vivo* assessment of TC, as required in clinics. Nevertheless, we included histology-based measurement of TC volume as a reference comparison. Errors in this approach originated from shrinkage, tearing and folding of the tissue sections (Sun *et al.*). These effects were minimized by performing the histology preparation and analysis of the samples right after the CT scans. In this study, we followed this approach and visual evaluation indicated that the shrinkage of temporal bone was negligible in the analyzed samples. However, accroding to the data in Table II, reference measurements appeared to be slightly lower, which may be due to the variations in clearly delinating especially the lower and upper borders of the TC as well as the measurement volume being small.

In vivo examination of temporal bone is possible with the current imaging equipment used in clinical radiology. Within all, multi slice CT scanner provides the best quality and high resolution images, necessary for visualizing and differenting bony structures including TC (Rau *et al.*). Given its relatively small size, we opted imaging TC with CT in newborn cadavers. CT–based volumetric measurement depends on the complexity of anatomy and quality of the scans, as well as performance of the assessor. The acquired data however allowed measuring the TC size accurately and repetitively (Table II), which indicated that CT is a well-suited imaging modality for this purpose. Dice and Jaccard

similarity values in Table IV and plots in Figure 5 implied minimal differences between the ROI markings between the assessors, meaning that the selected traces of the TC boundary were in very close proximity. This provided additional evidence on the validity and applicability of our image processing protocol and selection criterions for segmenting TC. Our findings based on the Dice and Jaccard analysis were in agrement with those of the previous studies who sized other body organs or anatomical structures (Chang *et al.*, 2009; Duda *et al.*, 2001; Lee *et al.*, 2003; La Macchia *et al.*, 2012).

Previously, TC size in adults has been studied and found to range from 500 to 1000 mm^3 (Werner *et al.*, 2011; Whittemore *et al.*, 1998). However, only few studies reported TC size in newborns and no study has investigated the relationsip between the TC volume and head dimensions. In a study with 14 normal humans; 6 infants who were under 1 year of age and 8 adults, Ikui *et al.* reported TC size in adults as 640.1 ± 69.1 mm^3 which was about 1.5 times larger than that of the infants 451.7 ± 68.2 mm^3. In a 22 day old infant, TC volume was reported as 330 mm^3. In the current study, the TC volume in newborns was found to range between 155 and 380 mm^3 when measured with the histological analysis (Table I). In addition, TC size has increased with increase in head circumference. This was in agreement with a previous report which indicated that the TC size increases in proportion to pneumatization in bone surrounding the cavity (Ikui *et al.*).

Ahn *et al.* (2008), found that chronic otitis media causes reduced middle ear volume compared to those contralateral normal ears. A smaller middle-ear cavity

increases the stiffness of the middle-ear at ear-canal just in front of the tympanic membrane, because the membrane motion drives against the volume compliance (Werner *et al*.). Whether it is an anatomical abnormality or postnatal origin, the change in TC size may have clinical implications for surgical and medical treatment planning, but its accurate measurement can be more critical for the prior diagnostics.

One of the limitations of this study was the small sample size. Finding newborn cadavers is a common problem faced in many anatomy departments. Analysis based on the availability of more cadavers would further strengthen the findings presented above. The other limitation was the manual segmentation of TC. Automation would speed up the evaluation process. But this would require using computer-aided artificial intelligence to identify the lower section of the TC axial limit, which often requires expert judgement or involvement because of insufficient tissue contrast to make the required distiction. From clinical perspective, our image acquisition protocol may not be appropriate since CT scans on children are avoided to limit the radiation exposure.

An interesting future project would be to study the potential of the method to automatically and objectively discriminate the black and white areas within the identified cochlea region using contrast differences in CT images. Taking into account these limitations, this is the first newborn postmortem study to provide quantitative evidence. We will continue our research on more accurate and efficient to calculate other anatomical structures. This work demonstrates that the presented method fills the gap between the basic biological research relying on postmortem histologic material and clinical applications relying on *in-vivo* CT-based image analysis. The measurement of TC volume in newborn cadavers is feasible with high resolution CT data. Volume computations from simple slice-by-slice manual segmentation of the TC boundary provide accurate, reproducible and reliable results provided the assessors are expert anatomists or the selection criteria is well defined prior to starting the segmentation process. Correlations exist among the TC volume and head circumference and head length, which may offer value in disease diagnosis and treatment or surgical intervention. The Dice and Jaccard values are simple and useful measures of spatial overlap, which can be applied to studies of accuracy and reproducibility in image comparison. The developed software can potentially be applied to measure other anatomical volumes from radiological images.

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RESUMEN: El objetivo fue determinar el volumen de la cavidad timpánica (CT) en recién nacidos, información no encontrada en la literatura. Se realizaron escaners a través de tomografia computadorizada (TC) y estudios histológicos en el hueso temporal; los datos se analizaron utilizando un software desarrollado en nuestra institución. Se obtuvieron imágenes de secciones de TC, de 0,5 mm de grosor, a partir de 5 cadáveres de recién nacidos, los que fueron analizados de forma independiente por dos investigadores expertos. El margen de los cortes de TC fue delineado manualmente y la medición del área de interés se estimó sobre imágenes ocultas. Después, se añadieron las mediciones de área de todas las secciones para estimar el volumen total. Las concordancias entre el estudio histológico y los hallazgos de la TC se compararon en cuanto a precisión, repetibilidad y confiabilidad. Se utilizaron las medidas de coeficiente de similitud de Jaccard y Dice como métrica de validación estadística para evaluar el desempeño del asesor en la medición manual del volumen. Se observó una buena correlación del evaluador con los valores medios de Dice, por encima de 0,8 indicando que es factible obtener mediciones coherentes y confiables de volumen. El protocolo propuesto ha demostrado ser preciso para calcular el volumen de la CT, y por lo tanto se puede utilizar para la planificación prequirúrgica asistida o para el diagnóstico de alteraciones estructurales en la CT.

PALABRAS CLAVE: Tomografía computadorizada; Cavidad timpánica; Volumen de cavidad timpánica; Índice de similitud.

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