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Descriptive growth model of the height of stapes in the fetus: a histopathological study of the temporal bone

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Abstract Temporal bone histological findings can be evaluated from several points of view. The most basic consists of a description of the characteristics and abnormalities of particular temporal bones. The second one is the measurement of various structures in a larger set of temporal bones and the monitoring of these structures over time. The height of stapes was measured in a set of 40 temporal bones from 27 fetuses, and the growth of stapes from the 13th to 36th weeks of pregnancy was determined. A computer-assisted nonlinear regression analysis of diagnostics enabling simultaneous examination of data (influential points, i.e., outliers and leverages) was carried out, a growth curve model proposed and a mathematical method with Ratkowski criteria for estimation applied to find the best descriptive model of the height of stapes versus time $y=f(x)$ growth curve; the results of 13 growth models were examined. It was found that the maximum growth of the height of stapes was between the 13th and the 24th weeks of pregnancy. The average height of stapes was 1.05 mm in the 13th week and 2.6 mm in the 24th week. Later, after the 25th week, the growth of the height of stapes was slower, and the average height in the 30th week was 3.0 mm.

Keywords Temporal bone · Regression · Growth curve · Stapes · Embryonic development

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Introduction

Histological assessment of the temporal bones of fetuses and newborns can bring new ideas into clinical practice. Besides the traditional methodological approach of the microscopic examination of temporal bones, some mathematical methods such as statistics may also be applied to obtain better knowledge. For example, determination of the height of stapes enables the design of the prothesis used in middle ear surgery [18].

In the embryonic development of stapes [2, 17], the primordial form of stapes is clearly apparent in the embryo at 6.5 weeks. The stapes has two developmental origins, the first being Reichert's cartilage (for the suprastructure and the tympanic part of the footplate) and the second the otic capsule (for the vestibular part of the footplate). The stapes is largely formed by true cartilage by 7.5 weeks, and changes from an annular (embryonic) to a stapedial (adult) form between 2 and 4 months. Ossification starts in the 19th week. The sole ossification center is present on the footplate and continues upward along each crus. The stapes attains its maximal growth at the midfetal stage of development.

Recently, there has been considerable interest in developing a nonlinear growth model to summarize the pattern in stature during pregnancy and childhood. Following the triple-logistic model of Bock and Thissen [4], Jolicoeur, Pontier, Pernin and Sempé [10] described an elegant model with seven parameters covering the entire childhood and showed that it performed considerably better than the models of Preece and Baines [20] or Shohoji and Sasaki [29]. Ledford and Cole [13] presented growth models for predicting child stature summarizing both the pattern and timing of growth in individuals.

Generally, regression growth model building is among the most complex problems solved in biometrics and clinical practice today [3, 6, 7, 12, 26, 30]. An interactive approach to model building can be divided into the following four steps: (1) selection of the set of provisional models, (2) extension and modification of the models proposed, (3) analysis of model assumptions and model rele-

Table 1 Selection of non-linear regression models of the growth curve

Model	Growth curve models proposed	Reference
A	$\beta_1/(1+\beta_4 \exp(\beta_3\ \beta_2\text{-}x))^{1/\beta_4}$	Schnute [27]
B	$\beta_1\ (1\text{-}\exp(\beta_3\ \beta_2\text{-}\beta_3\ x))$	Mitscherlich [16]
C	$\beta_1/(1+\exp(\beta_2\text{-}\beta_3\ x))^{1/\beta_4}$	Richards [25]
D	$\beta_1 \exp(\text{-}\exp(\beta_2\text{-}\beta_3\ x))$	Gompertz [9]
E	$\beta_1/(1+\exp(\beta_2\text{-}\beta_3\ x))$	Logistic [22]
F	$\beta_1\text{-}\beta_2 \exp(\text{-}\beta_3\ x)$	[15]
G	$\beta_1\ (1\text{-}\exp(\text{-}x\text{-}\beta_2)\ \beta_3)$	[15]
H	$\beta_1\text{-}\exp(\text{-}\beta_2\text{-}\beta_3\ x)$	[15]
I	$\beta_1\text{-}\exp(\text{-}\beta_2\ \beta_3\ x)$	[15]
J	$\beta_1+(\beta_2\ x)^{-1/\beta_3}$	[24]
K	$1/(\beta_1+\beta_2\ x\beta_3)$	[8]
L	$(\beta_2\ \beta_3+\beta_1\ x\beta_4)/(\beta_3+x\beta_4)$	[15]
M	$\beta_1\text{-}\beta_2 \exp(\text{-}\beta_3\ x\beta_4)$	[15]

vance, regression diagnostics in the classical sense and (4) testing of model quality and prediction capability [1, 14]. According to earlier studies (e.g., Weber [32], Peschel [19], Todorovic [31], Prodan [21], Wenk [33] and Kuzmitschev [11]), a true growth model must meet the following criteria [5]: (1) it must have a zero-point; (2) it must be increasing; (3) it must have an asymptote that is parallel to the time (age) axis; (4) it must have one inflection point. The present study was limited to growth models that describe growth as a function of time only, $y=f(t)$. The most important sigmoidal growth models selected from the literature and tested in this paper are given in Table 1. They contain between three and five parameters and describe the growth curve as the change of the height of stapes measures with the time of gestation. This table clearly shows the diversity of the available models. The choice of the most convenient growth regression model is not a trivial task and is best made respecting the five Ratkowski criteria: parsimony, parametrization, range of applicability, stochastic specification and interpretability (for details see [22, 23]). The aims of this study were (1) to review different growth functions and estimation methods to find a solution that can be proposed as a standard procedure of growth model building and (2) on the basis of the Ratkowski criteria to develop a predictive regression model and to predict the growth of the height of stapes in gestation.

Materials and methods

Subjects and data

The 40 temporal bones from 27 fetuses were removed within 24 h, usually after spontaneous abortion. After fixation in 10% formaldehyde solution, specimens were decalcified in 10% formic acid, embedded in paraffin wax and sectioned horizontally (midmodiolar plane) at a thickness of 10 µm. Every tenth section was stained with hematoxylin-eosin and examined microscopically.

Measurement of the height of stapes

The height of stapes is shown in Fig. 1 and was measured in the set of 40 temporal bones from fetuses from the 13th to 36th weeks of gestation. A microscopic ruler with a precision of 0.1 mm was used. The age of fetuses was determined on the basis of knowledge of the week of gestation, the weight and the height. All fetuses used for this study were without any malformation or developmental or genetic defects. The average outcomes of measured parameters in millimeters in particular weeks of gestation are shown in Table 2. The measured values were used in regression analysis, and results are shown in a graph of the growth curve (Figs. 2, 3).

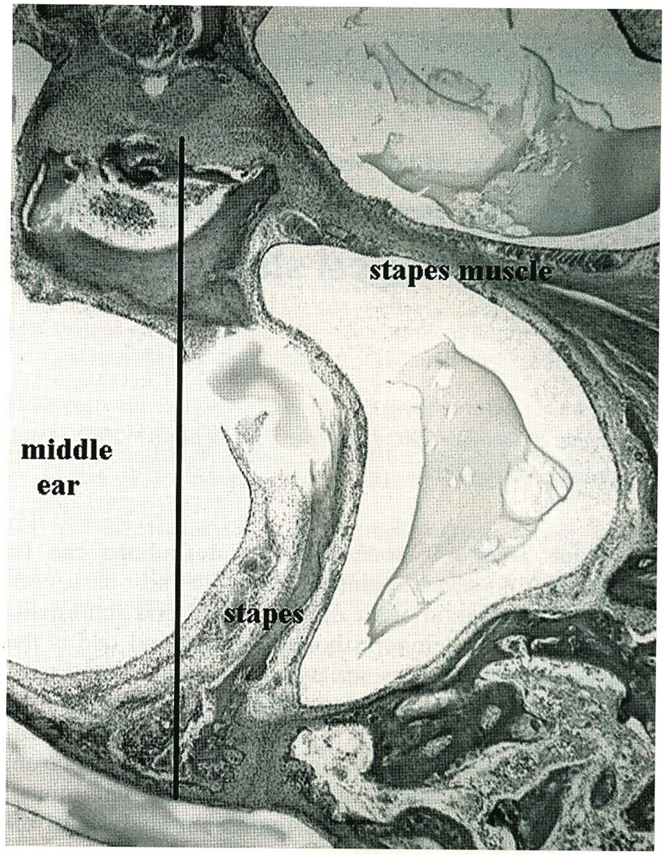

Fig. 1 Histological picture of the height of stapes

Growth curve model fitting

In searching for the best growth model, the nonlinear regression model $y=f(x\ \beta)+\varepsilon$ was first considered, where y is the response (dependent) variable or regresand and is an $n\times1$ vector of observations, x is a fixed $n\times1$ regressors vector of time, ($n>m$), β is the $m\times1$ vector of unknown parameters and ε is the $n\times1$ vector of random errors, which are assumed to be independent and identically distributed with mean zero and an unknown variance σ^2 [14]. Using the least-squares estimation method, we obtain the vector of fitted values $\hat{y}_{P,i} = f\ (x_i; b)$ and the vector of residuals $\hat{e}$

$$RSS(\beta) = \sum_{i=1}^{n} (y_i - f(x_i, \hat{\beta}))^2 \quad (1)$$

where $\hat{\beta} = \mathrm{b}$ and the quantity

$$s^2 = RSS(\beta)/(n-m) \quad (2)$$

is an unbiased estimator of σ^2, and $n\text{–}m$ is the number of degrees of freedom (DF) of a model fit, where n is the number of data points and m the number of model parameters. The number of data points n affects the residual sum of the squares (RSS) of a fit. Moreover, a model with more parameters is likely to yield a better fit than a model with fewer parameters. In the literature many

Table 2 Data of the growth curve expressing the height of stapes y (mm) dependent on time of gestation x (weeks), measured for 40 temporal bones from 27 fetuses; data are $\{x\ y;\}$

Right side											
13	1.1;	19	2.2;	20	2.5;	20	2.2;	21	2.7;	21	2.8;
22	1.8;	22	2.4;	23	2.5;	24	2.5;	24	2.5;	24	2.5;
27	2.3;	28	2.6;	29	2.6;	29	2.9;	30	2.7;	31	2.9;
32	2.7;										
Left side											
13	1.0;	19	2.2;	20	2.4;	20	2.5;	20	2.1;	21	2.7;
21	2.6;	21	2.6;	22	2.3;	22	2.5;	23	2.3;	23	2.4;
24	2.6;	24	2.6;	24	2.8;	28	2.8;	29	2.8;	30	3.3;
31	3.1;	32	2.5;	36	2.4;						

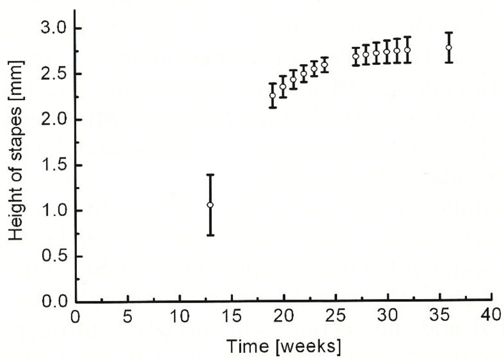

Fig. 2 The Mitscherlich growth curve (model B in Table 1) fitted to the data set of the height of stapes (*mm*) on gestation time (*weeks*) from Table 2 plus confidence band

growth curve models were found and tested, a selection of most suitable models being given in Table 1.

Resolution criteria for model selection

Various criteria for testing regression model quality can be used [14]. One of the most efficient is the mean quadratic error of prediction, *MEP*, being defined by the relationship:

$$MEP = \frac{\sum_{i=1}^{n} \left(y_i - \mathrm{x}_i^{\mathrm{T}} \mathrm{b}_{(i)}\right)^2}{\mathrm{n}} \tag{3}$$

where $b_{(i)}$ is the estimate of regression parameters when all points except the ith one were used and x_i is the ith row. The *MEP* can be used to express the predicted determination coefficient:

$$\hat{R}_P^2 = 1 - \frac{n\ MEP}{\sum_{i=1}^{n} y_i^2 - n\ \bar{y}^2} \tag{4}$$

where $\bar{y}$ is the mean of all n values y_i, i=1, ..., n. Another statistical characteristic in quite general use is derived from information entropy theory [14] and is known as the Akaike information criterion:

$$AIC = n\ \ln\left(\frac{RSS(\mathrm{b})}{\mathrm{n}}\right) + 2\mathrm{m} \tag{5}$$

The most suitable model is that which gives the lowest value of the mean quadratic error of prediction (*MEP*) and Akaike information criterion (*AIC*) and the highest value of the predicted determination coefficient, R_p^2. The mean of the absolute values of relative residuals (*MR*) and the residual standard deviation $s(e)$ should be of similar magnitude to the experimental error $s(\varepsilon)$ of the depen-

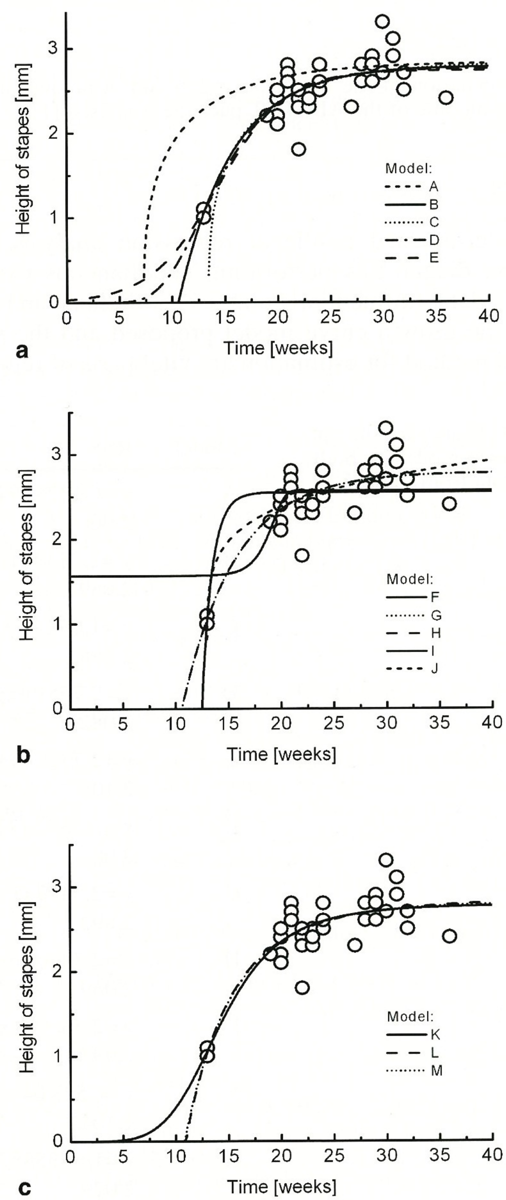

Fig. 3 Graphical presentation of a search for the optimal regression growth model for the data set of the height of stapes (*mm*) on gestation time (*weeks*) (both sides' data from Table 2) when (**a**) models A, B, C, D and E, (**b**) models F, G, H, I and J and (**c**) models K, L, M, N and P were tested. Statistical diagnostics of the fitness test are in Table 3

dent variable y, i.e., $s(e) \approx s(\varepsilon)$ or the relative value of this error $MR \approx s_{rel}(\varepsilon)$.

Experimental method

Procedure of regression model building

The procedure for construction of a growth curve using nonlinear regression model building consists of the following steps: (1) the proposal of a model, and the procedure should always start from the simplest model, (2) examination of the statistical significance of the parameter estimates and (3) construction of a more accurate model; on a basis of *MEP* or *AIC* the most convenient regression model of the data is determined. If some parameters are statistically insignificant the model is revised.

Software used

For computation of the nonlinear regression, a unique linear regression module of the ADSTAT package was used [1].

Results

Computer-assisted nonlinear regression analysis of regression diagnostics performing simultaneous examination of data (influential points, i.e., outliers and leverages), the growth curve model proposed and the mathematical method for estimation are vital parts of regression model building. For resolving of these types of problems, the MINOPT nonlinear regression procedure [1] and the procedure of regression model building according to the Ratkowski criteria [22, 23] can be used (Table 2).

With an initial estimate of parameters $b_1^{(0)}=1$, $b_2^{(0)}=1$ and $b_3^{(0)}=1$ for the Mitscherlich model and the data set of the height of stapes versus time of gestation from Table 2, the residual sum of squares $RSS(b)$ reached at the minimum was 2.0687, with the best estimates of parameters $b_1=$ 2.784 (0.098), b_2=10.575 (0.761) and b_3=0.1963 (0.0400). The low values of the parametric standard deviation (in parentheses) proves that all of the parameters are statistically significant.

Figure 2 proves that the Mitscherlich model closely resembles the data. This is also obvious from the regression curve fitting with 95% confidence intervals. The goodness-of-fit is proven by the low value of the residual standard deviation $s(e)$=0.2365 for model B (Mitscherlich) in Table 3, the regression rabat D=71.106%, and the mean of relative residuals MR=7.054%.

The employed regression method will find one of the growth curve models in two steps. The first step investigates whether some of the less complex models [Schnutte's (A), Mitscherlich's (B), Richard's (C), Gompertz's (D) or logistic (E)] and the second step models F through M of Table 1 could be used to describe the data satisfactorily. If

Table 3 Search for the optimal growth model for both sides' data from Table 2

Model	RSS	D (%)	AIC	MR (%)	s(e)	MEP ×100	Outliers
A	y =0.13282/(1–1.9472 exp(0.15129 (7.5505-x)))^(1/-1.9472)						
	2.066	71.16	-110.55	7.07	0.2395	6.2477	7, 37
B	y =2.7840 (1-exp((0.19627 10.575)-(0.19627 x)))						
	2.069	71.106	-112.48	7.054	0.2365	5.8505	7, 37
C	y =1.0/(1+(exp((-8.1455-(0.20182 x)))))^(1/2.4343E-05)						
	2.158	69.86	-108.79	7.592	0.2448	6.8497	7, 36, 37
D	y =2.7551 exp(-exp(3.2084-(0.25082 x)))						
	2.082	70.92	-112.22	7.04	0.2372	5.8688	7, 37
E	y =2.7328/(1+(exp(4.4723-(0.31162 x))))						
	2.106	70.59	-111.76	7.047	0.2386	5.9285	7, 37
F	y =2.7840-(22.140 (exp(-0.19624 x)))						
	2.069	71.11	-112.48	7.056	0.2365	5.8509	7, 37
G	y =2.5479 (1-(exp(- x +4.3303) 3426.8))						
	2.892	59.61	-99.08	8.176	0.2796	5.227	7, 37
H	y =2.7840-(exp(3.0973-(0.19617 x)))						
	2.069	71.11	-112.48	7.054	0.2365	5.8508	7, 37
I	y =2.5669-(exp(-5.5950E-08 (2.3754^ x)))						
	2.987	58.28	-97.79	9.505	0.2841	8.2824	7, 37
J	y =(-528.04+(40.724 x))^(1/6.5297)						
	2.104	70.61	-111.79	7.133	0.2385	6.0286	7, 37
K	y =1/(0.35887+(2.0855E+05 (x ^-4.9867)))						
	2.079	70.97	-112.29	7.044	0.237	5.8645	7, 37
L	y =((-8.7664 745.71)+(2.8637 (x ^3.2372)))/(745.71+(x ^3.2372))						
	2.069	71.1	-110.47	7.067	0.2398	6.2735	7, 37
M	y =2.8098-(106.91 (exp(-0.76370 (x ^0.65597))))						
	2.068	71.12	-110.5	7.06	0.2397	6.3488	7, 37
	3.272	54.3	-86.14	10.42	0.3149	0.1271	

so, its parameters are refined and other regression diagnostics computed. The growth curve is fitted through given data plus confidence band and the residual plot against the independent variable (here time of gestation) or against prediction is plotted (Fig. 3).

The authors were able to identify the maximum growth of the height of stapes, which was from the 13th to 24th week of pregnancy. The average height of stapes was 1.05 mm in the 13th week and 2.6 mm in the 24th week. The later growth of the height of stapes after the 25th week was slower, and the average height in the 30th week was 3.0 mm. Schuknecht [28] has published the average height of adult stapes as being 3.26 mm. The adult stapes showed considerable variations in size, the minimum height being 2.56 mm and the maximum height 3.78 mm.

In this study the authors were aware of possible inaccuracies in the measurement of particular parameters as a result of the processing of every tenth sample, which might influence the results. The other factor that influenced accuracy was the difficulty of ensuring the ideal horizontal plane of some cuts of the temporal bone. It is supposed, however, that with computer-assisted nonlinear regression analysis and respecting all Ratkowski criteria it is possible to use the Mitscherlich model for the normal fetal development of stapes height.

Conclusion

The use of mathematical methods and regression analysis brings new possibilities for the assessment of histological findings in the temporal bone. The measurement of the size of middle ear ossicles helps to design surgical tools and middle ear implants. Knowledge of the normal development and the normal size of stapes can help to distinguish the abnormal development and stapes malformations.

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