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Original Article

Growth Pattern of the Tympanic Ring in Human Fetuses

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OBJECTIVES: The study aimed to display the growth pattern of the tympanic ring in fetal cadavers of 20-30 weeks of gestation.

METHODS: In the study, 32 temporal bones of 16 fetuses (8 males, 8 females) of 24.62 ± 3.44 weeks of gestation were dissected to measure the height (TRH), width (TRW), and perimeter (TRP) of the annulus, and the width (TNW) of the opening part of the annulus at the level of the tympanic notch.

RESULTS: The TRH, TRW, TRP, and TNW were measured as 8.25 ± 1.04 mm, 7.64 ± 1.08 mm, 24.93 ± 3.40 mm, and 4.01 ± 0.91 mm, respectively. The parameters were similar at the seventh and eighth months of gestation; therefore, there was no variation from the seventh month. Linear function was calculated as $y = 1.328 + 0.281$ x weeks ($P < .001$) for the TRH, $y = 1.284 + 0.258$ x weeks ($P < .001$) for the TRW, $y = 3.367 + 0.876$ x weeks ($P < .001$) for the TRP, and $y = -0.603 + 0.188$ x weeks ($P < .001$) for the TNW.

CONCLUSION: The parameters (TRH, TRW, TRP, and TNW) did not alter from the seventh month in utero. The linear functions (which represent the growth pattern of the parameters) of the tympanic ring may be useful for ear professionals to guess the annulus diameters, and to diagnose growth retardation and probable congenital anomalies in utero during sonographic imaging.

KEYWORDS: Tympanic membrane, tympanic ring, tympanic annulus, fetus, linear function

INTRODUCTION

The tympanic annulus or ring (TR), a fibrocartilaginous thickening around the rim of the major-taut part (the pars tensa) of the tympanic membrane, surrounds the membrane with its horseshoe-like shape. The superior edge of the TR remains open at the tympanic notch (or the notch of Rivinus), where the minor-loose part (the pars flaccida) of the membrane settles. Fitting into the tympanic sulcus, a shallow groove, the TR connects the membrane to the wall of the external auditory canal.[1,2] The tympanic membrane conveys sound waves from the external auditory canal to the ossicular chain (malleus, incus, and stapes).[1] Thus, the TR surrounding the membrane plays an important role in voice transmission.[2] The morphology of the TR, including its diameter and thickness, attracts the attention of otologists, primarily as an anatomical landmark for otoscopic examination. Secondly, it is a prominent landmark in the implementation of transcanal approaches, and thirdly, it is a sign (e.g., aplasia or dysplasia of the TR) for detection of congenital anomalies (congenital aural atresia, microtia, etc.).[2-6] Despite this clinical importance, fetal morphometric studies on the TR, including its diameter, thickness, and perimeter are very limited in the literature.[2,3,7] In this context, we think that further examinations conducted on fetuses are needed for otologists to estimate the TR size.

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One of the 2 main functions of the TR is to be a scaffold for the tympanic membrane to fit into the tympanic sulcus, and the other is to form the bone and cartilage parts of the external auditory canal.[7,8] Therefore, knowledge related to the TR in fetuses is useful for understanding congenital ear anomalies.[3,8] In the 28-mm fetus (8 weeks of gestation), a minor field of mesenchymal condensation represents the TR, which is located just ventral to Meckel's cartilage. With the appearance of a preosseous small nodule (primary ossification center) at 9 weeks, ossification begins in the TR head. At 10 and 11 weeks, the draft of the C-shaped form appears, with the increase of the ossification centers. The ossification process of the TR completes by 19 weeks of gestation, but it continues to expand until 35 weeks of gestation. In the 315-mm fetus (35 weeks), the TR reaches its final size.[7] An error in the process of fetal development can lead to congenital malformations (e.g., congenital cholesteatoma and congenital aural atresia).[3,9] From this perspective, an extended quantitative dataset related to the TR dimensions in normal fetuses may be useful for surgeons to understand congenital anomalies. This study aimed to show the normal growth pattern of the TR in fetuses aged between 20 and 30 weeks.

MATERIAL AND METHODS

The university review board granted ethical approval of this fetal work. The fetal population with no structural abnormalities consisted of 16 subjects, 8 boys and 8 girls, at 24.62 ± 3.44 weeks of gestation (range, 20-30 weeks). After the placing of fetal heads in proper position for otologic surgery, a senior otologist (DÜT) dissected 32 temporal bones in the anatomy laboratory. Following the retroauricular incision, the auricle was reflected forward. Under a surgical microscope (Carl Zeiss f170, Carl Zeiss Meditec AG, Germany), the connections of the TR with the tympanic membrane were cut and the annulus was removed. The TR was placed on a paper that was scaled millimetrically, and photographed with a digital camera (Nikon D3300 digital camera, Nikon, Tokyo, Japan) attached to the microscope at the same position. To perform measurements, the TR photos were transferred to a digital image analysis software (Rasband WS, ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA, https://imagej.nih.gov/ij/, 1997-2018). On the other hand, to estimate the gestational age of the fetuses (weeks or months), the foot length was measured with a digital caliper (0.01 mm precision, Mahr, 16 ER, Göttingen, Germany). The determined parameters related to the TR in fetuses were as follows (Figure 1):

- The TRH: the TR height (the vertical diameter at the longest level)
- The TRW: the TR width (the horizontal diameter at the widest level)
- The TRP: the TR perimeter
- The TNW: the width of the opening part of the TR at the level of the tympanic notch (or the notch of Rivinus)
- The TNW/TRP ratio and the TRW/TRH ratio.

The study was carried out on 10% formalin-fixed fetal cadavers, but possible shrinkage in tissues caused by fixation was underestimated in measurements, owing to the article of Beger et al.[10] (who found that 10% formalin did not shrink the tissues significantly). With 3 repeated measurements, 2 independent researchers checked the intraobserver reproducibility through the repeated measures ANOVA (post-hoc Tukey's test) and interobserver reproducibility through intra-class correlation coefficients (ICC). The normality analysis of the dataset was made with the Shapiro–Wilk test. According to the age in weeks (between 20 and 30 weeks) and months (between fifth and eighth month) of gestation, the change in measurements of the TR parameters was evaluated with one-way ANOVA, along with the Bonferroni test. Student's *t*-tests were used to determine sex difference (the independent samples *t*-test), side difference (the paired samples *t*-test), and the TRH-TRW difference (the paired samples *t*-test). Pearson's correlation coefficient was utilized to determine correlations between the TRH, TRW, TRP, and TNW. The linear functions (regression equations) of these 4 parameters were calculated with simple linear regression. The statistical *P* value was .05.

RESULTS

The analyses of intraobserver reproducibility ($P > .05$) and interobserver reproducibility (ICC score: 0.990-0.998, $P < .001$) displayed that repeatability of measurements was excellent. The findings of this work focused on the fetal TR were as follows:

- The TRH, TRW, TRP, and TNW increased with the growth of the fetuses aged between 20 and 30 weeks ($P < .001$) (Table 1).
- The TNW/TRP ratio ($P = .092$) and the TRW/TRH ratio ($P = .320$) did not change according to gestational weeks (Table 1).
- The measurements of the parameters were not statistically different between boys and girls or between the right and left sides ($P > .05$) (Table 2).

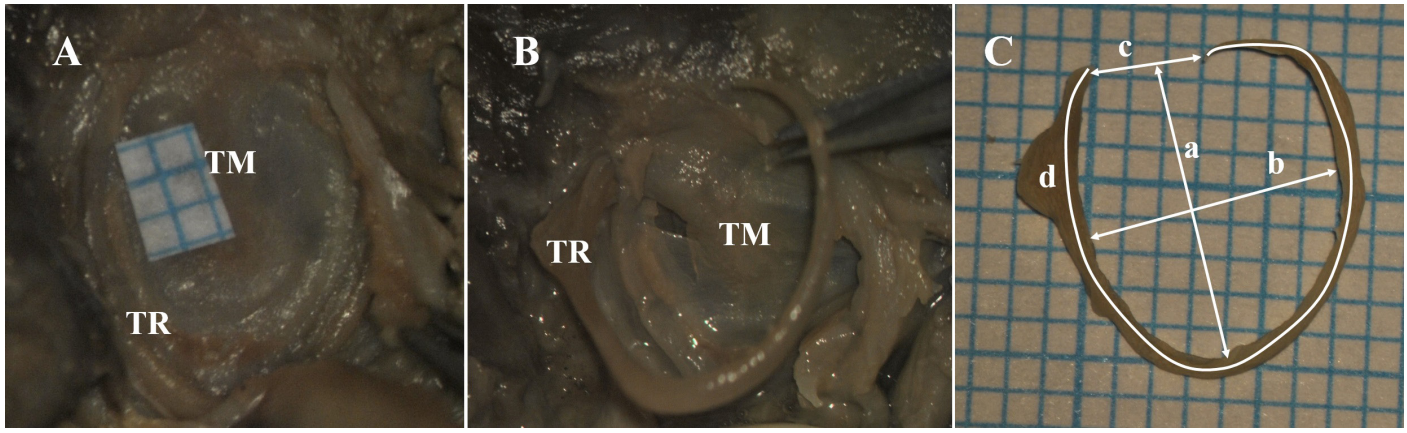

Figure 1. (A) Photograph showing the fetal tympanic ring (TR) and membrane (TM). (B) Photograph showing the removal of the tympanic ring. (C) Photograph showing the parameters. a: the TRH, b: the TRW, c: the TNW, and d: the TRP.

Table 1. The Demographic Data of Fetal Cadavers and Morphometric Data of the Parameters

Months	Weeks	Foot Lengths (mm)	Male (N)	Female (N)	Side (N)	TRH (mm)	TRW (mm)	TRP (mm)	TNW (mm)	TNW/TRP Ratio	TRW/TRH Ratio
Five	20	30.54 ± 0.40	1	1	4	6.81 ± 0.18	5.82 ± 0.54	19.08 ± 0.88	2.52 ± 0.61	0.13 ± 0.02	0.85 ± 0.09
Six	21	32.66 ± 0.86	1	1	4	7.14 ± 0.23	7.28 ± 0.42	21.84 ± 0.75	3.42 ± 0.32	0.15 ± 0.02	1.02 ± 0.08
	22	35.37 ± 0.63	0	2	4	7.52 ± 0.48	7.25 ± 0.33	23.35 ± 1.08	3.47 ± 0.14	0.14 ± 0.01	0.96 ± 0.02
	23	37.08 ± 0.15	1	0	2	8.55 ± 0.27	7.58 ± 0.72	25.45 ± 2.71	3.61 ± 0.39	0.14 ± 0.03	0.88 ± 0.05
	24	39.97 ± 0.84	1	0	2	7.79 ± 1.01	6.72 ± 0.46	25.93 ± 2.39	4.20 ± 0.26	0.16 ± 0.01	0.87 ± 0.17
Seven	25	41.61 ± 0.38	1	0	2	8.34 ± 0.51	7.44 ± 0.39	25.46 ± 0.35	5.28 ± 0.48	0.20 ± 0.02	0.89 ± 0.01
	26	45.61 ± 0.43	1	1	4	8.64 ± 0.49	7.78 ± 0.83	25.09 ± 0.50	4.71 ± 0.52	0.18 ± 0.02	0.90 ± 0.14
	27	48.22 ± 0.15	0	1	2	9.12 ± 0.09	9.20 ± 0.17	29.97 ± 1.27	4.93 ± 1.40	0.16 ± 0.05	1.01 ± 0.03
	28	51.64 ± 0.19	0	1	2	9.13 ± 0.38	8.55 ± 0.49	27.92 ± 0.53	4.58 ± 0.25	0.16 ± 0.01	0.93 ± 0.01
Eight	29	53.50 ± 0.12	1	0	2	9.49 ± 0.12	8.62 ± 0.38	27.60 ± 0.40	4.84 ± 0.24	0.17 ± 0.01	0.90 ± 0.05
	30	53.92 ± 0.60	1	1	4	9.65 ± 0.12	8.93 ± 0.20	28.95 ± 0.43	4.28 ± 0.22	0.14 ± 0.01	0.92 ± 0.03
-	24.62 ± 3.44	41.76 ± 8.53	8	8	32	8.25 ± 1.04	7.64 ± 1.08	24.93 ± 3.40	4.01 ± 0.91	0.16 ± 0.03	0.92 ± 0.08
P						<.001	<.001	<.001	<.001	.092	.32

- The TRH, TRW, TRP, and TNW did not alter from the seventh month in utero ($P > .05$) (Table 3).
- The parameters displayed positive correlations with each other ($P < .001$) (Table 4).
- The TRW was smaller than the TRH ($P < .001$).
- Linear functions for the TRH, TRW, TRP, and TNW were found as y = 1.328 + 0.281 x weeks ($P < .001$), y = 1.284 + 0.258 x weeks ($P < .001$), y = 3.367 + 0.876 x weeks ($P < .001$), and y = −0.603 + 0.188 x weeks ($P < .001$), respectively (Figure 2).

DISCUSSION

The abnormalities (e.g., dysplasia, and aplasia) of the TR may be associated with microtia, Treacher Collins syndrome, congenital aural atresia, Crouzon syndrome, congenital cholesteatoma, Goldenhar syndrome, congenital aural stenosis, and Pierre Robin syndrome.[3,5,6,9,11-13] Some authors have reported that a strong relation was observed between the TR anomalies (e.g., aplasia) and microtia or congenital aural atresia.[3,5,6,14] Due to anomalies or pathologies of the TR, congenital conductive hearing loss may occur in the postnatal period.[14] In this regard, Leibovitz et al.[3] studied 80 healthy fetal specimens aged from 12 to 32 weeks of gestation using ultrasonographic imaging, and suggested that sonographic imaging of the TR might be used to diagnose hearing loss due to its high demonstration rates (80% for 23 weeks and 90% for 16 weeks) in the second trimester. Considering limited papers focused on the TR size (e.g., its diameter),[3,7] we thought that an extended quantitative dataset to enrich the poor information pool related to the annulus in fetal cadavers with no malformations might be beneficial for otologists. The linear functions (which represent the growth pattern of the TR in fetuses aged between 20 and 30 weeks) of this work might be used for estimation of the TR diameters.

In this work, the TRH, TRW, TRP, and TNW were measured as 8.25 ± 1.04 mm, 7.64 ± 1.08 mm, 24.93 ± 3.40 mm, and 4.01 ± 0.91 mm, respectively. As far as we know, the TRP and TNW were measured with this study for the first time in the literature available in English. The TR diameters in the literature are presented in Table 5,[3,7] which shows that the measurements in this study are compatible with previous articles,[3,7] when considering the corresponding weeks. Similar to our work (8.55 ± 0.27 mm for the TRH,

Table 2. Sex and Side Comparisons

Parameters	Male (N = 16)	Female (N = 16)	*P*	Right (N = 16)	Left (N = 16)	*P*
TRH (mm)	8.25 ± 1.01	8.24 ± 1.10	.989	8.31 ± 1.01	8.18 ± 1.11	.749
TRW (mm)	7.62 ± 1.01	7.65 ± 1.19	.941	7.58 ± 1.21	7.69 ± 0.97	.794
TRP (mm)	24.99 ± 3.03	24.87 ± 3.84	.921	25.08 ± 3.58	24.78 ± 3.33	.810
TNW (mm)	4.14 ± 0.78	3.89 ± 1.04	.453	4.13 ± 0.99	3.90 ± 0.84	.498

Table 3. Growth Dynamics of the Tympanic Ring According to Gestational Months

Parameters	Fifth Month (N = 4)	Sixth Month (N = 14)	Seventh Month (N = 8)	Eighth Month (N = 6)	*P*
TRH (mm)	6.81±0.18[a, b, c]	7.71±0.67[b, c]	8.88±0.45	9.60±0.13	<.001
TRW (mm)	5.82±0.54[a, b, c]	7.25±0.45[b, c]	8.32±0.86	8.82±0.28	<.001
TRP (mm)	19.08±0.88[a, b, c]	23.89±2.04[b, c]	27.02±2.28	28.50±0.79	<.001
TNW (mm)	2.52±0.61[a, b, c]	3.84±0.71[b]	4.73±0.65	4.47±0.35	<.001

[a]Comparison to the sixth month, [b]Comparison to the seventh month, [c]Comparison to the eighth month, $P < .05$

Table 4. The Correlations Between the Parameters

Parameters	TNW (mm)	TRP (mm)	TRH (mm)
TRW (mm)	0.646**	0.866**	0.778**
	<0.001	<0.001	<0.001
TNW (mm)		0.708**	0.670**
		<0.001	<0.001
TRP (mm)			0.891**
			<0.001

**$P < .01$

7.58 ± 0.72 mm for the TRW), Leibovitz et al.[3] measured the TRH as 8.3 ± 0.7 mm and the TRW as 7 ± 0.6 mm in fetuses at 23 weeks. In addition, similar to this study (6.81 ± 0.18 mm for 20 weeks, 7.52 ± 0.48 mm for 22 weeks, and 7.79 ± 1.01 mm for 24 weeks), Anson et al.[7] measured the TRH as 7.4 mm at 19 weeks, 7.9 mm at 22 weeks, and 8.2 mm at 24 weeks. Anson et al.[7] reported that the ossification process of the TR completed in the 161-mm fetus at 19 weeks and the annulus continued to expand until 35 weeks of gestation (315-mm fetus). In this study, our findings suggested that the TRH, TRW, TRP, and TNW were similar at the seventh and eighth months; thus, they seemed to reach adult size in the seventh month in utero. This information may be important for clinicians to estimate the TRH and TRW during the diagnosis of congenital hearing loss using sonographic imaging.

Leibovitz et al.[3] stated that the TRH and TRW corresponded to the vertical (height) and horizontal (width) diameters of the tympanic membrane, respectively (Table 5).[15-20] Beger et al.[20] compared the tympanic membrane dimensions of 18 fetuses (24.27 ± 3.24 weeks) and 10 adults (75.70 ± 14.11 years), and found that the membrane diameters did not alter from the seventh month in utero, in accordance with this work (which showed that the parameters of the TR did not change from the seventh month). The literature data focused on the tympanic membrane diameters (Table 5) displayed that the numerical values were different in the studies. In Gray's Anatomy,[1] the classical textbook, the height and width of the membrane have been given as 9-10 mm and 8-9 mm, respectively. However,[21,22] these measurements were reported by some otorhinolaryngologists as 10 mm and 5 mm, respectively. The possible reasons for differences in the diameters of the tympanic membrane or the TR were listed as follows: methodology (e.g., in situ or ex situ measurements), demographic data (e.g., region), and c) technique (e.g., dissection or ultrasonographic imaging).[3,7,15,17] For instance, the height (9.40 ± 1.50 mm) and width (8.60 ± 0.90 mm) of the membrane in the work (in situ measurement in Italian adult specimens) of Salvinelli et al.[17] were larger than those (7.50 ± 0.50 mm for the height, and 7.90 ± 0.80 mm for the width) in the study (ex situ measurement in Japanese adult specimens) of Kirikae.[15] Salvinelli et al.[17] suggested that the difference was due to the measurement technique, not the regional differences. Leibovitz et al.[3] explained that their measurements related to the TR (prenatal ultrasound study) supported previous studies focused on the membrane and annulus (dissection studies).[7,23] However, their measurements (11.8 ± 1.2 mm for the TRH and 9.4 ± 1.2 mm for the TRW)[3] in 32-week-old fetuses were greater than the measurements (9.5 mm in the 35-week-old fetus, 10 mm in the newborn and

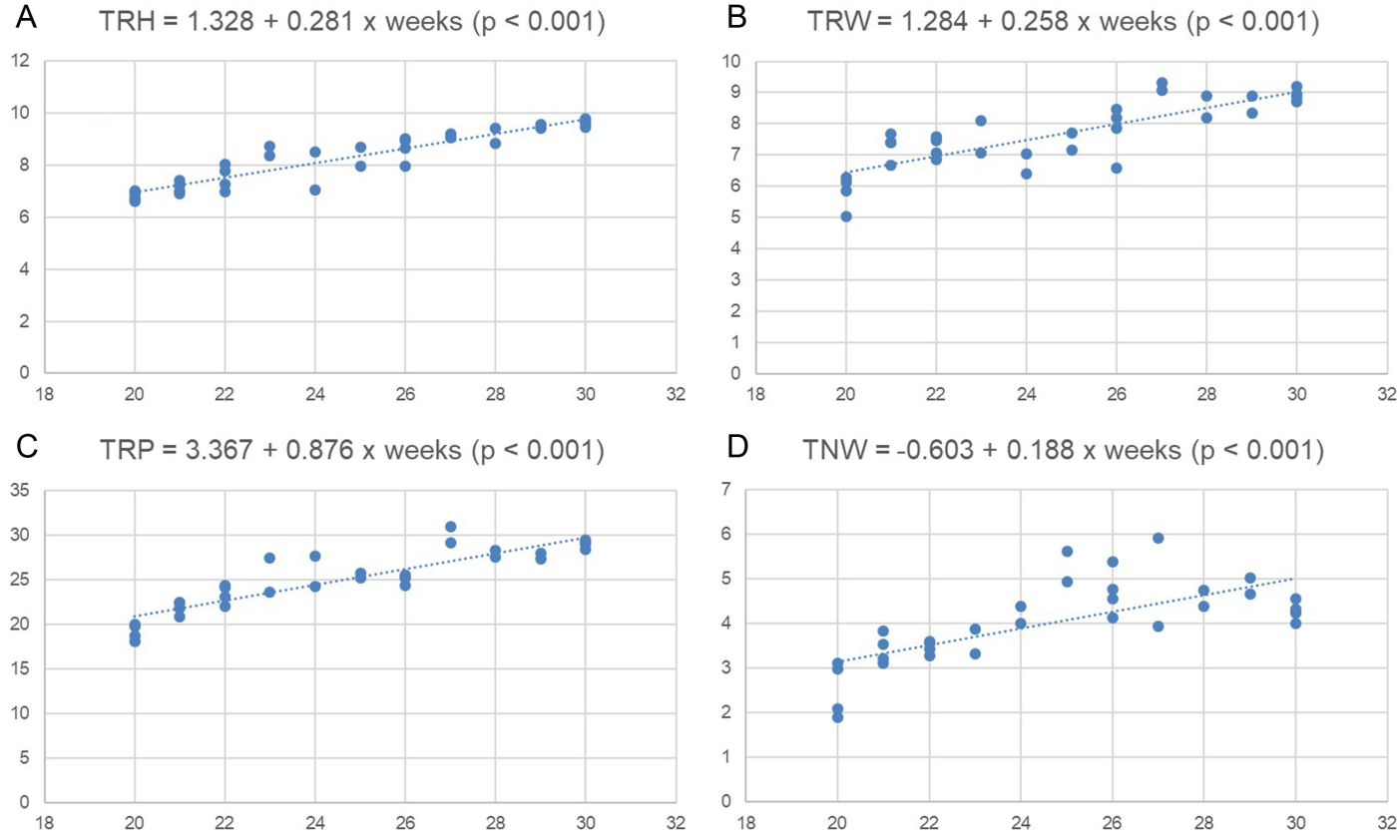

Figure 2. Charts showing the linear functions for the TRH (A), TRW (B), TRP (C), and TNW (D) of the tympanic ring.

Table 5. The Literature Data Related to the Tympanic Ring

Studies	Year	Region	N	Technique	Specimens	Age	TRH (mm)	TRW (mm)	TRP (mm)	TNW (mm)
Kirikae[15*]	1960	Japan	25	Dissection	Cadavers	Adult	7.50 ± 0.50	7.90 ± 0.80	-	-
Lim[16*]	1970	USA	20	Dissection	Cadavers	Adult	9-10.20	8.50-9	-	-
Salvinelli et al.[17*]	1991	Italy	280	Dissection	Cadavers	Adult	9.40 ± 1.50	8.60 ± 0.90	-	-
Wajnberg[18*]	1987	Israel	28	Dissection	Cadavers	Adult	8-9	9-10	-	-
Beger et al.[20*]	2020	Turkey	36	Dissection	Fetuses	24.27 ± 3.24 weeks	8.22 ± 1.12	7.25 ± 1.15	-	-
			20	Dissection	Cadaver	75.70 ± 14.11 years	9.06 ± 1.33	8.10 ± 1.43	-	-
Dahm et al.[19*]	1993	Australia	3	Dissection	Cadavers	0<0.5 years	9.3 ± 0.3	8.7 ± 0.6	-	-
			4			2<4 years	9.1 ± 0.6	9 ± 0.7	-	-
			5			4<6 years	8.9 ± 0.4	9.4 ± 0.2	-	-
			3			6<8 years	9.5 ± 0.5	9 ± 0.9	-	-
			3			8<10 years	8.8 ± 0.3	9 ± 0.9	-	-
			2			10<14 years	8.8 ± 0.4	9.5	-	-
			7			14<18 years	9.4 ± 0.3	9.3 ± 0.6	-	-
			3			>18 years	9	9.3 ± 0.4	-	-
Leibovitz et al.[3]	2013	Israel	20	Sonography	Fetuses	12 weeks	2.2 ± 0.5	2 ± 0.4	-	-
			20			16 weeks	5.4 ± 0.6	4.3 ± 0.6	-	-
			20			23 weeks	8.3 ± 0.7	7 ± 0.6	-	-
			20			32 weeks	11.8 ± 1.2	9.4 ± 1.2	-	-
Anson et al.[7]	1955	USA	1	Dissection	84-mm fetus	12 weeks	4.2	-	-	-
			1		120-mm fetus	16 weeks	6.15	-	-	-
			1		161-mm fetus	19 weeks	7.4	-	-	-
			1		190-mm fetus	22 weeks	7.9	-	-	-
			1		210-mm fetus	24 weeks	8.2	-	-	-
			1		310-mm fetus	34 weeks	9.75	-	-	-
			1		325-mm fetus	35 weeks	9.5	-	-	-
			1		350-mm fetus	38 weeks	10.0	-	-	-
			3		Term fetuses	-	9.25-10.0	-	-	-
			1		Newborn	-	10.0	-	-	-
			1		Infant	One year	10.0	-	-	-
This study	2020	Turkey	32	Dissection	Fetuses	24.62 ± 3.44 weeks	8.25 ± 1.04	7.64 ± 1.08	24.93 ± 3.40	4.01 ± 0.91

*tympanic membrane measurements, N, numbers of the temporal bones

infant) of Anson et al.[7] On the other hand, one of the reasons for the differences between the measurements may be the tympanic membrane shape. Wajnberg[18] suggested that the height (8-9 mm) of the membrane was smaller than its width (9-10 mm). In contrast to the suggestion of Wajnberg,[18] the other studies[3,16,17,23] and this work showed that the vertical elongated shape of the tympanic membrane is constant in fetuses and adults.

CONCLUSION

From the seventh month in utero, the parameters (the TRH, TRW, TRP, and TNW) of the TR did not alter. The linear functions (which represent the growth pattern of the parameters) of the TR may be beneficial for otologists to guess its diameters, and to diagnose growth retardation and probable congenital anomalies in utero during sonographic imaging.

Ethics Committee Approval: The Clinical Research Ethics Committee of Mersin University granted approval for this fetal work (dated 22.03.2018, no. 2018/150).

Informed Consent: Approval from the Institutional Review Board was obtained and informed consent was not required.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – O.B., A.B.Ö., O.D., D.Ü.T.; Design - O.B., A.B.Ö., O.D., D.Ü.T.; Supervision – O.B., Y.V., A.B.Ö., O.D., D.Ü.T.; Resource – O.B., Y.V., D.Ü.T.; Data Collection and/or Processing – O.B., D.L.Ö., F.M., P.T., S.Ç., Ş.A.; Analysis and/or Interpretation – O.B., D.L.Ö., F.M., P.T., S.Ç., Ş.A.; Literature Search/Writing – O.B., D.L.Ö., F.M., P.T., S.Ç., Ş.A.; Critical Reviews – O.B., Y.V., D.Ü.T.

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