Kymographic characteristics of voice in women with Polycystic ovary syndrome

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Keywords
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ABSTRACT

Purpose: Polycystic ovary syndrome (PCOS) is a heterogenous condition with commonly associated symptoms include irregular menstrual cycle, hirsutism, baldness, adult acne, and weight gain. There have been few attempts at profiling the voice characteristics of women with polycystic ovary syndrome. Videokymography enables to detect even subtle variations in vocal fold vibrations. The aim of the present study was to study the videokymographic characteristics among women with PCOS. Methods: A cross-sectional study was carried out among 50 women with and without PCOS diagnosed on ultrasonography. Videokymography was carried out and the characteristics were perceptually analyzed using a vocal fold kymographic rating scale. The analysis of the kymogram was done for the following characteristics: presence of vocal fold vibration, interference of surroundings, cycle to cycle variability, left-right asymmetry, cycle aberrations, and shape of lateral peaks. The kymographic images were obtained for all the participants of both the groups and a subjective consensus evaluation was done by two clinicians. The percentage of participants with the listed kymographic characteristics were tabulated. Chi Square test was also done to decide if there was a significant difference between the two groups for different kymographic features of vocal fold vibration. Results: Six of the 25 women with PCOS were found to have abnormal kymographic features such as surrounding structural interference, presence of cycle to cycle variability, and the shape of lateral peaks. Conclusion: Early detection of the vocal abnormalities in individuals with PCOS would help in the vocal rehabilitation especially for professional voice users.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous condition seen in one out of fifteen women of reproductive age\(^1\). The condition is often diagnosed based on the Rotterdam criteria in which women with any of the two out of three conditions are diagnosed to have PCOS. The conditions include oligo/anovulation, hyperandrogenism and polycystic ovaries\(^2\). Hyperandrogenism is one of the most important features to be noted in the women with PCOS in which the excessive level of androgen leads to a deepening of voice. The excessive level of androgen level of more than 200µg/dL affects the larynx, mainly the vocal folds\(^3\). Some of the commonly associated symptoms include irregular menstrual cycle, hirsutism, baldness, adult acne, and weight gain. It is also a leading cause of infertility; if left untreated can lead to long-term risks of diabetes, endometrial cancer, cardiovascular diseases and stroke\(^4,5\).

Voice is one of the characteristics that makes each individual unique from each other. Voice is produced as a result of vocal fold vibration of the larynx and the hormonal environment inside and outside the body plays a critical role in determining the quality of voice of an individual\(^6\). These laryngeal structures notably respond to the sex hormones namely estrogen, progesterone as well as androgens. The mucosa of the vocal folds has hormonal receptors. Excessive androgen level in the human body causes cells to be hypotrophied, dehydrated and secretions of glands to be reduced. This leads to the evolution of vocal characteristics that are masculine and irreversible if the androgen level exceeds 200µg/dL.\(^3\) Hannoun et al.\(^7\) reported certain vocal symptoms such as a sensation of a lump in the throat, clearing of throat, deepened voice as well as reduced loudness range in women with PCOS.

Despite the higher prevalence of PCOS, very little is known about the voice measures in affected women. Gugatschka et al.\(^8\) studied the vocal changes in 24 individuals with confirmed PCOS and 10 normals using videolaryngostroboscopy, acoustical analysis and German version of the Voice Handicap Index. The results revealed no significant changes in subjective and objective parameters of voice except for the decrease in fundamental frequency. VHI also did not show any differences between the clinical and the control groups suggesting that women with PCOS had no effects on the speaker’s voice-related quality of life. The results of basic parameters and endocrinologic serum values revealed that differences in androgen and associated testosterone levels were not high enough to result in virilization associated with voice change. Gugatschka et al.\(^8\) described a trend toward lower mean fundamental frequency. The subtle variations in the vocal fold vibration are yet to be undiscovered. Aydin et al.\(^9\) observed abnormal muscle tension patterns with impaired vocal fold vibration in women with PCOS using videostroboscopy.

In early studies, they have studied the vocal fold vibratory motions using videostroboscopy. However, it is not a real time measure and hence measures such as kymographic characteristics offers the study of complex motions of vibratory mucosa in real time. Svec et al.\(^10\) explored over 7000 videokymographic images across a wide range of voice disorders to provide objective documentation for monitoring vocal fold behaviour. Videokymography measures are expected to identify subtle variations in the vocal fold vibratory pattern and is yet to be explored in affected women with PCOS. Qiu and Schutte\(^11\) have provided further evidence to support that videokymography provides a simple and quick method to investigate vocal fold vibration and emerges as an important tool for inclusion in the routine clinical vocal fold examination. Till date, no studies have explored the kymographic characteristics of women diagnosed with PCOS. The present was planned in this direction with the aim of investigating the kymographic characteristics in women diagnosed with PCOS.

METHODS

The study was carried out after obtaining approval from the Institutional Ethical Committee (IEC KMC-MLR-10/16-278). An informed consent was taken from each participant after explaining the study.

Participants

The study was carried out among 50 women with and without PCOS. They were divided into 2 groups. Group I comprised of the 25 women (Mean age 22.3 ± 1.28 years, range 18 – 30 years) in the clinical group diagnosed with PCOS on ultrasound, while Group II comprised of 25 typical women as controls (Mean age 22.9 ± 0.91 years, range 18-30 years). The inclusion criteria for selection of Group I participants was based on the polycystic ovaries on ultrasound. The inclusion criteria for Group II was women rated to have a normal voice as assessed using Consensus Auditory-Perceptual Evaluation of Voice (CAPE-V). Two voice experts with over five years of clinical and research experience in the area of voice and voice disorders served as experts for the auditory perceptual evaluation of voice. The standardized CAPE-V tasks were used for carrying out the auditory perceptual evaluation of voice to ascertain perceptually normal voice quality. Further, these women had a regular menstrual cycles and no PCOS on ultrasound. Women with hypersensitive gag reflex, alcohol consumption, smoking, reflux symptoms and vocal abuse were excluded in both groups.

Instrumentation

Videokymographic system (Model 2156, CYMO, Netherlands) consisting of videokymographic camera to capture the image, a laryngoscope with standard C-mount, objective adapter for the camera and a continuous light source of high intensity. A standard video recording system was used to record the video signal.

Procedure

The participants were seated comfortably on a chair and were asked to phonate the sustained vowel /i/ at habitual pitch and loudness, while a 70-degree ATMOS rigid endoscope of 8mm diameter was inverted and correctly positioned in the laryngopharynx exactly above the vocal folds, in order to select the line of measurement to be examined. The line of

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measurement was perpendicular to the glottal axis in the middle of the vocal fold. The vocal folds were scanned from anterior to posterior to capture the vibrations along the whole glottal length. All the samples with even a slight deviation as compared to normal, were repeated for a re-evaluation. Further, 10% of samples in both groups were repeated for assessing intra-rater and inter-rater agreement. A 100% intra-rater and 90% inter-rater agreement was noted.

**Analysis of the kymogram**

Approach of systematic visual analysis for the subtle variations in the vocal fold vibrations was carried out through subjective consensus by two experienced clinicians. Modified Vocal fold kymographic image analysis – Rating scale\(^{(12)}\) was used to analyze the kymogram. Following are the features analysed in the kymograms of both the groups.

a) Presence of vocal fold vibration: This parameter provides information about the vibration of the vocal folds. i.e. whether both the vocal folds are vibrating or not. The rating is as follows; ‘0’ indicates no vibration of the vocal fold, ‘1’ when the vocal fold is vibrating partially and ‘2’ when the vocal fold is vibrating completely. The right and left vocal folds are scored separately.

b) Interference of surroundings: This parameter mainly deals with the structural interference such as arytenoid cartilages, aryepiglottic folds as well as epiglottis, if any. If there is a significant interference there are less chances of obtaining a kymogram. This is rated as ‘0’ if there is no or a negligible interference, ‘1’ if a small interference and ‘2’ if a significantly large interference.

c) Cycle to cycle variability: This mainly deals with the variability of changes in the consecutive cycles in terms of overall shape as well as the amplitude and is rated as ‘0’ for no variability, ‘1’ for small variability, ‘2’ for medium variability and ‘3’ if the variability is significantly large.

d) Left – Right asymmetry: This parameter is analyzed on the basis of frequency differences as well as phase differences between right and left vocal folds. Frequency differences are rated in such a way that ‘0’ if there is no difference, ‘1’ if there is a small difference, ‘2’ if a medium difference, ‘3’ if there is a large difference. The phase difference is also rated in a similar way such that ‘0’ if there is no difference, ‘1’ if there is a small difference, ‘2’ if a medium difference, ‘3’ if there is a large difference.

e) Cycle aberrations: Cycle aberrations are rated as ‘0’ if there is no ripple, double medial peaks or medium unsmoothness and ‘1’ if these are present which will affect the regular oscillations which are periodic in nature.

f) Shape of lateral peaks: This is based on the vertical phase differences. This is rated as ‘0’ if the peaks are sharp, ‘1’ if somewhat sharp, ‘2’ if somewhat rounded, ‘3’ if rounded/ disturbed/others.

**Statistical analysis**

The kymographic images were obtained for all the participants of both the groups and a subjective consensus evaluation was done by two clinicians based on the approach of systematic visual analysis for the subtle variations in the vocal fold vibrations if any. The percentage of participants who had shown the listed kymographic characteristics were tabulated. Chi Square test was also done to decide if there was a significant difference between the two groups for different kymographic features of vocal fold vibration.

**RESULTS**

The present study aimed at characterizing the kymographic waveforms in 25 women with PCOS and 25 women controls. The selected features were expressed in percentages for both the group of women. The percentage of participants who have shown the listed kymographic characteristics have been tabulated in the Table 1.

From the above Table 1, it can be noted that the shape of the lateral peaks, cycle to cycle variability, left-right asymmetry with respect to phase and interference of surroundings showed good difference between the normal and women with PCOS indicating that these parameters could be abnormal in women with PCOS. The following Figures (Figures 1-4) depicts the laryngoscopic and kymographic images of women with PCOS obtained during the phonation of high pitch sustained vowel /i/.

The results obtained using systematic visual analysis through consensus evaluation by two clinicians were statistically analyzed using Chi Square test. This was performed to find the significant difference if any between the groups for the kymographic parameters selected. Cycle to cycle variability, left right asymmetry, and the lateral peaks showed significant difference between the groups at p< 0.01. However, the other parameters did not show any significant difference at p>0.05.

**DISCUSSION**

Aydin et al.\(^{(9)}\) observed abnormal muscle tension patterns and impaired vocal fold vibration based on stroboscopic findings in women with PCOS. Stroboscopy and VKG differ in terms of the type of camera apparatus used as well as the amount of pixel data that is collected. While stroboscopy involves 30 frames per second, VKG technique makes use of 8,000 lines per second. Stroboscopic examination makes it difficult to visualize aperiodicity or fluctuating fundamental frequency\(^{(13)}\). While VKG makes it possible to have a qualitative and quantitative description of both periodic and aperiodic vocal fold movement\(^{(14)}\). Thus, kymographic features were investigated in women with PCOS. The findings have been discussed across the different kymographic features.

Vocal fold vibrations were present bilaterally in all the participants (100%) in both the groups. However, the small interference of surroundings was present for 24% of the PCOS participants involving the epiglottis and arytenoid adduction in comparison to 8% in normal women. The interference of...
Table 1. Percentage of participants with following kymographic characteristics

| Characteristics                     | Rating                  | Percentage of normal participants | Percentage of PCOS participants | Chi square, df, p-value |
|-------------------------------------|-------------------------|-----------------------------------|---------------------------------|------------------------|
|                                     |                         | Right (0%) | Left (0%) | Right (100%) | Left (100%) |                                     |
| Presence of vocal fold vibration    | Vibrating               | 0          | 0%        | 0%          | 0%         | -                                    |
|                                     | Vibrating partially     | 1          | 0%        | 0%          | 0%         | -                                    |
|                                     | Not vibrating           | 2          | 0%        | 0%          | 0%         | -                                    |
| Surrounding structural interference | Negligible              | 0          | 92%       | 92%         | 76%        | 76%                                  |
|                                     | Smaller                 | 1          | 8%        | 8%          | 24%        | 24%                                  |
|                                     | Larger                  | 2          | 0%        | 0%          | 0%         | 0%                                   |
| Cycle to cycle variability          | Negligible              | 0          | 92%       | 92%         | 60%        | 56%                                  |
|                                     | Small                   | 1          | 8%        | 8%          | 16%        | 20%                                  |
|                                     | Medium                  | 2          | 0%        | 0%          | 16%        | 16%                                  |
|                                     | Large                   | 3          | 0%        | 0%          | 8%         | 8%                                   |
| Synchronicity                       | Synchronous             | 0          | 100%      | 72%         |            |                                      |
|                                     | Nonsynchronous           | 1          | 0%        | 8%          |            |                                      |
| Left-right asymmetry                | Negligible              | 0          | 100%      | 100%        |            | -                                    |
|                                     | Small                   | 1          | 0%        | 0%          |            | -                                    |
|                                     | Medium                  | 2          | 0%        | 0%          |            | -                                    |
|                                     | Large                   | 3          | 0%        | 0%          |            | -                                    |
| Phase differences                   | Negligible              | 0          | 88%       | 24%         |            |                                      |
|                                     | Small                   | 1          | 12%       | 60%         | Chi value= 19.01, 3, 0.00            |
|                                     | Medium                  | 2          | 0%        | 12%         |            |                                      |
|                                     | Large                   | 3          | 0%        | 4%          |            |                                      |
| Cycle aberrations                   | None                    | 0          | 100%      | 100%        | -          |                                      |
| Ripple/medial unsmoothness/ double  | Present                 | 1          | 0%        | 0%          | -          |                                      |
| medial peak                         | Sharp                   | 0          | 88%       | 16%         | 16%        | 24.47, 3, p< 0.001                  |
|                                     | Somewhat sharp          | 1          | 12%       | 56%         | 56%        |                                      |
|                                     | Somewhat rounded        | 2          | 0%        | 20%         | 12%        |                                      |
|                                     | Rounded/disturb/others  | 3          | 0%        | 8%          | 16%        |                                      |

Figure 1. Interference of surroundings
surrounding structures is often characterized by supraglottic hyperfunction and occurs as a compensation for the underlying pathology. With the presence of such interferences, it is difficult for the visualization of the periodic vibration of vocal folds. However, the other participants with PCOS and normal controls did not exhibit interference of surrounding structures. Moreover, no organic lesions were observed in both the group of participants.

The cycle-to-cycle variability refers to the discrepancies in the consecutive glottal cycles. This is assessed for both the folds separately because it may vary for one vocal fold with
respect to other. The results of the present study revealed that 16% of women with PCOS had small variability, another 16% with medium variability and 8% with large variability. However, medium and large variability were not evident in normal controls. These variabilities are often due to the differences in tension, stiffness, and mass of the vocal folds. In these individuals with PCOS, there is a possibility of increased androgen level, which would have increased the mass of the vocal fold unevenly and led to the large cycle-to-cycle variability in 8% of the participants and the other 16% with medium variability.

Left-right asymmetry parameter mainly highlights the differences in the stiffness, mass, and tension between the pairs of the vocal folds, which was analyzed under frequency, and phase difference. The frequency difference between the pairs of vocal folds is assessed. Such differences occur when there is high asymmetry here, the right or left vocal fold may vibrate faster or slower with respect to each other. This variation would result in slight roughness in voice. However, no participants from both the groups exhibited any frequency difference. Previous studies have indicated that presence of testosterone hormones such as androgen has a significant influence on the vibratory patterns of vocal fold vibration\(^3,15,16\). However, individuals with PCOS did not exhibit any frequency differences across the vocal folds.

The phase differences mainly characterize the vibration of vocal folds at different speeds. It leads to the opening of the faster vibrating folds to reach the maximum opening earlier even if there are synchronous vibratory cycles\(^17\) In this study, 60% of the participants had small phase difference and 12% with medium phase difference and only one client had large phase difference which only constitutes 4% of the population. Haben et al\(^18\) have reported that small phase difference has been noted even in normal individuals. There are different factors that cause asymmetry, which includes differences in the fundamental frequency\(^19\) subglottal pressure\(^19,21\) vocal fold mass and stiffness characteristics, which differ from individual to individual\(^22\) and vocal loading\(^22\) respectively. All these factors would have caused right left asymmetry in the vocal fold vibrations. A study done by Jiang and Titze\(^24\) has revealed that the mass in the vocal folds can lead to amplitude, frequency as well as phase differences. In this view, it can be considered that the participants who have exhibited the phase differences may be due to the increased mass in the vocal folds due to hyperandrogenism.

The shape of lateral peaks deals with the vertical phase difference. Shapes of the lateral peaks vary from sharp to round depending on the movement of upper vocal fold margins with respect to the lower margins. The sharp peak is the result of sudden movement that occurs from the upper margin to lower margin. Whereas the rounded peaks are the result of vertical phase difference\(^27\). In the present study, 16% of participants in PCOS had sharp lateral peaks, 56% of the participants had somewhat sharp peaks, 20% with somewhat rounded shape and 8% with rounded lateral peaks. This difference may be due to the differences in the vocal fold characteristics such as a change in mass, stiffness and tension characteristics. The reduced sharpness may be due to the reduced lateral excursion of vocal folds.

Cycle aberrations parameter mainly analyzes any variation in the glottal cycles without interruption to the periodicity of vocal fold vibration. These aberrations are mainly observed as either ripples, medial unsmoothness or double medial peak. These are mainly observed in cases such as any focal lesions like sulcus vocalis or when the vocal folds are weak\(^40\). These aberrations were not observed in any of the participants in both the groups.

The results of the present study partially find support from Hannoun et al\(^7\) who assessed the prevalence of different vocal symptoms including loss of voice, deepening of voice etc. in women with PCOS when compared to normal women with no PCOS. There was only statistically significant difference in the symptoms of namely, reduced loudness, sensation of lump in throat and deepening of voice. No significant difference was observed with respect to acoustic parameters but a rise in perturbation measures and lowering of maximum phonation time. The possible reasons they have mentioned for this difference is the factor of hyperandrogenism in women with PCOS, which have affected the hormonal atmosphere in larynx and would have led to the increase in the thickness of the vocal muscle. This would have led to the decrease in fundamental frequency of vocal fold vibration. Also, hyperandrogenism leads to hypertrophy of vocal muscle cells, dehydration, and decrement in secretions of glands. This occurs when the androgen level exceeds more than 200µg/dl which leads to a masculine voice characteristic\(^26\).

Gugatschka et al\(^8\) could not find any significant differences in subjective and objective parameters except for lowered fundamental frequency which was not statistically significant. The possible reason they have quoted is smaller sample size and also the levels of androgen, as well as the testosterone levels, did not have much difference to show a noticeable change associated with voice. Therefore, it is assumed that only six participants of the current study would have crossed this androgen level which could have led to the deviant kymographic characteristics.

Thus, the results of the present study indicate that some individuals with PCOS may exhibit vocal abnormalities, which need attention from the voice pathologist. These changed vocal fold vibratory motions may be so subtle that it can be detected only by kymograms. However, the interpretations of the study should be taken with caution as videokymographic data alone might not indicate deviation vocal quality. Future studies can be carried out to analyze and correlate the acoustic findings, hormone levels, day of the menstrual cycle and kymographic features. Further, there is a need for a detailed investigation to explore the impact of these subtle vibratory changes in the vocal folds on the self-reported, acoustic and perceptual characteristics of voice of women with PCOS. The study also encourages further research in this population with respect to vocal characteristics.

The present study included women diagnosed with PCOS based on ultrasonography alone, and verification with blood hormone levels was not included. Further, the data collection was carried out based on the convenience of the participant and investigator; the day of the menstrual cycle was not considered. The subjective bias of the kymographic ratings was overcome by including two expert raters.
CONCLUSION

This study helps one to differentiate any subtle variation in vocal fold vibratory patterns in women with PCOS and women in the control group, thereby facilitating early detection of voice problems in women with PCOS. These subtle kymographic variations may be so significant in professional voice users and therefore kymographic evaluation would serve as an early detection tool for the identification of voice problems in women with PCOS.

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Authors contributions
SB: data collection, analysis and interpretation and writing of first draft, DRG: analysis, interpretation of data and writing, RKB: idealization of the study, analysis, interpretation of data and writing.