Influence of Reduced Saliva Production on Phonation in Patients With Ectodermal Dysplasia

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SUMMARY: Objective. Patients with ectodermal dysplasia (ED) suffer from an inherited disorder in the development of the ectodermal structures. Besides the main symptoms, i.e. significantly reduced formation/expression of teeth, hair and sweat glands, a decreased saliva production is objectively accounted. In addition to difficulties with chewing/swallowing, ED patients frequently report on the subjective impression of rough and hoarse voices. A correlation between the reduced production of saliva and an affliction of the voice has not yet been investigated objectively for this rare disease.

Methods. Following an established measurement protocol, a study has been conducted on 31 patients with ED and 47 controls (no ED, healthy voice). Additionally, the vocal fold oscillations were recorded by high-speed videoendoscopy (HSV@4 kHz). The glottal area waveform was determined by segmentation and objective glottal dynamic parameters were calculated. The generated acoustic signal was evaluated by objective and subjective measures. The individual impairment was documented by a standardized questionnaire (VHI). Additionally, the amount of generated saliva was measured for a defined period of time.

Results. ED patients displayed a significantly reduced saliva production compared to the control group. Furthermore, the auditory-perceptual evaluation yielded significantly higher ratings for breathiness and hoarseness in the voices of male ED patients compared to male controls. The majority of male ED patients (67%) indicated at least minor impairment in the self-evaluation. Objective acoustic measures like Jitter and Shimmer confirmed the decreased acoustic quality in male ED patients, whereas none of the investigated HSV parameters showed significant differences between the test groups. Statistical analysis did not confirm a statistically significant correlation between reduced voice quality and amount of saliva.

Conclusions. An objective impairment of the acoustic outcome was demonstrated for male ED patients. However, the vocal folds dynamics in HSV recordings seem unaffected.

Key words: Ectodermal Dysplasia−Standardized Voice Evaluation−Saliva Production−Evidence-based Medicine.

INTRODUCTION

Communication is a major part of human interaction.[1] An impairment of the voice is directly linked to a limitation of social interaction and may even affect the perceived quality of life for concerned individuals.[2,3] Despite the immanent relevance of the human voice, the phonation process is not yet fully understood.[4,5] The airflow from the lungs and the oscillating vocal folds in the larynx form the primary signal of voice in a complex fluid-structure-acoustic interaction.[6−9] Lately, the influence of hydration on the viscosity of the laryngeal mucus, as well as in salivary flow and viscosity have increasingly been recognized as essential players in allowing adequate vibration of the vocal folds and vocal tract structures, respectively.[10−12]

The rare disease of ectodermal dysplasia (ED) offers a unique opportunity to investigate this correlation. Patients with ED suffer from an inherited disorder in the development of the ectodermal structures.[13,14] The most frequent form of ED is an X-linked recessive inheritance, which is why males are typically more strongly affected than females.[15] Alongside the main symptoms, i.e. significantly reduced formation and expression of teeth, hair and sweat glands, a decreased saliva production is objectively accounted.[16] Besides difficulties with chewing and swallowing, ED patients frequently report on the subjective impression of rough and hoarse voices.[17] An objective and differentiated analysis with standardized voice-related diagnostic tools has not yet been done for this rare disease. Except for individual case studies, most studies rely on the patient's self-evaluation or expert opinions commenting on dryness of the mouth and voice only in general without further details.[16,17]

In order to fill this gap, we designed this study on 31 ED patients and 47 controls based on the established multi-dimensional protocol by the European Laryngological Society for functional voice assessment.[18] The individual impairment was documented by a standardized questionnaire (VHI).[19] The generated acoustic signal was evaluated by objective parameters, auditory-perceptive measures for roughness, breathiness, hoarseness (RBH) and by the performance in the phonetogram.[20,21] The visual inspection of the vocal folds by videoendoscopy was complemented with high-speed video (HSV) recordings and

computer-calculated glottal dynamic parameters.[22,23] By this, an impairment of the voice iin ED patients could be analyzed objectively. More specifically, the differences between males and females were revealed and the different involvement of the vocal fold dynamics and the acoustic outcome could be discussed in detail.

Moreover, the production of saliva was measured in all participants of the study for a defined period of time (5 minutes of saliva measurement after 5 minutes chewing of neutral gum). This enabled a systematic investigation of correlations between the reduced amount of saliva and the different factors involved in voice production (vocal fold oscillation dynamics, acoustic signal, performance in phonetogram etc) which offers new possibilities to conduct fundamental research on the phonation process.

Besides the general relevance of gaining further overall knowledge on the role of laryngeal mucus and saliva in phonation, we are convinced that this work will be even more significant for personally affected ED patients. We obtained a reliable confirmation of an objective impairment of the voice in ED patients and thereby achieve recognition of their subjective impression of a hoarse voice. This will be highly valuable for optimal patient care and focused therapy for the rare disease ED. A profound knowledge of the underlying correlations might even trigger further developments regarding synthetic mucus and saliva.[24]

METHODS

Study participants

In total, 31 patients with a diagnosed form of the rare disease ectodermal dysplasia (ED) were investigated at the annual meeting of the largest German support group for ED, "Ektodermale Dysplasie e.V." in 2018. 18 male patients (ED_m) and 13 female patients (ED_f) participated. At this occasion 11 children were recorded, as well, but not included in the present study because of their large diversity in age/gender and unclear status of voice break. The healthy control group (no ED and no current or previous voice pathologies) contained 26 male test subjects (C_m) and 21 female test subjects (C_f), who were recruited at the Friedrich-Alexander University Erlangen-Nürnberg. The age of the control group covers a similar range to ED patients from 19 to 65 years, even so the medians differ by 10-15 years between the gender-specific subgroups. The age distribution with median, minimum and maximum values in every subgroup is given in Table 1. All measurements in this study are covered by a votum of the local ethics commission (ref. N° 61_18B).

Data acquisition and measurement protocol

This study followed the recommendations of the measurement protocol established by the European Laryngological Society (ELS) for a functional assessment of voice pathologies.[18] This multimodal diagnostic scheme included a standardized questionnaire for self-evaluation, i.e. voice

TABLE 1.
Age Distribution of All Subjects in This Study.

Group Age (yrs)	ED_m N = 18	C_m N = 26	ED_f N = 13	C_f N = 21
Median	40.0	24.0	35.5	24.0
Minimum	19	20	28	19
Maximum	65	36	62	63

handicap index (VHI) documenting the individual voice-related impairment.[19]

The acoustic quality was evaluated by SLPs on the basis of the phonetogram, recording the maximally achieveable pitch and intensity range[20,21] and with an auditory-perceptive evaluation of roughness, breathiness and hoarseness (RBH) on a standardized German reading text ("Der Nordwind und die Sonne"). This was done using the software tool Lingwaves by Wevosys (Baunach, Germany) with default settings. The acoustic evaluation was completed by a recording of sustained phonation of the vowel /a/ for computer-based analysis, which is further specified in the following paragraph.

For structural and functional assessment, the vocal fold oscillations *were* recorded by high-speed videoendoscopy (HSV). The imaging unit comprised a rigid PENTAX Medical (Montvale, NJ) laryngoscope (70° optic) which was connected to the Photron FASTCAM MC2 high-speed camera via Precision Optics Corporation (Gardner, MA) zoom coupler. The high-speed videos *were* recorded with 512×256 Px at 4 kHz.

In addition to the voice-related diagnostic techniques, the amount of generated saliva was measured. All participants were asked to refrain from eating 2 hours prior to the measurement. The subjects were given a neutral paraffin pellet (Ivoclar Vivadent, Schaan, Liechtenstein) to chew for 5 minutes in order to stimulate salivation. Subsequently, all generated saliva was then collected by spitting into a cup for further 5 minutes and weighed.

Data processing and analysis

To increase the reliability of the auditory-perceptive evaluation, the assessment of roughness, breathiness and hoarseness was performed by three trained SLPs on anonymized and blinded data sets. The results were averaged over the three raters and mean values were used in the subsequent statistical analysis.

The objective acoustic parameters were determined with a well-established software tool, known as Glottis Analysis Tools (GAT), that was developed in-house.[23,25,26] A sequence of 5 seconds in the acoustic signal was analyzed in each recording, which fulfills established recommendations of using at least 100 cycles.[27] The GAT software provides an automated cycle-based determination of the fundamental frequency f_0 and calculation of commonly used parameters reflecting the acoustic signal quality, i.e. Jitter, Shimmer, and cepstral peak prominence (CPP).[28,29]

The automated analysis of the vocal fold dynamics was performed on a signal sequence of 25 consecutive oscillation cycles in each HSV recording. For the glottal area waveform (GAW), a minimum signal length of 20 cycles is recommended for parameter stability.[30] The GAT software enables an automatic segmentation of the glottal area waveform, cycle detection and calculation of glottal dynamic parameters.[24−26] The characteristic features for healthy phonation are represented in the chosen parameters: glottal gap index (GGI)[31] and closing quotient (CQ)[32] reflect closure of the vocal folds, amplitude periodicity (AP)[33] and time periodicity (TP)[33] quantify the regularity of the complete GAW oscillation, while the phase asymmetry index (PAI)[33] and the amplitude symmetry index (ASI)[34] measure temporal and spatial differences between the behavior of left and right vocal folds.

Statistical tests were employed to reveal significant differences between ED patients and controls. Considering the gender-specific expression of the disease, all comparisons and tests were performed separately within the male (ED_m <> C_m) and within the female subgroup (ED_f <> C_f). A Shapiro-Wilk test with a significance level of $P = 0.05$ was applied to test the two subgroups ($N_m = 44$, $N_f = 34$) for normal distribution. Accounting for small-sized subgroups, which were mostly not normally distributed, the nonparametric Mann-Whitney U test for comparison of two groups was applied with a significance level of $P = 0.05$ in order to find statistically significant differences between (ED_m <> C_m) and (ED_f <> C_f). All statistics were performed using SPSS version 24 (IBM, Armonk, NY). Detailed results are provided in the Supplementary Materials in Table S1 and Table S2.

RESULTS

The total number of 78 data sets did not enter the statistical analysis in its entirety. In the auditory-perceptive evaluation, three recordings of the reading text had to be excluded due to their speaker's foreign accents inhibiting an unbiased assessment. One acoustic recording for calculation of objective parameters had to be excluded for environmental background noise. Finally, the largest losses were registered among the HSV recordings, where nine recordings did not meet the necessary quality or length for further processing, which is typically due to the gagging reflex or fogging. A detailed account of the analyzed data sets in each subgroup is given in Table 2.

An overview on the mean values and standard deviations of all evaluated parameters is presented in Table 3. The individual parameters are grouped under the corresponding measurements. The investigated subgroups in the columns were sorted for separate comparison of males (left: ED_m <> C_m) and females (right: ED_f <> C_f). The pairs of mean values with statistically significant difference according to the Mann-Whitney U test were highlighted by red cells. Further details on the test results of the statistical analysis can be found in the Supplementary Materials (Table S2).

TABLE 2.
Available Data Sets Per Group That are Suitable for Evaluation and Comparison.

Group Measurement	# ED_m	# C_m	# ED_f	# C_f
Saliva measurement	18	26	13	21
Voice handicap index	18	26	13	21
Phonetogram	18	26	13	21
Auditory-perceptive evaluation	17	26	12	20
Objective audio parameters on /a/	17	26	13	21
Objective HSV parameters on /i/	16	23	11	19

For visualization and intuitive comparison, a series of box-whisker-plots was generated displaying the median values as well as the interquartile ranges (IQR) limited by the lower and upper quartiles, eg in Figure 1. The whiskers indicate the highest and lowest values within 1.5 IQR. In order to reflect the complete range of data points, the outliers (>1.5 IQR) were included as circles and extreme outliers (>3 IQR) as stars. Statistically significant differences within each gender were indicated by brackets ($P < 0.05$). For the sake of completeness, the box-whisker-plots of parameter groups that display no statistically significant differences between patients with ED and controls are provided in the Supplementary Materials (Figure S1-S2).

Saliva production
The mean values of the measured weight of saliva for all subgroups are shown in Table 3. The Mann-Whitney U test in the male group showed that the difference between the mean saliva weight of 1.2 ± 0.8 g for ED_m and 4.4 ± 2.2g for C_m was statistically significant ($Z = -5.063$, $P < 0.001$). The small difference between ED_f (2.5 ± 1.3g) and C_f (3.4 ± 2.1g) was not statistically significant.

The distribution of saliva over the test groups is illustrated in Figure 1 (left). The interquartile ranges (IQR) and whiskers of the control groups C_m and C_f were considerably larger than for ED_m and ED_f. Despite the reduced median and range of saliva in ED_f compared to C_f, the data lies within the IQR of C_f reflecting the results of Mann-Whitney U test.

Self-evaluation by VHI
In the group comparison of male test subjects (Table 3), a statistically significant increase in the mean values of VHI for ED_m (28.8 ± 22.7) compared to C_m (5.1 ± 5.9) was found (Mann-Whitney U: $Z = -4.676$, $P < 0.001$). The enlarged whiskers and outliers (VHI = 93) in the boxplot illustration in Figure 1 (right) attest to the widely extended data distribution covering almost the complete scale to the maximum of VHI at 120.

TABLE 3.
Mean Values and Standard Deviations of all Investigated Parameters Averaged for all Test Groups. Statistically Significant Differences ($P < 0.05$ in Mann-Whitney U test) Between ED Patients and Controls are Highlighted in Bold Style.

Parameter (unit)	ED_m mean ± std	C_m mean ± std	ED_f mean ± std	C_f mean ± std
Saliva (g)	**1.2 ± 0.8**	**4.4 ± 2.2**	2.5 ± 1.3	3.4 ± 2.1
Patient's self-evaluation				
VHI (a.u.)	**28.8 ± 22.7**	**5.1 ± 5.9**	**13.1 ± 12.3**	**4.4 ± 5.0**
Phonetogram				
Lowest f_0 (Hz)	87.3 ± 10.4	86.1 ± 12.0	131.5 ± 34.4	135.4 ± 19.6
Highest f_0 (Hz)	**384.8 ± 100.7**	**503.6 ± 167.6**	673.2 ± 208.7	745.7 ± 309.8
Pitch range (Hz)	**297.6 ± 103.5**	**419.2 ± 166.6**	541.7 ± 206.3	610.2 ± 308.6
Lowest SPL (dB)	52.2 ± 7.6	53.1 ± 7.1	52.8 ± 5.4	49.2 ± 5.8
Highest SPL (dB)	92.2 ± 9.6	94.0 ± 7.0	89.5 ± 8.8	90.7 ± 10.1
Intensity range (dB)	40.1 ± 13.0	40.9 ± 8.7	36.8 ± 11.0	41.4 ± 13.6
Auditory-perceptive evaluation				
Roughness R (a.u.)	0.78 ± 0.64	0.50 ± 0.45	0.50 ± 0.54	0.23 ± 0.29
Breathiness B (a.u.)	**0.55 ± 0.55**	**0.14 ± 0.21**	0.25 ± 0.25	0.22 ± 0.29
Hoarseness H (a.u.)	**1.00 ± 0.62**	**0.54 ± 0.44**	0.56 ± 0.50	0.37 ± 0.34
Objective audio parameters on /a/ (5 sec)				
CPP (dB)	29.4 ± 3.8	31.3 ± 2.8	33.4 ± 5.0	32.8 ± 2.9
Shimmer (%)	**16.9 ± 15.4**	**10.3 ± 11.2**	18.5 ± 27.6	10.1 ± 6.4
Jitter (%)	**2.0 ± 1.6**	**0.9 ± 1.1**	1.9 ± 1.4	1.4 ± 0.7
Objective HSV parameters on /i/ (25 cycles)				
Closing Quotient (a.u.)	0.36 ± 0.10	0.34 ± 0.10	0.39 ± 0.12	0.46 ± 0.08
Glottal Gap Index (a.u.)	0.05 ± 0.09	0.03 ± 0.06	0.08 ± 0.16	0.08 ± 0.09
Amp. Periodicity (a.u.)	0.98 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	0.98 ± 0.01
Time Periodicity (a.u.)	0.97 ± 0.01	0.96 ± 0.02	0.96 ± 0.02	0.96 ± 0.02
Phase Asymm. Index (a.u.)	0.03 ± 0.04	0.01 ± 0.03	0.03 ± 0.04	0.03 ± 0.04
Amp. Symm. Index (a.u.)	0.91 ± 0.04	0.91 ± 0.04	0.92 ± 0.04	0.92 ± 0.06

In the female test group, there was a statistically significant difference in the mean values of subjective self-evaluation by VHI questionnaire between 13.1 ± 12.3 for ED_f and 4.4 ± 5.0 for C_f (Mann-Whitney U: $Z = -3.173$, $P = 0.002$). It is notable that this was the only significant difference in the comparison of female participants in the investigated parameters of this study (Table 3).
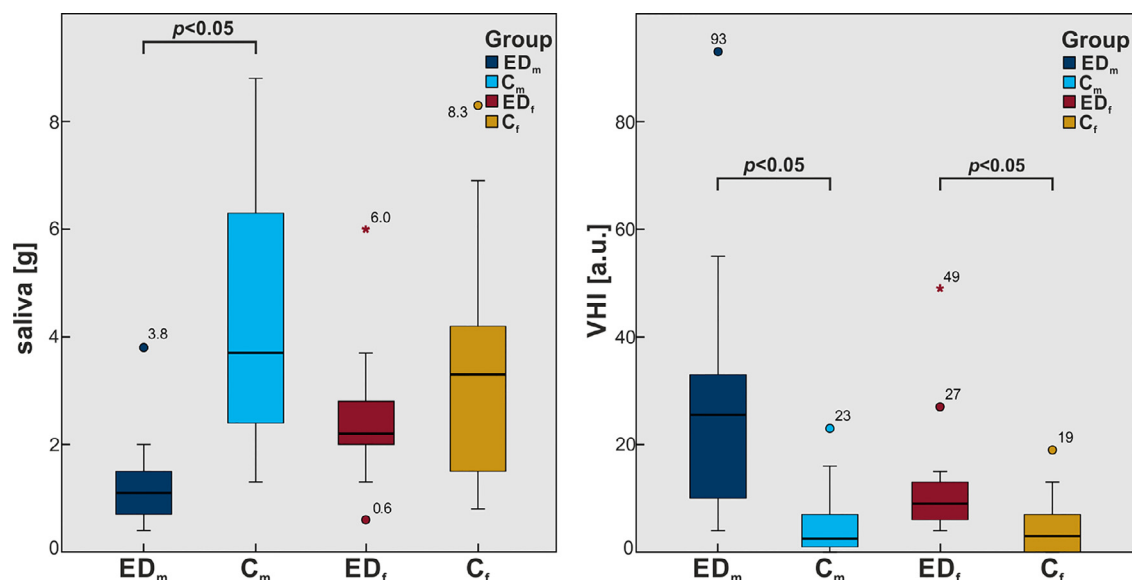

FIGURE 1. Boxplot of saliva (left) and VHI (right) for all four test groups. Statistically significant difference within each gender group is indicated by brackets ($P < 0.05$).

Auditory-perceptive measures

The professional assessment of the standardized reading passage determined increased mean values in all auditory-perceptive measures for both ED groups with respect to their control group (Table 3). However, only breathiness and hoarseness in the comparison of ED_m to C_m proved to be statistically significant in the Mann-Whitney U test (breathiness: $Z = -2.727$, $P = $ x003D 0.006; hoarseness: $Z = -2.356$, $P = $ x003D 0.018). The roughness values of ED_m were slightly increased with respect to C_m, but yielded no significant P-values.

All boxplots in Figure 2 illustrate that the covered range of values is widest for the experimental group ED_m. For the female test group ED_f, the boxplots of roughness and hoarseness showed small widening of the covered ranges compared to C_f. However, this trend did not prove to be statistically significant.

Phonetogram

As part of the phonetogram the lowest and highest achievable frequencies and the resulting pitch range of each test subject as measured by a trained SLP are given in Table 3. Naturally, the male ($f_{0,min} \approx 87$ Hz) and female ($f_{0,min} \approx 133$ Hz) test groups were clearly separated. However, within each gender group, the mean values of the lowest obtainable frequency differed by less than 5 Hz between ED patients and controls, which was equally reflected in the non-significant statistics and in the boxplot with medians, see Figure 3 (left). The Mann-Whitney U test on the mean values showed, that the difference in the highest possible frequency between males' groups ED_m (384.8 ± 100.7 Hz) and C_m (503.6 ± 167.6 Hz) was statistically significant ($Z = -2.244$, $P = 0.025$). The difference between the females ED_f (673.2 ± 208.7 Hz) and C_f (745.7 ± 309.8 Hz) was not statistically significant ($Z = -0.408$, $P = 0.683$). The boxplot depiction on medians and quartiles was less clear for this distribution, see Figure 3 (middle). As a result of lowest and highest achievable frequency, the difference in pitch range between

ED_m (297.6 ± 103.5 Hz) and C_m (419.2 ± 166.6 Hz) was statistically significant ($Z = -2.292$, $P = 0.022$) but not between ED_f and C_f.

In addition to the range of frequencies, the maximum range of attainable intensity was ascertained by the lowest and highest sound pressure level (SPL). The resulting mean values are remarkably homogeneous over all four test groups (Table 3). The lowest SPL over all four test groups was around 50 dB, the highest SPL was around 90 dB and the resulting intensity range at 40 dB. The mean values of lowest SPL, highest SPL and intensity range differed by less than 2 dB between the male groups ED_m and C_m and less than 5 dB between the female groups ED_f and C_f. None of the comparisons displayed statistical significance. The corresponding boxplots of the intensity ranges along with further details on the statistic tests are given in the Supplementary Materials (Figure S1).

Objective acoustic parameters

The acoustic parameters are calculated on the basis of sustained phonation of the vowel /a/. In contrast to the phonetogram, the acoustic signal is recorded at comfortable pitch and loudness. The fundamental frequency is determined with the GAT software by minimum-based cycle detection. The mean values for each test group are given in Table 4. The mean values in the male subgroups differ only marginally by 4 Hz while the female subgroups display 30 Hz difference in the fundamental frequency of the acoustic recording.

The mean values of the objective acoustic parameters CPP, Shimmer and Jitter for all subgroups are shown in Table 3. The mean values of CPP were slightly higher for the female subgroups around 33 dB than for the male subgroups around 30 dB. The differences between ED patients and controls were not statistically significant, which was also reflected in the rather homogeneous distribution in the boxplots of Figure 4 (left).

The mean Shimmer values were larger in both ED groups (ca. 17%) compared to their controls (ca. 10%) in Table 3.
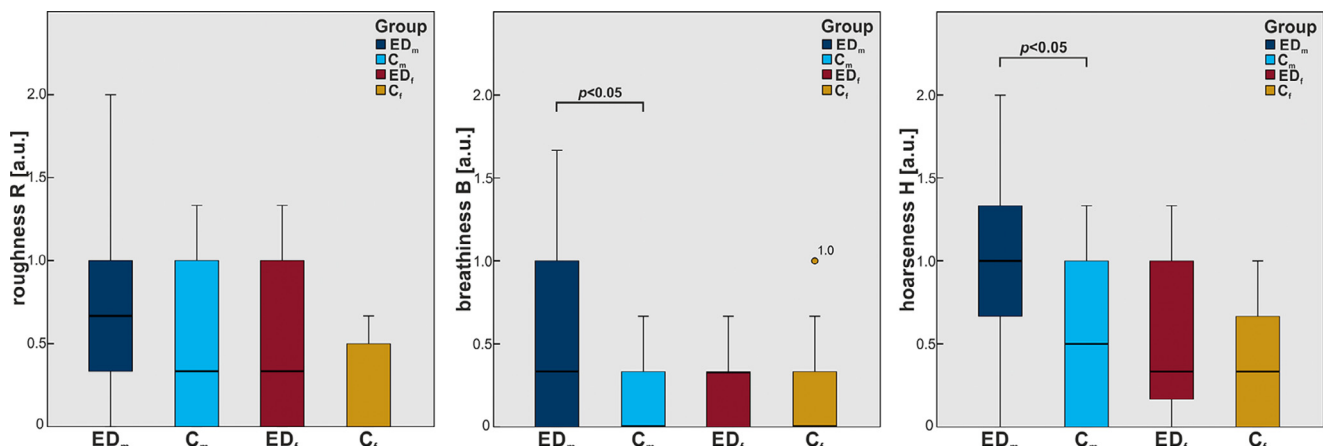

FIGURE 2. Boxplot of roughness, breathiness and hoarseness for all four test groups. Statistically significant difference within each gender group is indicated by brackets ($P < 0.05$).
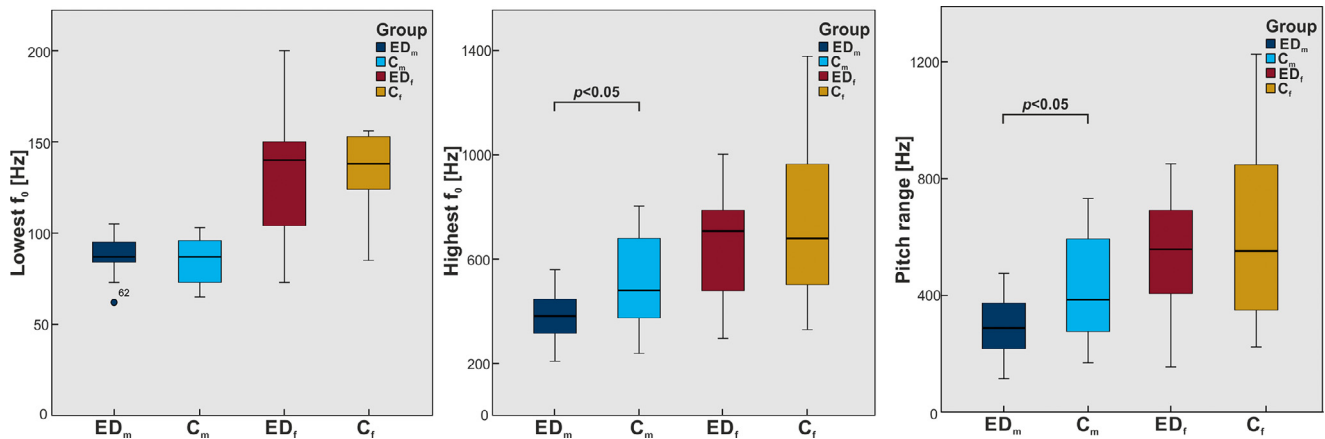
FIGURE 3. Boxplot of achieved frequencies in phonetogram: lowest f_0, highest f_0 and pitch range for all four test groups. Statistically significant difference within each gender group is indicated by brackets ($P < 0.05$).

Despite relatively large standard deviations in both male subgroups (see Figure 4 [middle]), the differences between the mean Shimmer values of ED_m (16.9 ± 15.4%) and C_m (10.3 ± 11.2%) were statistically significant according to the Mann-Whitney U test ($Z = -2.385$, $P = 0.017$). Apart from one extreme outlier in ED_f, the distribution of the female Shimmer values displayed a smaller variation and no statistical significance was found.

In Figure 4 (right), the Jitter values behaved similarly but more pronounced than the Shimmer values. As shown in Table 3, the mean values in the ED groups were both larger than the corresponding control group. The differences between the mean Jitter values of ED_m (2.0 ± 1.6%) and C_m (0.9 ± 1.1%) were statistically significant according to the Mann-Whitney U test ($Z = -3.055$, $P = 0.002$), whereas the differences in the female subgroups were not statistically significant.

Objective HSV parameters
The HSV parameters were calculated on the basis of sustained phonation of the vowel /i/. Just as for the computed acoustic parameters, the HSV was recorded at comfortable pitch and loudness. The fundamental frequency was determined with the GAT software by maximum-based cycle detection. The mean values for each test group are given in Table 4. The mean values in the male subgroups were about 100 Hz smaller than in the female recordings. Both ED groups were 25−30 Hz below their gender-specific control groups.

Objective HSV parameters were calculated for three different characteristics, namely closure, periodicity and symmetry, Table 3. Mean values and standard deviations of all investigated parameters averaged for all test groups. Statistically significant differences ($P < 0.05$ in Mann-Whitney U test) between ED patients and controls are highlighted in red cells. Apart from the two closure values CQ and GGI, the determined mean values were almost identical over all subgroups: AP = 0.98, TP ≈ 0.96, PAI ≈ 0.03, ASI ≈ 0.91. The only noticeable deviation occurred between the closure parameters of males and females, however independent from the experimental group (ED or C). Females displayed slightly larger closing quotients and higher glottal gap indices (CQ ≈ 0.43, GGI = 0.08) than males (CQ ≈ 0.35, GGI = 0.04). According to the Mann-Whitney U test, none of the objective HSV parameters proved to be statistically different between the experimental groups (ED or C). More details on the statistics and the boxplot for all HSV parameters are provided in the Supplementary Materials (Figure S2).

DISCUSSION
The findings on the reduced salivation concur with other studies on patients with ED, showing significantly reduced saliva in ED_m compared to C_m.[16,35] The generated amount of saliva was reduced for ED_f compared to C_f, as well, but the effect was not statistically significant. This is in line with literature reporting on microsymptoms in female ED patients.[15,36]

TABLE 4.
Fundamental Frequency Determined by Cycle Detection on Sustained Phonation of /a/ in Acoustic Signal and of /i/ in HSV Recordings. Mean Values and Standard Deviations are Calculated Separately for Each of the Four Test Group.

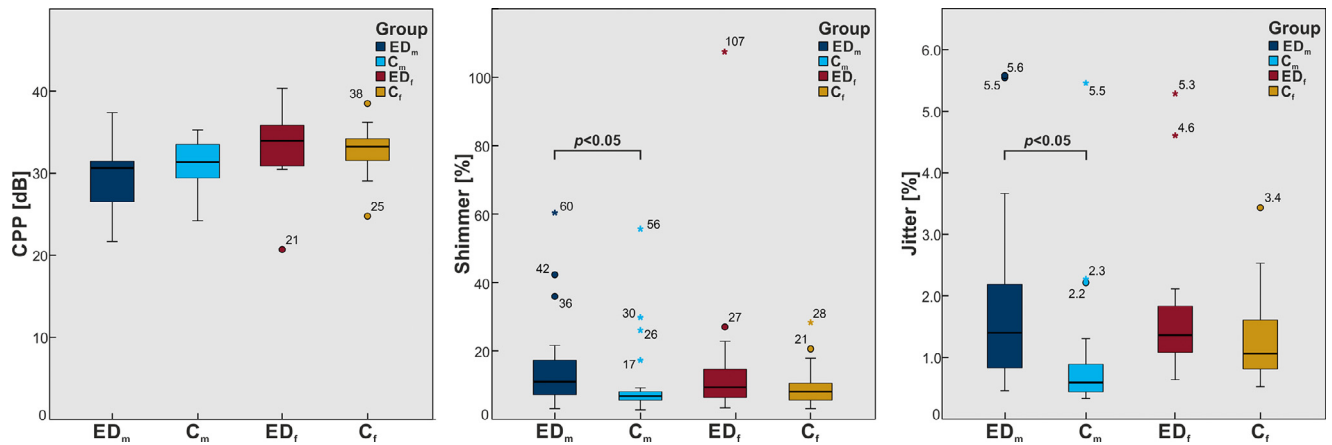| Group Parameter	ED_m mean ± std	C_m mean ± std	ED_f mean ± std	C_f mean ± std
/a/: $f_{0,audio}$ (Hz)	126.5 ± 18.8	122.7 ± 29.6	239.5 ± 51.5	209.6 ± 33.4
/i/: $f_{0,HSV}$ (Hz)	170.5 ± 43.0	196.4 ± 79.1	260.8 ± 44.1	292.4 ± 63.5

FIGURE 4. Boxplot of objective acoustic parameters calculated on sustained phonation of vowel /a/: CPP, Shimmer and Jitter for all four test groups. Statistically significant difference within each gender group is indicated by brackets ($P < 0.05$).

The different elements of the ELS protocol enabled an objective and differentiated assessment of the voice in ED patients compared to vocally healthy controls.

Clinical parameters

The VHI questionnaire documented functional, physical and emotional aspects resulting from the impaired voice in daily life on a scale 0-120. The determined mean values of both ED groups compared to their gender-specific control groups were significantly increased. 67% of the male ED patients and 23% of the female ED patients stated at least "minor" impairment of the voice (VHI > 14) compared to less than 8% in C_m and 2% in C_f.[37] In the ED_m group, even four cases with "high degrees" of voice impairment (VHI > 50) occurred. It is noteworthy, that this was the only parameter in the ELS protocol that was tested statistically significant between ED_f and C_f, even though the expression of ED symptoms is typically greatly reduced in females.[36]

In the auditory-perceptive evaluation, only the parameters breathiness and consequently hoarseness were affected in the male ED group, confirming earlier studies.[17] With less than 30% of ED_m displaying more than H = 1 (minor limitations) and no cases of H>2 (severe limitation), the perceived voice quality was not drastically affected but the effect was still statistically significant.

In order to put the self-evaluation via VHI questionnaire and the external assessment on the RBH scale by trained SLPs into perspective, a scatter plot with the data of all test subjects and the mean values over all subgroups is provided in Figure 5. Both control groups were mainly located around VHI = 5 and up to H = 1. The ED_f group was only slightly shifted towards higher VHI values (13.1) covering a larger area than C_f up to VHI = 49. The mean value and distribution of ED_m was distinctly shifted towards higher values of VHI and hoarseness. It is remarkable that some test subjects (VHI < 30; H = 2) with the subjective impression of a minor impairment

displayed the highest H values in this distribution, whereas the highest VHI values occurred with best SLP ratings for hoarseness (VHI = 93; H = 0.3).

The phonetogram showed that only the highest achievable frequency was affected in male ED patients, but not the lowest frequency. Normative values for $f_{0,max}$ in healthy males are inconsistent in literature ranging from around 300 Hz[38] to over 600 Hz.[39] Even though the highest f_0 was significantly lower in ED_m compared to C_m, the obtained pitch range is not pathological.[38] It has been shown that the observed decrease of the highest reachable frequency is also typical for functional dysphonia.[39]

Objective acoustic parameters

Based on a 5 sec sequence of sustained phonation (/a/), three objective acoustic parameters were calculated, i.e. CPP, Shimmer und Jitter. The mean CPP values of around 30 dB for all groups were higher than the typically reported normative range of healthy adults, which could be attributed to different recording techniques.[40] The statistical test and graphic evaluation displayed no clear trends between ED patients and control. As a measure of the harmonic content, the parameter seemed to be unaffected by this disease. As opposed to this, the Jitter and Shimmer quantifying the variability of the periodic length and amplitude in the acoustic signal were significantly increased for ED_m compared to C_m. The increased values of Jitter and Shimmer indicated lower regularity of the signal for ED_m and by this lower acoustic quality. Curiously, the decreased regularity is usually accompanied by increased values for roughness in the perceptive evaluation, which did not prove to be statistically significant in this study.

From the results of the phonetogram with significantly decreased $f_{0,max}$ for the ED_m group, it could be assumed that the acoustic parameters might be dependent on the fundamental frequency, which would distort the statistical effects due to collinearities. In order to exclude this kind of bias on the acoustic parameters, the fundamental
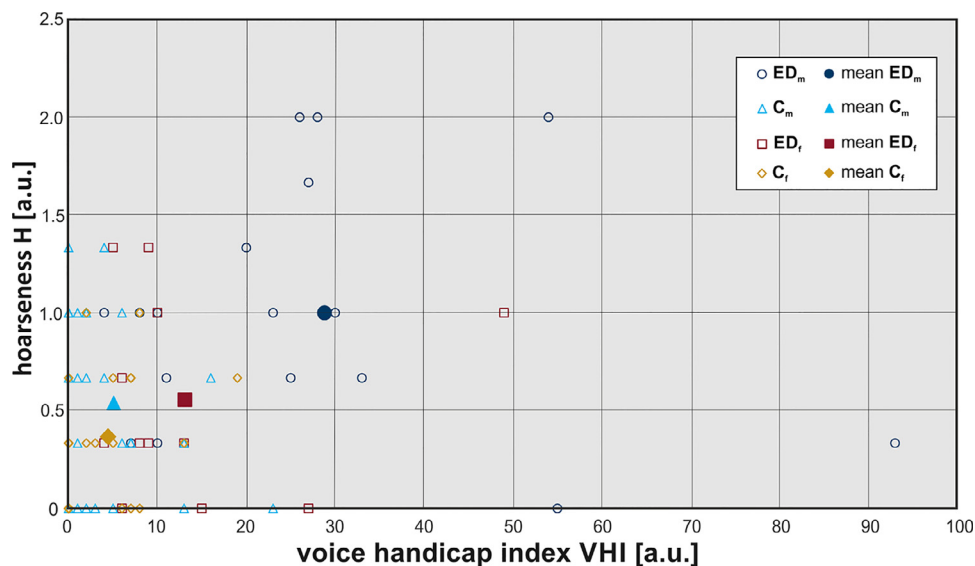
FIGURE 5. Distribution of hoarseness over VHI for four test groups. Mean values calculated separately over each test group are indicated by corresponding filled marker points.

frequencies of all acoustic recordings were evaluated which yielded no significant difference, as shown in Table 4.

In addition to the Mann-Whitney U tests between two subgroups, an investigation on the influence of saliva on the parameters with statistically significant differences between ED_m and C_m would be highly desirable. Further statistical tests on the underlying correlations are thought to provide a deeper understanding of the role of saliva (and vocal fold hydration in general) on the phonation process. However, due to small samples sizes and insufficient homogeneity in the subgroups, we refrained from further tests like AN(C) OVA or mixed models, until further data is recorded.

As a preliminary measure, a graphic evaluation of the parameters with prominent differences between the data sets of ED_m and C_m was chosen. Representatively, the distribution of Jitter% over saliva in Figure 6 allows a comparison of male ED patients and male controls. Further distributions of breathiness, hoarseness and $f_{0,max}$ from the phonetogram is provided in the Supplementary Materials (Figure S3-S5).

In Figure 6, the two data sets ED_m and C_m are clearly separated along the x-axis displaying the measured weight of saliva. Moreover, the offset of the ED_m group and its mean value towards higher Jitter values is obvious, as well. However, there was no indication within either subgroup that smaller amounts of saliva generally result in higher Jitter values. Based on the graphic evaluation, it was not conclusive that the reduced salivation causes the reduced voice quality. Other properties than the sheer weight of the saliva, like viscosity or rheology,[11] may contribute to the phonatory process and the acoustic outcome.

Objective HSV parameters
Based on the endoscopic HSV recordings of sustained phonation of /i/, objective HSV parameters were calculated to

reflect the essential properties of vocal fold dynamics being closure, symmetry and periodicity. However, none of the determined parameters showed statistically significant differences between EDs and controls. All parameters lay within the normative range of healthy adults.[41,42] Only, the GGI was slightly increased for the female subgroups compared to the male subgroups, which is known in physiological phonation of women and children.[43−45] Moreover, even though not statistically significant, the GGI was slightly increased for ED_m (0.05 ± 0.09) to C_m (0.03 ± 0.06), which might be associated to the increased breathiness in the ED_m subgroup.[46]

In line with literature, the evident decrease of saliva production in ED patients (Table 3, Figure 1) insinuated a similar reduction of the laryngeal mucus.[35,16] Furthermore, it was reported in numerous studies, that the hydration level of the vocal folds affects the glottal dynamics.[47,48] Therefore, it is remarkable that despite the decreased salivation in ED patients and despite the undeniable impairment of the acoustic outcome, there was no statistically significant effect on the vocal fold dynamics quantified by the HSV parameters in this study.

Nevertheless, there could still be a significant difference in male ED patients on the laryngeal level, which is simply not reflected in the chosen HSV parameters. These differences between male ED patients and controls could possibly arise from the interaction at the boundary layer between airflow and mucosal tissue surface. In the future, this should not only be further investigated in the larynx but in the complete vocal tract.

LIMITATIONS / OUTLOOK
A common drawback in studies on rare diseases like ectodermal dysplasia is the limited number of participants, which hampers the application of advanced statistical
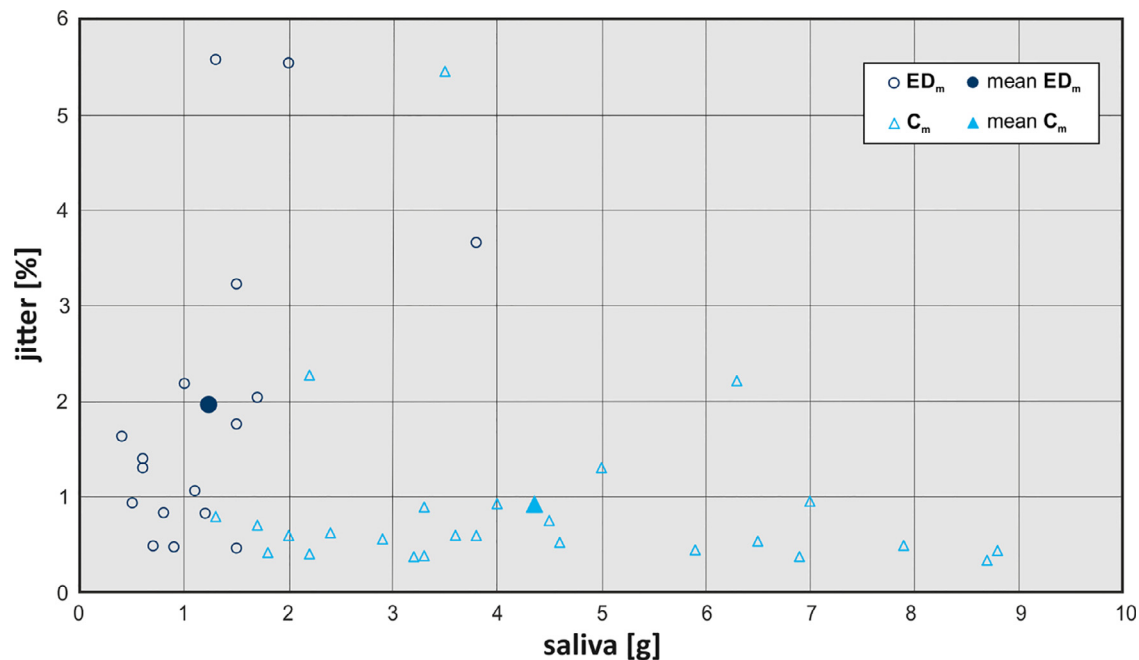
FIGURE 6. Distribution of Jitter% over saliva for male test groups. Mean values calculated separately over each test group are indicated by corresponding filled marker points.

models. Furthermore, in this preliminary study, the genetic expression of the ED patients could not be considered. It will be insightful to analyze if and how the different forms of ED are possibly affected in different ways.

Future studies should incorporate further details on the vocal tract by considering accompanying symptoms like the dental status and dryness of the oral cavity. Several conditions involving mucous glands like Sjögren's syndrome exhibit a proneness to gastrointestinal problems and laryngopharyngeal reflux, which could equally influence the voice quality.[49−51]

The high-speed videos were recorded in black/white images, which allows for automated computer-based analysis but only limited evaluation of inflammation, laryngitis, dryness etc. A second endoscopy with colored imaging would have exceeded the timeframe of this study. Furthermore, we deliberately concentrated on reliable measures and decided to evaluate the HSV recordings by objective parameters only. However, we plan to extend the objective analysis to saliva and laryngeal mucus. In addition to the mere weight of the generated saliva, future studies are planned to incorporate the composition (i.e. enzymes, proteins, etc) and consistency (i.e. elasticity, viscosity, etc) of the collected saliva. In order to ensure comparability of the data sets, the conditions of the saliva measurement should be further restricted with regard to drinking, teeth brushing and smoking prior to the measurements.[52] Even the time of the day should be considered as it might influence the saliva production.[53]

In order to gain further knowledge on the functional interaction in the larynx and the effect of hydration, it would be preferable to investigate the laryngeal mucus besides the saliva from the oral cavity. However, the laryngeal mucus is practically inaccessible without sedation of the patients,[11] which limits the availability severely. Therefore, further research on the general correlation of saliva and laryngeal mucus would be desirable even outside the context of ectodermal dysplasia.

CONCLUSIONS

The standardized ELS protocol for the functional assessment of the voice comprised subjective and objective evaluations of the phonation process, which were complemented by blinded ratings and statistical methods. Overall, this study demonstrated an unambiguous impairment of the acoustic outcome in male patients with ectodermal dysplasia (ED) but no affliction of the vocal fold dynamics in the HSV recordings. The impairment of the voice might seem subordinate compared to other symptoms of ED. However, since the voice contributes significantly to the perceived quality of life[3,54−56] it should be considered seriously within the diagnostic and therapeutic process. Further investigations are required to determine the role of hydration within the fluid-structure-acoustic interaction of the phonation process.

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