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ORIGINAL ARTICLE

Evaluation of the stapedial tendon growth dynamic in human fetuses

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Abstract

Purpose The main objective of the study was to investigate the morphometric properties of the stapedial tendon (ST) for pediatric otosurgeons and anatomists.

Methods The present study was placed on 15 fetuses (8 females, 7 males) aged from 20 to 30 weeks of gestation (at mean, 24.27 ± 3.24 weeks) using the collection of the Anatomy Department of Medicine Faculty, Mersin University. All measurements were obtained with a digital image analysis software.

Results In terms of male/female or right/left comparisons, no statistically significant difference was found in relation with the numerical data of ST. The surface area, length, and width of ST were detected as follows: 0.61 ± 0.15 mm^2, 1.27 ± 0.30 mm, and 0.45 ± 0.08 mm, respectively. The absence of ST was observed in two fetuses with and without severe malformations. In another fetus with cleft lip and polydactyly, multiple abnormalities were bilaterally identified in the middle ear: (1) the absence of the incudostapedial joint and (2) the presence of an abnormal tissue attaching to the stapes. The abnormal tissue was determined to be irregular dense connective tissue using light microscope and electron microscope.

Conclusion Our findings showed that ST did not proportionally grow according to increasing gestational weeks. In the light of the numerical data, we thought that similar to stapes, ST attains the adult size in the fetal period. As ST anomalies may accompany severe malformations (e.g., cleft lip, polydactyly or syndactyly) that can be easily detected on observation by clinicians, we suggest that the detailed examination of middle ear in newborns should be taken into account for early diagnosis of conductive hearing loss to prevent any management delays.

Keywords Congenital malformations · Fetus · Middle ear · Stapedial tendon · Stapedius muscle

Introduction

The stapedial tendon (ST) originates from the orifice at the apex of the pyramidal eminence and attaches to the posterior surface of the neck of the stapes [22]. By contractions of the stapedius muscle fibers found in the bony sheath of the pyramidal eminence, ST pulls the footplate of the stapes away from the oval window [8, 22]. This action, also known as the stapedial reflex, is important for protection of the inner ear against hazardous levels of sound and improving intelligibility of talk if there is background noise [8, 15, 22]. Despite this functional importance, ST can be cut during transaural tympanotomy, cochleostomy, stapedectomy, stapedotomy and cochlear implantation [10, 13, 14, 17]. Since these applications can be performed in early childhood period [14, 17], the dimension, location and variations of the tendon in fetal middle ears are

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important for pediatric otosurgeons to protect its integrity and function and in addition for anatomists to understand and interpret its developmental process [13, 15, 18–20].

In the previous studies [18, 20], the fetal development of ST is examined using histological techniques. Rodríguez-Vázquez [18] and Rodríguez-Vázquez et al. [20] studied on human embryos and fetuses aged from 38 days to 17 weeks of gestation and reported that two completely different anlagen form the musculo-tendinous structure of stapedius muscle. They observed that ST derives from the interhyale, while the muscular belly locates in the second pharyngeal arch medial to the facial nerve and near the interhyale [18, 20]. Forming an angle, the interhyale is composed of two segments: (a) the internal part, which forms the tendon and (b) external or lateral part, which attaches to the cranial end of the Reichert's cartilage, and normally disappears at the beginning of the fetal life [18, 20]. The deviations during the fetal development of the stapedius muscle can cause the tendon abnormalities such as absence or ossification. From this point of view, further information related to the size, location and variations of ST in fetal middle ears may be useful for pediatric otosurgeons and anatomists.

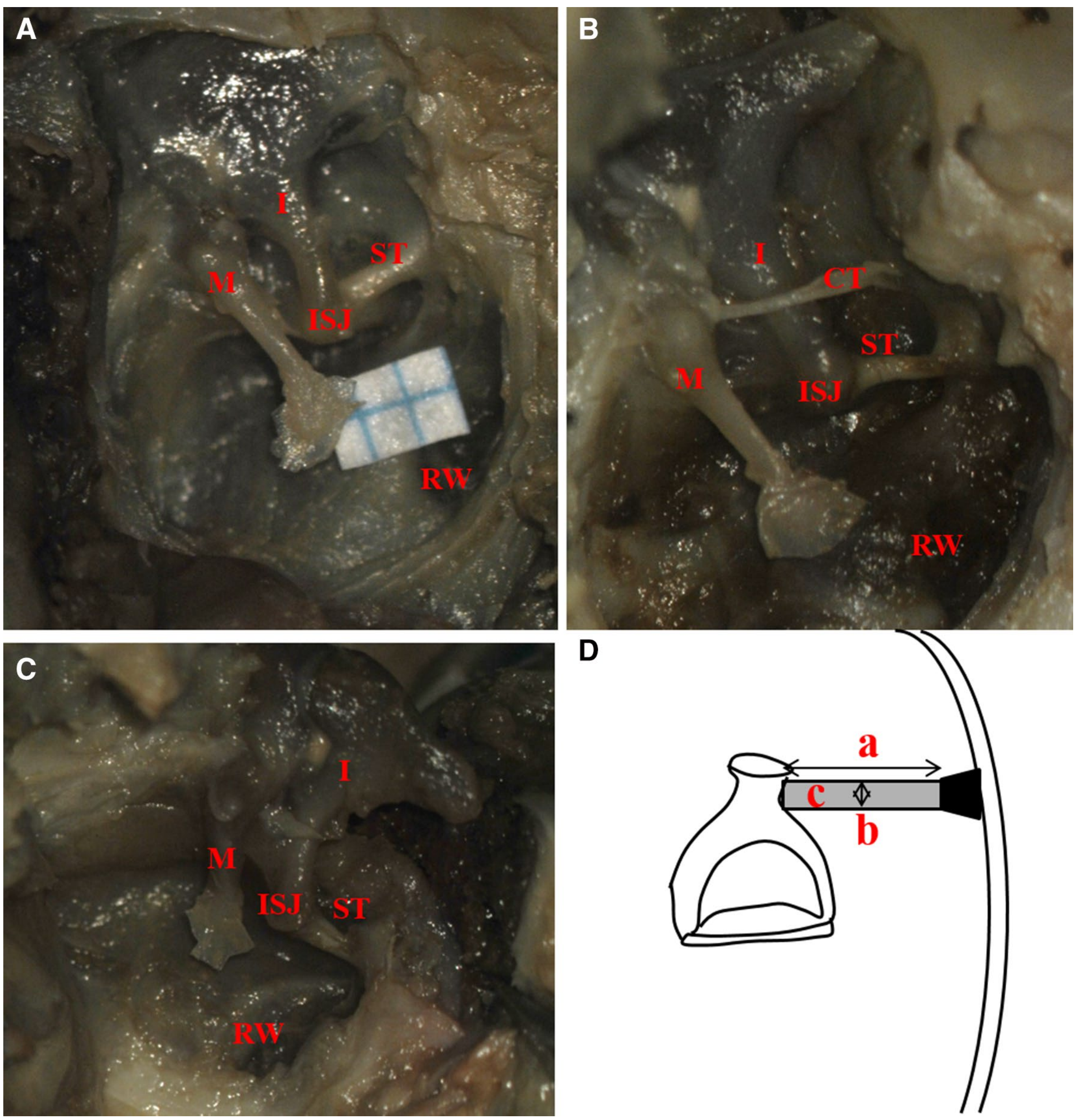

Fig. 1 The photographs show the stapedial tendon (ST) in the fetuses aged **a** 20 weeks, **b** 23 weeks and **c** 26 weeks of gestation. The photograph (**d**) show the parameters: a) the length, b) the width, and c) the surface area (the gray area indicates the two-dimensional surface of ST calculated from the photograph). *M* malleus, *I* incus, *ISJ* incudostapedial joint, *RW* round window, *CT* chorda tympani

Existing inventory based on the quantitative analysis of ST in adults is fairly limited [8]. Besides, the morphometric assessment of ST in fetal cadavers is not encountered in the current literature. Moreover, variations and anomalies related to ST in fetuses such as the ossified, shortened or absent tendons are reported at a quite limited number of investigations [12, 15, 25, 27]. In this regard, the main aim of the present study is to show the growth dynamics and algebraic anatomy of ST as well as its variations and anomalies.

Materials and methods

This study was conducted on 30 fetal temporal bones using the collection of the Anatomy Department of Medicine Faculty, Mersin University. The Clinical Research Ethics Committee approved the study ethically. Under a surgical microscope (Carl Zeiss f170, Carl Zeiss Meditec AG, Germany), ST was exposed via the classical tympanoplasty approach. In the same environmental conditions, all dissections were performed by the same otosurgeon (D.Ü.T.). Upon that, the following parameters were determined (Fig. 1).

- Length of ST (from the apex of the pyramidal eminence to the neck of the stapes),
- Width of ST (at the middle part),
- Surface area of ST (between the stapes and pyramidal eminence).

The present study was placed on 15 fetuses (8 females, 7 males) aged from 20 to 30 weeks of gestation (at mean, 24.27 ± 3.24 weeks). However, since there were anomalies in three fetuses, morphometric evaluation was made on 12 fetuses, bilaterally. Although the fetuses used in the study were fixed with 10% formalin, shrinkage caused by formalin fixation was underestimated during the measurements, since Cutts [9] reported that the ratio of reduction of plasticity in the tissue content was 0.5–1%. Using photography device of the surgical microscope, the specimen photographs were captured under the same distance and position with millimeter scales [24]. Photographs were transferred to a digital image analysis software (ImageJ software) and then the measurements repeated in three times by two independent investigators (OB and TK) at different times were recorded. The mean values of the recordings were utilized for the data processing. To evaluate the reliability of the measurements, intra-observer reproducibility (ANOVA with repeated measures and post hoc RIR Tukey tests) and inter-observer reproducibility (ICC: intra-class correlation coefficients) were used. Foot lengths of fetuses were measured to determine the gestational ages. Normality of the data was checked with Shapiro–Wilk test. The variance homogeneity was tested by Levene test. Statistically significant differences were evaluated in term of side (the paired sample t test) and sex (the independent sample t test). Alterations in the recordings of ST according to the gestational weeks were examined with one-way ANOVA and post hoc Bonferroni tests. Statistical significance was set as $p < 0.05$.

Results

In Table 1, the demographic values (sex, age, foot length, number of sides) of fetuses are presented. Due to the measurements made by the independent researchers (ICC = 0.996–0.998, $p < 0.001$) and the same researcher (OB or TK, $p > 0.05$), it was accepted that the reliability of the data was excellent. The mean values of the recordings of the parameters of ST considering the gestational weeks are given in Table 2. In term of sex or side, no statistically significant difference was found in relation with the numerical data of ST. The scatter plots of parameters versus gestational ages are offered in Fig. 2. In three fetuses, variations and abnormalities related to ST were observed.

Case 1: During the dissections in a 30-week-old female fetus with cleft lip and polydactyly on left hand (Fig. 3a), multiple abnormalities were bilaterally identified in the middle ear: (1) the absence of the incudostapedial joint, (2) the shortened long crus of the incus, and (3) the presence of an abnormal tissue, which attaches the neck of the stapes (Fig. 4). Using light microscope (Olympus BX50, Olympus GmBH, Germany) and electron microscope (SEM, Supra 55, Zeiss, Germany), the abnormal tissue stained by toluidin blue (semithin sections: 2 μm) and uranyl acetate—lead citrate (thin sections: 70 nm) was evaluated. As a result of histological evaluation, the abnormal tissue was found to be irregular dense connective tissue (Fig. 5). ST and the belly

Table 1 The demographic data of fetuses

Gestational weeks	Foot length (mm)	Sides (N)	Male	Female
20	30.99 ± 0.50	4	2	0
21	32.71 ± 0.70	4	1	1
22	35.12 ± 0.72	4	1	1
23	36.83 ± 0.19	2	0	1
24	39.97 ± 0.84	2	1	0
25	42.11 ± 0.31	2	1	0
26	45.36 ± 0.74	4	1	1
27	47.72 ± 0.55	2	0	1
28	52.14 ± 0.50	2	0	1
29	53.30 ± 0.15	2	0	1
30	54.20 ± 0.22	2	0	1
24.27 ± 3.24	40.97 ± 8.14	30	7	8

Table 2 Morphometric data related to ST

Gestational weeks	N	Length (mm)	Width (mm)	Surface area (mm^2)
20	2	1.42 ± 0.45	0.52 ± 0.05	0.64 ± 0.09
21	4	1.42 ± 0.48	0.43 ± 0.02	0.58 ± 0.19
22	4	1.30 ± 0.25	0.54 ± 0.06	0.75 ± 0.16
23	2	1.26 ± 0.26	0.41 ± 0.01	0.55 ± 0.10
24	2	1.17 ± 0.19	0.50 ± 0.04	0.78 ± 0.01
25	2	1.25 ± 0.48	0.43 ± 0.02	0.60 ± 0.11
26	4	1.10 ± 0.29	0.41 ± 0.08	0.51 ± 0.15
27	2	1.22 ± 0.19	0.34 ± 0.09	0.48 ± 0.15
28	2	1.30 ± 0.41	0.51 ± 0.14	0.65 ± 0.14
Total	24	1.27 ± 0.30	0.45 ± 0.08	0.61 ± 0.15
p		0.965	0.08	0.362

of the stapedius muscle were normal, bilaterally. The surface area, length and width of ST for the left/right sides were as follows: 0.45/0.63 mm^2, 1.02/1.12 mm, and 0.35/0.46 mm, respectively.

Case 2: During the dissections in a 20-week-old male fetus with syndactyly on right foot (Fig. 3b), absence of ST was identified in the right middle ear (Fig. 6). Using light microscope, the remnant tissue stained by toluidin blue (semithin sections: 2 µm) was evaluated. It was observed that the belly of the stapedius muscle and pyramidal eminence were present (Fig. 7). In the left middle ear, the surface area, length and width of ST were as follows: 0.57 mm^2, 1.24 mm, and 0.56 mm, respectively.

Case 3: Similar to case 2, the absence of ST with normal muscle belly and pyramidal eminence was identified in the left middle ear during the dissections in a 29-week-old female fetus without severe malformations. In the right middle ear, the surface area, length and width of ST were as follows: 0.65 mm^2, 1.15 mm, and 0.41 mm, respectively.

Discussion

Our findings delineating that ST did not proportionally grow in fetal life during stapes ossification period, contribute to the current literature on its algebraic anatomy.

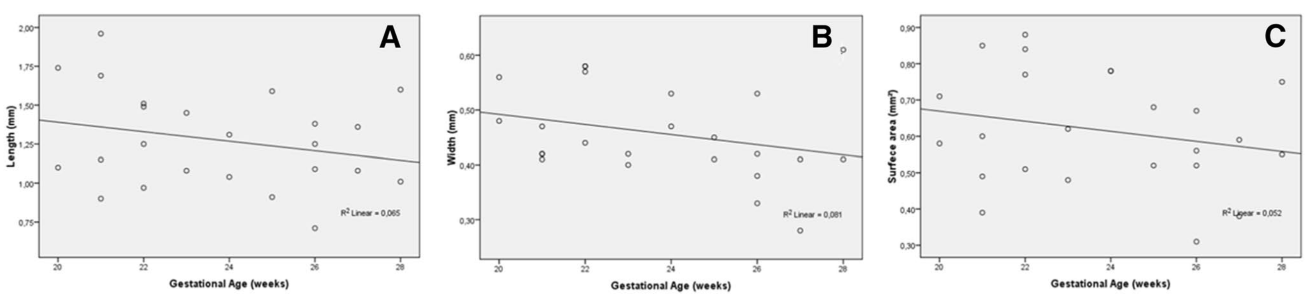

Fig. 2 The scatter plots of **a** the lengths, **b** the width and **c** the surface area of ST

Fig. 3 The asterisks show **a** the cleft lip and polydactyly on left hand on the fetus aged 30 weeks, **b** syndactyly on right foot on the fetus aged 20 weeks of gestation

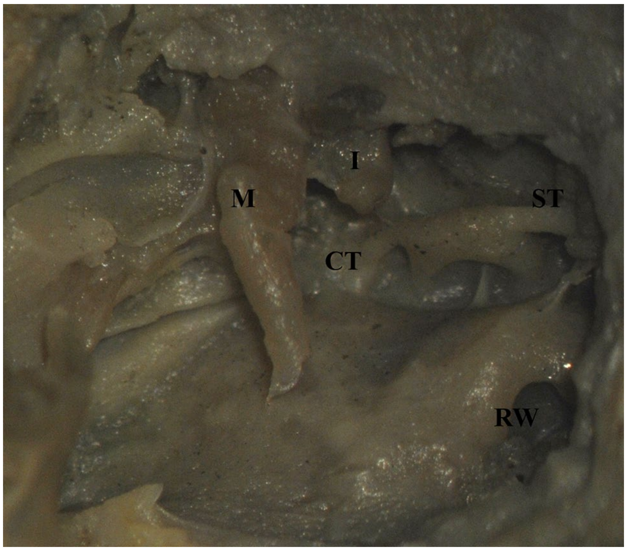

Fig. 4 The photograph shows the abnormal connective tissue (CT) and the absence of the incudostapedial joint. *ST* stapedial tendon, *M* malleus, *I* incus, *RW* round window

High incidence of ST anomalies in the present study may indicate a very detailed investigation for internal structures of the temporal bone (e.g. middle ear bones and ligaments) in the presence of observable external fetal abnormalities.

The length of ST from too short to extremely long shows quite variations in adults [13]. In this study, the surface area, length and width of ST were as follows: 0.61 ± 0.15 mm^2, 1.27 ± 0.30 mm, and 0.45 ± 0.08 mm, respectively. The values of ST in our study is compatible with the length (0.99 ± 0.09 mm) and width (0.40 ± 0.07 mm) of Cheng and Gan's [8] mechanical study placed on 12 fresh-frozen human temporal bones aged from 51 to 92 years. Considering the study of Anson [1], who reported that auditory ossicles reach adult size and shape in fetal life, we thought that similar to stapes, ST attains the adult dimension in the fetal period. Similar to the previous studies conducted on different muscles on human fetuses [2–5, 7], no statistically significant difference in terms of sex or side was found in relation with the numerical data of ST. However, unlike those studies [2–5, 7], our numerical values showed that ST did not proportionally grow according to increasing gestational weeks.

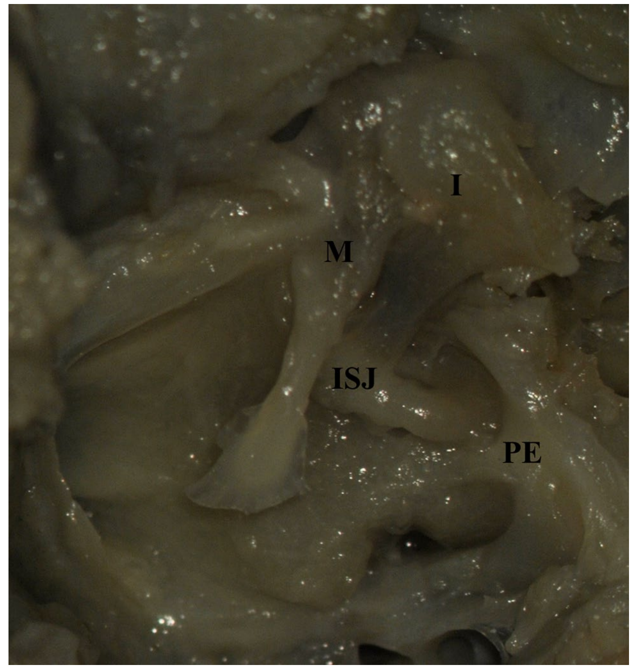

Fig. 6 The photograph shows the absence of the stapedial tendon. *M* malleus, *I* incus, *ISJ* incudostapedial joint, *PE* pyramidal eminence

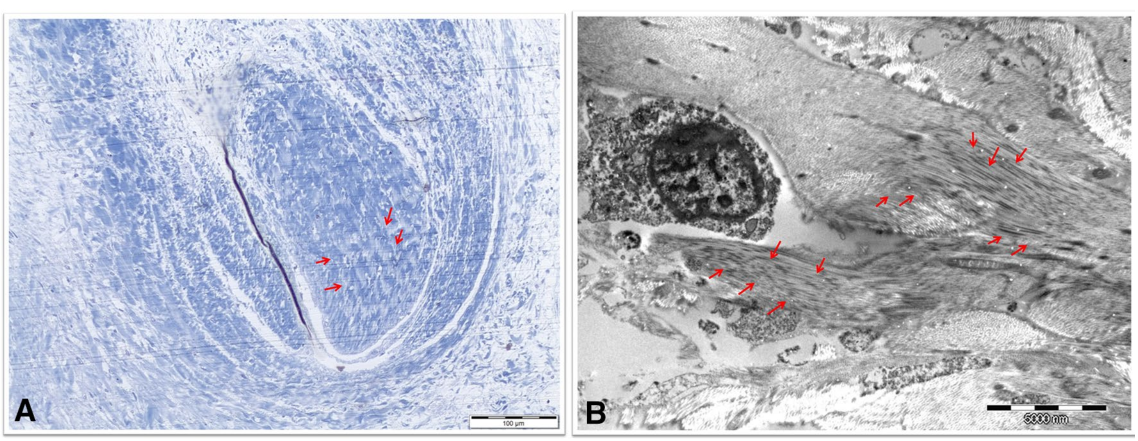

Fig. 5 The photographs show irregular dense connective tissue formations under **a** the light microsope (×100) and **b** electron microscope (× 5000). The abnormal tissue was stained with **a** toluidin blue (semithin sections: 2 µm) and **b** uranyl acetate—lead citrate (thin sections: 70 nm). Red arrows show the collagen fibers

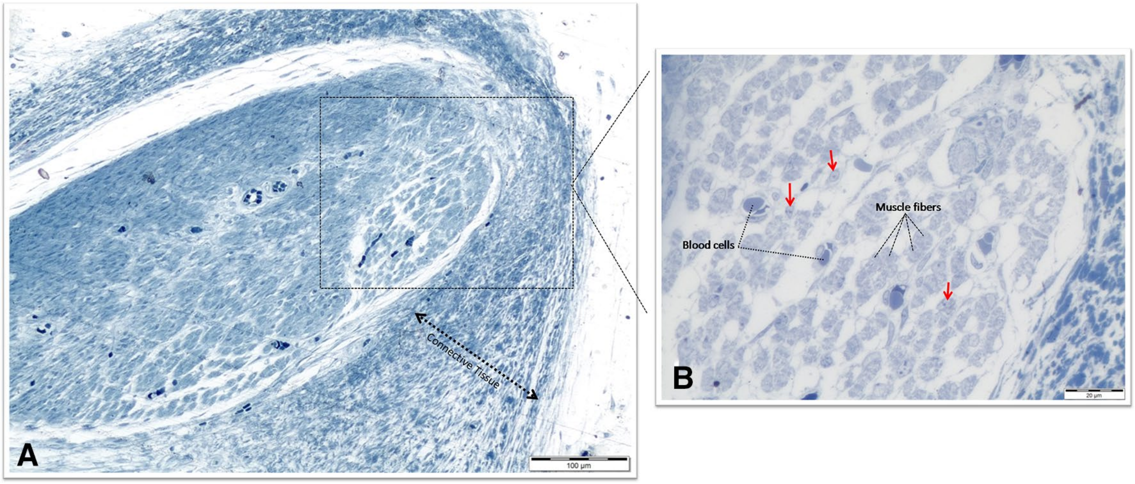

Fig. 7 The photographs show the muscle fibers, muscle nuclei (red arrow) and blood cells in **a** × 100 and **b** × 400. The tissue was stained with toluidin blue (semithin sections: 2 µm)

Rodríguez-Vázquez [18] explained that this situation is due to the degree of angulation persistence between ST and the muscular fibers. Moreover, the variations of ST length depend on the attachment site of muscular fibers in the interhyale, the internal segment of which forms its length [18]. However, we investigate the growth dynamic of ST in human fetuses aged from 20 to 28 weeks according to increasing gestational ages during stapes ossification period. Therefore, the development of ST in fetuses in a wider age range from 8 to 40 weeks is better to be studied in future investigations.

The abnormalities of ST may be associated with congenital hearing loss, myoclonus, otosclerosis, CHARGE syndrome, and trisomy 18 syndrome [11, 14–16, 23]. Since different procedures such as cutting or preservation can be performed under these conditions [17, 21], the size and location of ST are clinically important. Roberson et al. [17] studied on 23 ears of 19 patients (at mean ages, 11.9 months) for cochlear implantation, and reported that due to the inferior localization of ST, it was cut in seven ears to facilitate access to the round window. This anomaly was not observed in our series. Considering as a possible cause of conductive hearing loss, a shortened ST was declared by Zawawi et al. [27]. Hough [13] stated that because ST can be used to see certain slight movements of the head of the stapes during mobilization, an extremely short tendon might complicate the manipulation of the stapes. In this light, taking into account the limited knowledge related to the quantitative anatomy of ST, the investigation of its morphometric anatomy in fetal middle ears is important to determine the course, length and variation anomalies, due to supplying more sight compared to adult studies for infant surgeries.

The following variations are identified related to ST in the literature: the ossified [25], shortened [27] or absent [12, 15] tendon; presence of the muscular belly without the tendon [20]; doubled stapedius muscle [26]; and ectopic or supernumerary muscle tissue [12, 19]. These anatomic variations and abnormalities make ST in fetal middle ears an important area of interest for otosurgeons and anatomists [13, 15]. Among these variations and abnormalities, only the absence of ST was seen in this series. Hough [13], unlike what is thought, expressed that the tendon absence is not only part of the other severe malformations and can be seen in patients without any anomaly. The incidence of this variation was given as 0.5-1% [13, 15]. Rodríguez-Vázquez et al. [20] presented an isolated unilateral absence of ST in a fetus aged 14 weeks of gestation, initially. They explained that this variation is due to the lack of formation or the regression of the internal part of the interhyale [20]. In this study, the middle ear anomalies including the presence of abnormal connective tissue and the absence of ST were observed in two fetuses with severe malformations such as cleft lip, polydactyly or syndactyly. Zawawi et al. [27] argued that similar to ossified ST, shortened tendon can cause conductive hearing loss by limiting the movement of the stapes, theoretically. We thought that with a similar mechanism, the abnormal connective tissues observed in our cases may cause hearing loss and probable stapes immobilization. In our opinion, as ST anomalies may accompany severe malformations such as cleft lip, polydactyly or syndactyly in newborns, the detailed examination of middle ear is vital for early diagnosis of conductive hearing loss to prevent any management delays [6].

Conclusion

Our numerical data representing the growth dynamics of ST proved that it did not proportionally grow according to increasing gestational weeks. Interestingly, similar to stapes, the tendon attains the adult size in the fetal period without statistically significant differences in terms of sex and side. The findings of the present study may be suggested as a preliminary step for further fetal and adult investigations emphasizing different ethnicities, gender and countries or regions on the world to determine stapedial tendon development patern.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest

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