PITCH AND AMPLITUDE PERTURBATIONS IN 10 YEARS OLD CHILDREN

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A DISSERTATION SUBMITTED AS PART FULFILMENT FOR FINAL YEAR M.Sc. (SPEECH AND HEARING) TO THE UNIVERSITY OF MYSORE TO WHOM I MEAN HIS LIFE

MY BELOVED FATHER

ΤO

CERTIFICATE

This is to certify that the dissertation entitled "PITCH AND AMPLITUDE PERTURBATIONS IN 10 YEARS OLD CHILDREN" is the bonafide work in part fulfilment for the degree of Master of Science (Speech and Hearing) of the student with Register No. M - 9001.

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This is to certify that the dissertation entitled "PITCH AND AMPLITUDE PERTURBATIONS IN 10 YEARS OLD CHILDREN" has been prepared under my supervision and guidance.

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- cull Dr. R.S. SHUKLA GUIDE

DECLARATION

This dissertation is the result of my own study under the guidance of Dr. R.S. Shukla, Lecturer, Department of Speech Pathology, All India Institute of Speech and Hearing, Mysore, and has not been submitted earlier at any University for any other Diploma or Degree.

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CONTENTS

			Page No.
I.	Introduction		1 - 7
II.	Review of Literature		8 - 29
III.	Methodology		30 - 35
IV.	Results and Discussions		36 - 50
V.	Summary and Conclusion		51 - 54
	<u> </u>		
	Bibliography	• • • •	55 - 64

LIST OF TABLES

No.		Page No.
1.	Jitter (m.sec) given by Sridhara (1986)	23
2.	Directional jitter by Sorenson & Horii (1984)	24
3.	Shimmer (dB) by Horii (1980,a)	25
4.	Shimmer (dB) By Sridhara (1986)	26
5.	Directional shimmer by Sorenson & Horii (1984)	27
6.	Mean and S.D. values for the 6 pitch and	
	amplitude perturabation measurements in	
	thirty 10 years old normal male children	37
7.	The results of one way ANOVA for the	
	6 parameters of the present study	38
8.	Pitch perturbation measurements in 10 years	
	old male and normal male adults	45
9.	Amplitude perturbation measurements in	
	10 years old male children and normal male	
	adults	46
10.	Jitter ratio and shimmer (dB) values for	
	three age groups	47
11.	Showing ANOVA Test results for jitter ratio	
	and shimmer (dB)	48

LIST OF FIGURES

No.

1.	Jitter ratio values in 3 age groups for	
	the three vowels /a/, /i/ and /u/	49

Page No.

INTRODUCTION

A voice disorder in a child presents a challenge to those concerned and many speech pathologists feel voice disorders among children are in the increase. The causes for voice disorders in children are many. Children may abuse or miuse their voice by excessive talking, screaming or by speaking at a high level of intensity and frequency which is not appropriate to their vocal cords. The abuse and/or misuse may cause vocal nodules, vocal polyps, hyperkeratosis, nonspecific laryngitis. Apart from these, congenital anomalies of laryngeal structures, neurological causes, trauma tumours, infections and developmental disorders such as hearing loss, mental retardation and cerebral palsy may also cause voice disturbances in children.

As in the case of adults, children with voice problems also require a comprehensive evaluation, for an effective treatment. A comprehensive diagnostic procedure for the voice disorders whether in adults or in children consists of the following

- 1. To evaluate the degree and the nature of the dysphonia.
- 2. To find out the etiology, its degree and extent.
- 3. To determine the prognosis and therapeutic procedures there on.

Thus a comprehensive evaluation of a voice disorder is a necessary antecedent step to a successful treatment. There are various objective methods such as aerodynamic, physiological, acoustic methods and subjective methods such as rating scales to evaluate the disordered voice.

The vocal function being a multi-dimensional one, there is no, one single measure either with which one can measure the entire aspects of it. Therefore, in a clinical situation a battery of tests are performed to evaluate the cause and the degree of dysphonia, among which acoustic measurements have received considerable attention in the recent past.

According to Kent (1976) "Although the physiologic and phonetic interpretation of acoustic data are sometime uncertain, acoustic analysis are appropriate to test certain hypothesis about developmental changes in anatomy, motor control and phonological function". These acoustic analysis have been considered to have application in identification, diagnosis and treatment of developmental disorders of communication.

Acoustic analysis of the voice signal is one of the most attractive methods for assessing phonatory behaviour or the laryngeal pathology because it is not only noninvasive but also provides objective and quantitative

data. Moreover, due to the advancement of microcomputers and microcomputer based instruments acoustic analysis is easy to perform, less time consuming and more reliable.

Michel and Wendhal (1971) were the first reserchers to list many acoustic and aerodynamic correlates of voice and they opined that these correlates of voice have potential to differentiate abnormal voice from normal voice and to differentiate many vocal pathologies from one another without looking into the larynx.

Since then many more acoustic correlates of voice have been identified and are being identified and several researchers have engaged themselves in determining the importance of these acoustic correlates in diagnosing and differentially diagnosing the voice disorders.

Probably of the many acoustic correlates pitch and amplitude measurements have been extensively studied. Pitch perturbation (jitter) is a parameter related to fundamental frequency. Amplitude perturbation (shimmer) is a parameter related to the vocal intensity. It is defined as variations of peak amplitude in successive glottal pulses.

The voice produced by the vibration of the vocal cords, though generally assumed to be periodic no two cycles in a

given vibration are identical. Therefore, in reality voice is quasiperiodic. Every speaker's vibratory cycle are erratic to some extent and this has been documented by several investigators. But abnormal larynx produces more erratic voice than a healthy one. Pitch and amplitude perturbations are acoustic correlates of these erratic vibratory patterns (Beckett, 1969; p 418), that result from diminished control over the phonatory system (Sorenson, Horii and Leonard, 1980).

Several researchers (Moore & Thompson, 1965; Moore & Von Leden, 1958) have demonstrated that speakers with vocal pathologies display greater pitch and amplitude perturbations. Frequency perturbation is sufficiently sensitive to pathological changes in the phonatory process and perhaps even to severe respiratory insufficiency (Cilbert, 1975). Similarly amplitude perturbations provide great deal of information on the disordered voice (Wendhahl, 1963; 1966 a; 1966 b; Takhashi & Koike, 1975; Horii, 1986).

Several investigators (Lieberman, 1961; Von heden, 1963; Moore & Timke, 1960; Hollien & Kreul 1971; Sorensen & Horii, 1984; Horii,1984; Hollien, Michel & Doherty, 1973; Murry & Doherty, 1980; Balaji, 1988; Venkatesh, Satya and Jeny, 1992; Venkatesh, Raghunath & Neelu, 1992.

have measured jitter and shimmer in normals and abnormals to evaluate the usefulness of jitter and shimmer in the diagnosis and differential diagnosis of voice disorders. These studies have generally shown that shimmer and jitter measurements not only help in the diagnosis of voice disorders, but also in early diagnosis of voice disorders. These authors while accumulating the data for jitter and shimmer used only adults as their subjects. Thus they have provided normative data for jitter and shimmer measurements for adults with which the adult dysphonic patients can be differentiated. To make the differential diagnosis easier they have also provided scores of jitter and shimmer for different types of dysphonic groups, such as vocal nodules, tumours, cancer and unilateral recurrent laryngeal nerve paralysis.

However, the data collected for the normal adults may not hold good for children because of the following reasons.

- Children's vocal cords differ to a great extent in morphology from that of adults.
- Speech is a neuromuscular activity. The acoustic characteristics of speech have been found to vary with age. These acoustic features on various aspects

of speech production indicate that the accuracy of motor control improves with age until adult like performance is achieved at about 11 or 12 years, somewhat after the age at which speech sounds acquisition usually judged to be complete (Kent, 1976). Thus children are expected to show greater variations when compared to adults.

3. Lastly some of the voice disorders such as hoarseness due to vocal nodules or polyps are more common in children than in adults. Therefore, there is greater need to identify the problem early in case of children.

The present study was designed, to

- To obtain the normative data for the following pitch and amplitude perturbation measurements, in 10 years old male children.
 - 1) Jitter ratio
 - 2) Directional perturbation quotient for jitter (DPQ)
 - 3) Relative average perturbation (three point)
 - 4) Shimmer (dB)
 - 5) Directional perturbation quotvent for shimmer (DPQ)
 - 6) Amplitude perturbation quotvent (APQ)

- 2. To compare the values of the above jitter and shimmer measurements with those of adult normative data (already available) in order to verify whether children exhibit variation as per theoritical expectation.
- 3. To compare the values of jitter and shimmer measurements with 7 and 8 year old children (study being conducted simultaneously by others) to see whether these voice parameters vary with age.

REVIEW OF LITERATURE

The vocal folds are part of an aero-acoustic oscillator that provides the acoustic excitation, source for voiced speech. The health of the vocal folds which affect the quality of the sound produced by the oscillator is a major concern. This concern makes acoustic measures of the quality of the oscillations produced by the vocal folds a matter of considerable interest.

The healthy vocal folds form a well balanced system that produces nearly periodic oscillations. The lungs which are source of energy for sustained phonation. provide adequate amount of air and this is done directly under the supervision of Central Nervous System with necessary neuro-muscular co-ordination. This helps in maintaining continuous vibration of the vocal folds. Though the voice produced by the healthy vocal folds is expected to be periodic, it is not so in reality. No two cycles in a given vibration are identical and hence voice is quasiperiodic in nature.

As early as in 1927, Simon reported that there are no tones of constant pitch in either vocal or instrumental sounds and suggested that the phonatory system is not a perfect machine and every speaker's vibratory

cycles are erratic to some extent. Even the most serious attempt by a speaker to produce steady phonation with constant pitch, loudness and quality results in perturbations in fundamental frequency, amplitude and wave shape of the speech signal. The small variations (perturbations) in amplitude and period-time from cycleto-cycle in the speech waveform are known to be natural ingredient in normal speech (Lieberman, 1961). These perturbations in fact are important for the natural quality of speech synthesis (Holms, 1962). The cycle-tocycle variation in frequency has been termed as jitter and cycle-to-cycle variation in amplitude has been termed as shimmer.

The variations in pitch and amplitude are probably due to the periodicity of the neuromuscular phonatory control system (Schultz-Coulon, Baltmer, & Fedders, 1979). The pitch and amplitude perturbations are displayed both in normal voices and pathological voices (Moore & Thompson, 1965, Moore & Von Leden, 1958). Additionally the speakers with vocal pathologies demonstrated greater perturbation values than normal speakers (Deal & Emanuel, 1978; Iwata & Von Leden, 1970). Its known that perturbations with large magnitude give rise to a rough voice quality. Many investigators have tried to explain the physiological processes behind the pitch and amplitude measurements. Structural and biomechanical asymmetries of the vocal folds are known to contribute perturbation (Hirano, Kiki, Imazzumi, Kakita & Matsushita 1979, Ishizaka and Isshiki, 1976; Isshiki, Tanabe, Ishizaka & Broad, 1977). The random effects of laryngeal mucous and airflow also contribute to perturbation (Broad, 1979; Titze, 19883a).

Baer (1978, 1980) has explained that vocal jitter results from the imperfect integration of the forces generated by individual laryngeal motor units and is thus associated with the inherent sloppiness of muscle excitation. Titze (1988 a,b), Larson & Kempster (1983) and Larson, Kempster & Kistler (1987) have supported the notion that slight changes in the vocal fold length and stiffness caused by intrinsic laryngeal muscle, single motor unit twitches can and do affect vocal fundamental frequency to vary to a great extent.

Some of the sourcess of perturbation which are listed by Askenfelt & Hammarberg (1986) Kempster (1984)are

 a) Randomness in the action potentials of laryngeal muscles, creating fluctuations in the muscle forces and configuration of the larynx.

- b) Randomness in the distribution of mucous on the folds and asymmetries in vocal fold structures.
- c) Randomness in the flow emerging from the glottis (Instability and turbulence).
- d) Irregularity in source and tract interactions that stem from nonstationary articulatory configurations.

Factors influencing jitter and shimmer

The perturbation measures are likely to be affected by a number of jitter and shimmer producing phonatory variables. Some of these are normal phenomena of voice production while others are of pathological origin.

Specific phonatory conditions such as soft versus hard voice initiation and termination, intensity, fundamental frequency and duration have been shown to affect the resulting jitter or shimmer magnitudes (Hollien et, al.,1973; Horii, 1979; Jacob, 1968; Koike, 1973; Montgomery, 1967). Voice onset and termination characteristics have much greater frequency perturbation than the midstream of a sustained phonation (Lieberman, 1961, Horii, 1973). If the middle 3 seconds interval of each phonation is analyzed there would be soft initiation and termination, Koike (1973) studied normal men and women and found that steady state phonation had a mean relative average pertur-bation of 0.0046, the first 17 glottal cycles of a normal soft vocal initiation had a frequency perturbation of 0.0276, when measured the same way. Unless voice onset itself is the phenomenon being examined, clinicians will want to evaluate sustained vowel no less than a half second or so, after voice initiation.

Horii's (1979) data show that dividing absolute frequency perturbation by the mean fundamental frequency tends to over compensate for the change in jitter with fundamental frequency, Hollien, Michel & Doherty (1973) have observed the same phenomenon in the jitter factor. Therefore, we should expect relative perturbation to be somewhat higher in higher frequency voices while absolute jitter magnitude should decrease with increasing fundamental frequency. The shimmer values for 2 different frequencies 100 Hz and 200 Hz in Horii's study didn't differ by more than 0.01 dB. Horii (1985) reported that fry phonation was characterized by considerably greater jitter than modal phonation.

Jacob (1968) found that jitter ratio tended to decrease with increasing vocal intensity. This aspect of relative pitch perturbation has not been explored in detail but it would seem prudent to do all measurements at a standard intensity level. Vocal intensity levels should neither be extremely low or extremely high i.e. they should be comfortable according to the subject's report and the required phonatory duration of 5 seconds should also be well within the subject's capability (Ramig,1980). If the subjects were to phonate at a specific intensity and/or as long as possible the jitter or shimmer values are expected to increase.

The question of if jitter varies systematically across different vowels is as yet unresolved. Wilcox and Horii (1980) and Horii (1980) found /a/ and /i/ had significantly greaterjitter than /u/. But Johnson and Michel (1969) observed a tendency for high vowels to show greater jitter than low ones. Horii (1982) failed to validate any significant differences in mean jitter across 10 English vowels. Sorenson and Horii (1983) found significantly more jitter for /i/ than for /u/ and /a/ as produced at comfortable pitch and loudness by women.

Shimmer values also change according to the vowels in which they are measured. Yoshiyuki and Horii (1980) studied vocal shimmer during the sustained phonation of /a/, /i/ and /u/ in 31 adults male speakers using an automatic analysis program. The average shimmer was the lowest for /u/ with 0.36 dB, highest for /a/ with 0.47dB and intermediate for /i/ with 0.37 dB. Overall average shimmer was 0.39 dB for the three vowels. Newman Keuls test showed that the observed shimmer for /a/ was greater than /i/ and that the jitter for /i/ was greater than /a/ and /u/. Zemblin reported a significantly greater jitter for /a/ than /i/. So comparisons of jitter and shimmer values are most safely done only for measurements of the same vowel.

Horii (1979) and Ban Craft (1979) discussed temporal and amplitude resolution and signal to noise ratios of the analysis systems are also important considerations in jitter and shimmer measurements.

The next factor is sex related one. Sorenson and Horii (1983) points to the possibility that adult females may normally have more vocal jitter than men, at least for some vowels. They studied jitter and shimmer in 20 adult females. The results showed overall average jitter of 0.84% and shimmer was 0.25 dB. Significant differences between male and females in terms of jitter and shimmer was found. The findings of this study says that normal adult female speakers have more jitter and less shimmer than normal adult male speakers. So even on such fine laryngeal behaviours such as jitter and shimmer there are significant differences between the sexes and this seems to indicate that normative data for both male and voices separately need to be developed.

Lorraine A. Ramig and Robert L. Ringel (1983) studied jitter and shimmer in the elderly and compared their values with the adult values. They studied 48 men representing 3 chronological age groupings (25-35, 45-55 and 65-75) and two levels of physical condition (good and poor). Subjects in good physical condition produced maximum vowel phonation duration with significantly less jitter and shimmer and had larger phonation ranges than did subjects of similar chronological ages who were in poor physical condition. These differences were more apparent in the productions of the elderly subjects (65-75 yrs). In their study shimmer was the only acoustic measure that varied significantly between younger (25-35 yrs) and the elderly (65-75 yrs) subjects. No significant age related differences were observed on mean fundamental frequency, jitter or maximum phonation with advancing chronological age. While chronological aging is undoubtedly a contributor to changes in the acoustic characteristics of voice, the results of this study suggest that age related changes in body physiology or physiological aging also must be considered. Changes in voice fundamental frequency, maximum phonation range, average jitter and shimmer which are believed to reflect age related physical changes in the laryngeal mechanism, have been well documented (Enders,

Bambach, & Flosser 1971; Hollien & Shipp, 1972; Mysak, 1959; Segre, 1971; Wilcox & Horii, 1980).

Hollien et.al.,(1973); Horii, (1979 & 1982), Sorenson and Horii (1983), Carper (1984) found jitter (%) in infant vocalizations is three to four times greater than that observed in adult vocalizations and roughly twice that reported for elderly subjects (Wilcox & Horii 1980).

Orilikoff and Baken (1989,b) have found that the heartbeat accounted for about 7% of the measured frequency perturbation in the voices of normal adult men ranging from approximately 0.5% to almost 20% for a given phonation. These data indicate that the reliability of jitter measurements is non-randomly influenced by heartbeat related phenomena. Titze (1988, 1989) said it would seem reasonable to assume that there may be heartbeat related modulation of the sound pressure of a prolonged phonation and consequently contamination of the shimmer measurements.

David Sorenson and Yoshiyuki, Horii, Rebecca Leonard (1980) studied laryngeal topical anesthesia on voice fundamental frequency perturbation. They studied five adult males. The results showed that the average jitter was significantly greater under the anesthesia than under normal conditions and that the jitter difference between

the two conditions was more prominent at high frequency phonations. In this study Sorenson and Horii tried to explain the laryngeal mechano receptors contributions to vocal fold tensions.

Could and Okamura (1974) reported increased glottal resistance under topical anesthesia of laryngeal mucosa. These equivocal findings resulted because some phonatory tasks did not tax the phonatory mechanisms enough or because the physiologic acoustic or perceptual variables examined were not sensitive enough to reveal subtle sensory contributions to phonation. These studies show evidence that the deprivation or reduction of laryngeal tactile feedback disrupts intricate frequency control mechanisms and results in deviation from normal voice.

Factors such as heredity (Bourtiere, 1970; Woodruff and Birrea, 1973) lifestyle and diet (Bourliere,1970; Mann, Shaffer, Anderson and Sanstead, 1964) and exercises (deVries, 1974; Smith and Bierman, 1973; Spirduso, 1980; Shepard and Sidney,1980) have reported to affect the oricess of physical change associated with aging and hence voice too.

Thus factors such as age, sex, fundamental frequency of voice, intensity of voice, voice initiation, termination, tactile feedback to the vocal folds, phonovascular factors, etc. influence the jitter and shimmer measurements.

The neurophysiological and perceptual significance of jitter and shimmer even with the recent acceleration of research in this area (Heiberger & Horii, 1980; Hollien, Michel & Doherty, 1973; Horii, 1979; 1980; Ludlow, Coulter & Cardano, 1979; Ramig, 1980; Sorenson, 1980) is not well understood. However, these measurements have been intensively studied in normals and dysphonics and recently being used extensively for diagnostic purposes.

The pitch and amplitude perturbations can be measured by acoustic analysis procedures. Acoustic analysis is objective, non-invasive and because of the advancement in technology in microcomputers, it is easy, less time consuming and more reliable.

Jitter is not a sole diagnostic criterion. It doesn't account for all of what the listener perceives in the disordered voice. Far from it factors such as amplitude perturbation (Wendahl,1963; 1966 a,b; Takhashi, & Koike, 1975; Horii, 1988), Spectral noise, glottal waveform changes, account for a great deal, perhaps most of what is heard as abnormality. But frequency perturbation is sufficiently sensitive to pathological changes in the phonatory process and perhaps even to severe respiratory insufficiency (Gilbert, 1975). Lieberman and his colleagues (Lieberman, 1961, 1963; Lieberman & Michaels, 1962; W.R. Smith & Lieberman, 1969) conducted the pioneering studies of detecting laryngeal pathology by waveform analysis. Based on the analysis of connected speech produced by normals and pathological subjects (with laryngeal polyps, nodules and cancer) Lieberman suggested a perturbation factor (PF) as an indicator of laryngeal pathologies. His study of frequency perturbation tended to confirm the observation of Von Leden, Moore, & Timeke (1960); that the normal vibratory patterns of the vocal folds are disrupted in the presence of laryngeal pathology and in particular that there is greatly increased tendency for rapid and frequent lapses of vibratory regularity.

Specifically, Lieberman (1963) reasoned that frequency perturbations reflect.

- 1. Changes in glottal periodicity
- 2. alterations of the glottal waveform
- 3. Variations of vocal tract

Configuration that result in phase shifts of the acoustic wave. The first of these was considered to produce cycleto-cycle period differences greater than 0.5 msec. Liberman therefore produced an index that he called the perturbation factor defined as integral of the frequency distribution of t > 0.5 ms i.e. the perturbation equal to or greater than a half millisecond.

He opined that the perturbation factor may well turn out to be useful as a screening measure for detection of laryngeal disorder since it is sensitive to the size and location of pathologic growths in the speaker's larynx. When growth occurs on the speaker's vocal cords the differences between the perturbation factors of the normal and pathologic larynges are proportional to the size of the pathologic growths as long as the growths don't interfere with normal closure of the vocal cords. Inflammatory conditions and very small nodules have in general comparatively small effect on either of the perturbation factor or on the acoustic waveform (Lieberman, 1963).

In the recent past there is a considerable body of literature that asserts the usefulness of frequency and amplitude perturbation measures in evaluation of laryngeal and vocal pathology (Kitajima, Tanabe, and Isshiki, 1975; Davis, 1976; Horii, 1970; Lieberman, 1961, 1963; Hecker & Kreul, 1971; Klingholz & Martin, 1983; Hartmann & Von Cramon,1984; Zyski, Bill, McDonald & Johns, 1984). Increased pitch and/or amplitude perturbations were also found to be associated with hoarseness and have been

positively correlated with severity of pathology and perceptual roughness ratings (Coleman, 1969; Deal & Emanuel, 1978). There are different jitter and shimmer measurements putforth by several authors like jitter ratio, jitter factor, DPF, APQ, FPQ. Hecker and Kreul (1971) found directional perturbation was sensitive enough to distinguish between normals and dysphonics. Murry & Doherty (1980) reported that directional perturbation was the single most effective parameter for separating two groups of normals and laryngeal cancer patients. Koike (1973) stated the clinical implication of relative average perturbation. In 1975, Takahashi and Koike (1975) introduced acoustic correlates such as amplitude perturbation quotient and frequency perturbation quotient. Deal & Emanuel (1978) suggested that cycle to cycle variations in amplitude may provide a better index of perceived roughness of voice than cycle to cycle variations in period.

Venkatesh, Satya and Jeny (1991) based on discriminant function analysis found that shimmer (dB) and amplitude perturbation measurements respectively and jitter ratio, relative average perturbation and deviation from linear trend among pitch perturbation measurements respectively were the best discriminating measurements between normals and dysphonics. The clinical implication of their study

is to use shimmer (dB) as a screening device for voice disorder to economize time.

Jacob (1968), Hollien, Michel & Doherty (1973), Koike (1973), Horii (1979) reported that nonpathologic speakers appear to have average perturbation (jitter) of 1% or less during the middle portions of the sustained vowel phonation. Hollien, Michel & Doherty (1973) studied jitter factor in 4 subjects and found it to be 0.48, 0.76, 0.85, 2.67 and fundamental frequency of 102 Hz, 142 Hz, 198 Hz and 276 Hz respectively. Murry & Doherty (1980) studied jitter factor in five male subjects and found mean fundamental frequency to be 115.3 and jitter factor to be 0.99. Horii(1984) studied jitter ratio in 6 normal subjects and found jitter ratio to vary from 5.3 to 7.6.

Zemblin (1962) investigated the variations that occured in the period (T - 1/f) of the vocal folds vibration during the production of prolonged sounds. In a population of 33 subjects he found that cycle-to-cycle differences in period ranged from 0.2 to 0.9 msec, with a mean of 0.41 msec for a sustained vowel /a/. While these variations are not large they suggest that very slight changes in the vocalfolds occur during the course of normal vibration. As long as the vibrations fall

within certain critical limits, a slight cycle-to-cycle differences in vibratory period (jitter) do not produce adverse effects in the perceived voice quality.

Sridhara (1986) studied young normal males and females for jitter in msec. for various vowels and gave the following results.

Subjects	J	itter (msec)	
	/a/	/i/	/u/
Men	0.065	0.11	0.067
Women	0.058	0.03	0.048

Table 1.: Jitter (msec.) given by Shridhara (1986)

Hecker and Kreul (1971) found directional perturbation (jitter) factors ranged from 27.7% to 39.2% with a mean of 33.3% for a group of non-pathological speakers. The mean directional jitter for the normal group was 58.5% with a range of 45.8% to 65.3%.

Sorenson & Horii (1984) found directional jitter values to be 47.3% which was averaged across the vowels was 47.3% for the men and 51.2% for the women. These values were lower than those calculated for the five normal subjects in the Murry and Doherty study. The differences in the values between the two studies has been attributed partly to the age differences in the subjects. Sorenson & Horii (1984) studied directional jitter in normal adults. Their results are tabuled below.

		Vowels	
	a	i	u
Men	46.24	49.26	46.37
Women	48.79	52.77	52.04

Table 2.Directional jitter by Sorenson & Horii (1984)

Murry and Doherty (1980) found directional jitter factor for the normal subjects was 58.5% with a range of 55.1% to 76.7%. The values from this study are substantially higher than the corresponding values of Hecker and Kreul (1971). The differences were attributed to the test materials utilized and to the analysis techniques of the researchers.

Relative average perturbation was studied by Koike (1973) for seven normal male subjects and was found to be 0.0051. Venkatesh, Satya and Jeny (1992) studied jitter ratio, directional perturbation quotient for jitter and relative average perturbation (3 point) in normal Indian adult males and females. They found jitter ratio to be 9.17, 7.8 and 8.5 for /a/, /i/ and /u/ respectively in males and 9.17, 7.8 and 8.5 for /a/, /i/ and /u/ respectively in females. Directional jitter was 58.28, 55.99, and 57.48 for /a/, /i/ and /u/ in males respectively and 58.28, 55.7, and 56.02 for /a/, /i/ and /u/ in females respectively. RAP (3.) was found to be 0.0058 for /a/, 0.0053 for /i/ and 0.0061 for /u/ in males and 0.0062 for /a/, 0.0054 for /i/ and 0.0058 for /u/ in females.

Shimmer measurements in normals have also been studied by various investigators. Vocal shimmer during the sustained phonation of /a/, /i/ and /u/ was investigated for 31 adult males using automatic analysis program by Horii (1980 a,b). He also studied 20 normal females (1980 b). The findings of his study is given in Table 3.

	/a/	/i/	/u/	Overall
Men	0.47	0.37	0.33	0.39
Women	0.33	0.23	0.19	0.25

Table 3. Data on shimmer (dB) by Horii (1980 a)

Sridhara (1986) studied young normal males and females for shimmer (dB) and reported as in Table 4.

Table 4. Data on shimmer dB by Shridhara

	/a/	/i/	/u/
Men	0.33	0.066	0.15
Women	0.7	0.37	0.44

Kitayama and Gould (1976) reported that average shimmer in normal phonation is in the order of 0.1 dB with a critical value of 0.19 dB.

Venkatesh, Satya and Jeny (1992) studied in 30 adults males and 30 adult females shimmer (dB), directional perturbation quotient for amplitude and amplitude perturbation measurements in normal adult males and females. They found shimmer (dB) to be 0.28 for /a/, 0.175 for /i/ and 0.215 for /u/ in males and 0.252 for /a/, 0.198 for /i/ and 0.184 for /u/ in females. DPQ (shimmer) for /a/, /i/ and /u/ were 60.24, 59.46 and 60.74 respectively in males and DPQ (shimmer) for /a/, /i/ and /u/ were 64.75, 65.85 and 66.06 respectively in females. APQ was found to be 1.873 for /a/, 1.70 for /i/ and 1.427 for /u/ in males and 1.799 for /a/, 1.367 for /i/ and 1.284 for /u/ in females. Sorenson and Horii (1984) studied directional perturbation factor for shimmer in adult normal males and females and gave the data as in Table 5.

Table 5. Directional shimmer data by Sorenson andHorii (1984).

Amplitude perturbation quotient was studied by Takahashi, Koike and Calcaterra (1977) and was found to be 40.3 in males and 32.9 in females. They studied 7 normal males and 2 normal females, Davis (1979) found APQ to be 5.97 in males and 6.81 in females.

Many researchers have studied the pitch and amplitude perturbation measurements in dysphonic subjects. Moore and Thompson (1965) gave jitter values of 0.3 msec. (4.9%) for several hoarse voice and 0.06 msec. (1.14%) for moderately hoarse voice. Wendhahl(1932) found very slight frequency variations as little as one cycle/second around the median sounded rough and the magnitude of judged roughness was directly related to the frequency differences between successful cycles.

Sonesson (1967) reported that patients with laryngeal hemi-paralysis show a large amount of shimmer values than normal jitter values. Kitajima and Gould (1979) reported that shimmer values vary from 0.08 to 3.23 dB in subjects with vocal polyps.

Balaji (1988) studied jitter and shimmer in 10 dysphonic males and 8 dysphonic females, and compared them with normals. Dysphonic male group and dysphonic female group exhibited greater jitter than normal male group and normal female group respectively. These results were in agreement with Sorenson (1967), Kitajima and Gould (1976) and Chandrashekar (1987). Greater jitter values were obtained in dysphonic males and females with narrow glottic chink than other types of pathologies such as recurrent laryngeal nerve (unilateral) palsy, vocal polyp, laryngitis and vocal Dysphonic males exhibited greater shimmer than nodules. dysphonic females. All dysphonic males exhibited greater shimmer when compared to normal males. Narrow glottic chink exhibited the greatest shimmer value of all types of dysphonic males. Dysphonic female group with any of the types of voice disorders exhibited greater shimmer than normal females but less than dysphonic males.

Hecker and Kreul (1971) studied directional perturbation factor for jitter in subjects with laryngeal cancer and found the mean directional factor to be 64.5% with a range of 55.1% to 76.7%. Joanne Robbins (1984) studied different variables in laryngeal oesophageal and TEP speakers and found that the esophageal group was significantly different from the TEP and laryngeal group.

In summary variations in jitter and shimmer are present in normal voice too. There seems to be many factors influencing pitch and amplitude perturbations, including sex,age frequency of voice etc. There are variations of basic pitch and amplitude perturbation factor like, jitter ratio, directional jitter, directional shimmer and APQ to name a few. All these acoustic correlates have be measured both in normal and pathological voices, with the aim of arriving at a quick screening device for the early detection of laryngeal pathologies. Though these parameters have been studied extensively in adult population yielding a desirable result, the review of literature suggest that data on various parameters of pitch and amplitude perturbation factors in children have not been documented. Hence, the present study.

METHODOLOGY

Several investigators have studied the pitch and amplitude perturbation measurements, both in normal subjects and in subjects with laryngeal pathologies. The results of these studies show that pitch and amplitude perturbations are larger in subjects with laryngeal pathologies. These finding suggest that perturbation measurements of frequency and amplitude can be used in the diagnosis of laryngeal disorders. So the need was felt to establish normative data for different age groups.

The present study was aimed at establishing normative data for the following pitch and amplitude perturbation measurements in thirty 10 years old male children, as there was no data available on these perturbation measurements in children.

I. Pitch Perturbation Measurements

(a) <u>Jitter Ratio</u> is the mean perturbation divided by the mean waveform duration when done in terms of period (Horii, 1979).

(b) <u>Directional perturbation factor for jitter</u> takes into account only the direction and not the magnitude. It is defined as the percentage of of the total number of differences in frequency for which there is a change in algebraic sign (Hecker & Kreul, 1971). (c) <u>Relative average perturbation</u> (three point) is defined as comparative average of change at three different points, It was given by Koike (1973).

II. Amplitude Perturbation Measurements:

(a) <u>Shimmer</u> (dB) is defined as cycle to cycle variation in amplitude measured in deciBels.

(b) <u>Directional perturbation factor for amplitude</u> takes into account only the direction and not the magnitude. It is defined as the percentage of the total number of differences in amplitude for which there is a change in algebraic sign (Hecker & Kreul, 1971).

(c)Amplitude perturbation quotient: It was given by Takahashi and Koike, (1971)and Calcaterra(1977). This measure is analogous to the RAP originally devised by Koike (1973). The function uses an 11 point average for smoothing and is defined as

$$APQ = \frac{1}{n-10} \sum_{i=6}^{n-5} / A_{i-5} + A_{i-4} + \dots + A_{i} / A_{i}$$
$$\frac{1}{n} \sum_{i=1}^{n} A_{i}$$

Where A_i = Peak amplitude of each wave n = number of waves measure.

Subjects

Thirty ten years old normal male children ranging from 10.2 yrs to 10.11 yrs served as subjects for the study. The subjects were chosen based on the following criteria.

- (i) Normal E.N.T. findings
- (ii) Normal audiological findings
- (iii) Normal intelligence
- (iv) No known history of voice problem, vocal abuse or other relevant vocal history.

Speech Sample:

Speech sample consisted of phonation of the vowels /a/, /i/ and /u/ for five seconds. The subjects were required to phonate the three vowels by keeping the voice as steady as possible and at habitual frequency during the phonation. They were required to phonate the three vowels /a/, /i/ and /u/, thrice and hence the speech sample consisted of 9 phonations of 5 seconds each per subject. It was intended to take middle 3 seconds phonation for pitch and amplitude perturbation analysis.

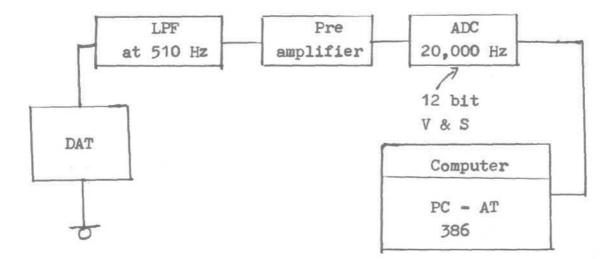
Recording

The subjects were seated comfortably in front of a microphone situated in a sound treated room. The microphone was connected to a digital tape recorder (Sony)

The subjects were instructed to phonate the vowels /a/, /i/ and /u/ for 5 seconds at habitual pitch and at comfortable loudness. There were also instructed not to move their head and neck during phonation. All the subjects were provided with a practice session of 5 to 7 minutes, with the aid of vocal II prior to the recording. This helped the children to produce steady phonations. The distance between the speaker's mouth and the microphone was 15 to 20 cms, during recording. For each phonation sufficient time gap was given for the intake of air for the next phonation.

Pitch and Amplitude Perturbation analysis

(Schematic diagram)



The output of the tape recorder was low pass filtered at 500 Hz and fed to an A/D converter for digitization. The digitization was done with a sampling frequency of 20 KHz using a 12 bit ADC cord. The digitized phonation were stored in a PC-AT 386 and was analyzed for the following perturbation measurements using Vagni software developed by voice and speech systems, Bangalore.

(i) Jitter Ratio

J.R. =
$$\frac{\frac{1}{n-1}}{\frac{1}{n}} \sqrt{\frac{p_{i}}{p_{i}}} \frac{P_{i}}{p_{i}} + 1} x 1000}{\frac{1}{n}} \sum_{i=1}^{n} P_{i}}$$

 P_i = Period of ith cycle in MS

n = Number of periods in the sample

(ii) Directional Perturbation Quotient for jitter

DPQ is the total is the percentage of total number of differences for which there is a change in algebraic sign.

DPF = sign change count Total number of differences in period

(iii) Relative average perturbation

$$= \frac{\frac{1}{n-2}}{\frac{1}{n} \sum_{i=1}^{n-1}} / \frac{\frac{1}{P_{i}-1} + P_{i} + P_{i} + 1 - P_{i}}{3} / \frac{\frac{1}{P_{i}-1} + P_{i}}{3}$$

(iv) Shimmer (dB)

$$S (dB) = \frac{\frac{n-1}{1-1}}{\frac{20(\log \frac{1}{4}A_{1}+1)}{n-1}}$$

(v) Directional perturbation quotient for shimmer

DPF = Sign change count Total number of differences in amplitude

$$APQ = \frac{1}{n-10} \frac{1}{1=6} / A_{i-5} + A_{i-4} + \dots + A_{i} + \dots + A_{i}$$
$$\frac{1}{n} \sum_{i=1}^{n} A_{i}$$

Analysis of all the above 6 parameters were done and the values were recorded.

Statistical Analysis

The following appropriate statistics were applied to the data obtained,

- (i) Mean
- (ii) Standard deviation
- (iii) Anova followed by DMRT.

RESULTS AND DISCUSSIONS

The purpose of the present study was to obtain pitch and amplitude perturbation values for thirty 10 years old normal male children. The values obtained for the pitch perturbation measurements such as jitter ratio DPQ for jitter, and RAP (3 pt) and for amplitude perturbation measurements such as shimmer (dB), DPQ for shimmer and APQ, for the three vowels /a/, /i/ and /u/ are shown in Table 6.

Since the values of the six pitch and amplitude perturbation were obtained, for 30 normal 10 year old male children, the obtained data which was given in Table 6, may be considered as normative data for that age group.

To know whether the six parameters differed with respect to each vowel (/a/, /i/ and /u/) one way ANOVA was administered separately for each parameter. The results of the six ANOVA tests have been summarized in Table 7. From the table 7 we may observe that all the 6 parameters differed with respect to vowels. The one way ANOVA test was followed by DMRT (Duncan's Multiple Range Test) to find out how the mean values of each vowel differed for each parameter studied.

perturbation		APQ	2.175	(0.68)	1.85	(0.45)	1.695	(0.52)
nd amplitude	ren.	DPQ	62.71	(3.9)	60.09	(5.02)	63.24	(6.2)
the 6 pitch a	year old normal male children	Shimmer (dB)	0.310	(0.0847)	0.244	(0.0678)	0.2422	(0.108)
on values for	year old norm	RAP (3 pt)	0.00585	(0.0023)	0.01282	(6600.0)	0.00785	(0.00199)
dard deviati	in thirty 10	DPF	62.78	(2.68)	68.53	(2.59)	68.43	(2.58)
Table 6. Mean and Standard deviation values for the 6 pitch and amplitude perturbation	measurements	Jitter ratio	9.11	(1.65)	14.54	(2.44)	13.36	(2.04)
Table 6.			Ю		-н		ב	

Table 7.	The results of one way ANOVA for the	six
	parameters of the present study.	

Parameters	DF	F-Ratio	Significant/Not significant
Jitter Ratio	2 87	57.40	Highly significant***
DPQ (jitter)	2 87	47.36	Highly significant***
RAP (3 pt)	2 87	10.79	Highly significant**
Shimmer (dB)	2 87	5.19	Highly significant**
DPQ (shimmer)	2 87	3.73	Significant*
APQ	2 87	5.78	Highly significant**

(i) Jitter ratio:

Jitter ratio for /a/ was 9.11, /i/ was 14.54, and /u/ was 13.36 in 10 year in 10 years old male children (Table 6). There was a highly significant difference between jitter ratio of /a/ as compared to jitter ratio of /i/ and /u/. There was no significant difference found between jitter ratio of /i/ and /u/. Similar pattern was observed in 7 years old normal male children (Neelu, 1992). On the other hand jitter ratio values were significantly different for all the three vowels /a/, /i/ and /u/ in the 8 years old normal male children (Sai Prasanna, 1992).

However, Venkatesh et.al., (1992) reported that jitter ratio was highest for /a/, intermediate for /u/ and lowest for /i/ for adult population. We note in the present study, the jitter ratio was highest for /i/, intermediate for /u/ and lowest for /a/.

The question of whether jitter varies systematically across different vowels is as yet unresolved. Wilcox and Horri (1980) and Horii (1980) found that /a/ and /i/ had significantly greater jitter than /u/, whereas Johnson and Michel (1969) observed a tendency for high vowels to show greater jitter than low ones, across 10 English vowels. The results of the present study support the observation of Johnson and Michel (1969), Very recently Sorenson and Horii (1983) while studying adult females, also observed that high vowels tend to have a higher jitter values.

(ii) Directional Perturbation Quotient (DPQ) for jitter:

BPQ (jitter) for /a/ was 62.78 /i/ was 68.54 and /u/ was 68.43 (Table 6). There was a highly significant difference between the DPQ values for /a/ as compared to /i/ and /u/. DPQ for /i/ and DPQ for /u/ are not significantly different from each other and they are greater than DPQ value for /a/. Similar patterns were observed in 7 years old normal male children (Neelu, 1992) and 8 years old normal male children (Sai Prasanna, 1992).

On the contrary, data on Indian adult normal males (Venkatesh et. al., 1992) show that DPQ for jitter was highest for /a/, intermediate for /u/ and lowest for /i/.

In addition, data on western normal adult male population (Sorenson and Horii, 1984) show that directional jitter was highest for /u/ as compared to /a/ and /i/ which contradicts this study. The reason for this discrepancy is not explored.

From these studies we may conclude that there

seem to be no systematic effect of vowel difference on directional jitter.

(iii) RAP (3 point)

RAP (3 pt) value for /a/ was 0.00585, /i/ was 0.01282 and /u/ was 0.00786 (Table 6). There was a highly significant difference between the RAP (3 pt) values of the three vowels /a/, /i/ and /u/. Similar pattern was observed both in 7 years old (Neelu, 1992) or 8 years old (Sai Prasanna, 1992) normal children. RAP (3 pt) was highest for /i/ in the present study where as it was observed that RAP value was highest for /a/ in Indian normal adult males (Venkatesh et, al., 1992).

Again here, children data do not agree with adult data.

(iv) Shimmer (dB)

Shimmer (dB) for /a/ was 0.31, /i/ was 0.24 and /u/ was 0.242 (Table 6). There was a highly significant difference for shimmer (dB) values for /a/ as compared to /i/ and /u/. It was found in present study that shimmer (dB) was highest for /a/ and a similar pattern was observed in Indian normal adult male population. (Venkatesh et. al., 1992). Shimmer (dB) for /a/ was observed to be higher in Horii's (1984) data on adult normal male population.

Thus, all the studies agree that vowel /a/ has the highest shimmer (dB) value, when compared to the vowels /i/ and /u/ and this probably may be related to the degree of opening of the oral cavity during the articulation of the vowel.

(v) Directional Perturbation Quotient for shimmer

DPQ for shimmer was 62.71 for /a/, 66.09 for /i/ and 63.24 for /u/ in 10 years old normal male children (Table 6). DPQ for shimmer is highest for /i/ and it is significantly different from DPQ for /a/ and /u/, in this study. Directonal shimmer was observed to be highest for /i/ in Sorenson and Horii's (1984) data which agrees with the present study. On the other hand in adult Indian normal male population (Venkatesh, et.al., 1992) it was observed that /u/ had the highest directional shimmer. Thus in all the studies the tense vowels /i/ and /u/ has shown greater directional shimmer than the lax vowel /a/.

(vi) Amplitude perturbation quotient

APQ for /a/ was 2.17, /i/ was 1.84 and /iV was 1.69 in 10 years old normal male children (Table 6). APQ for /a/ is found to be highest for /a/ as compared to /i/ and /u/ and it is significantly different from /i/ and /u/. It was also observed in Indian adult normal male population (Venkatesh et,al., 1992) that APQ was highest for /a/ as compared to /i/ and /u/. Similar patterns were observed in normal 8 year old male children (Sai Prasanna, 1992) and 7 year old normal male children (Neelu, 1992). Probably because amplitude perturbation quotient reflect intensity changes, APQ is greater for the vowel /a/, because it is an open vowel as opposed to /i/ and /u/ which are close vowels.

In summary the parameters which account for intensity variability show greater values for the open vowels and parameters which reflect frequency variations tend to show greater values for the tense vowels /i/ and /u/ as opposed to lax vowel /a/.

The pitch and amplitude perturbation measurements obtained in the present study were compared with normal adult males, to see how the children have perturbed on the six parameters (Table 8 and Table 9) as compared to the adults. From Table 8 and Table 9 it was found that the six perturbation measurements such as jitter ratio, directional jitter, relative average perturbation, shimmer (dB), directional shimmer and amplitude perturbation quotient obtained in thirty 10 years old normal male children studied in this study were higher as compared to the data obtained for these parameters in adult male population by Venkatesh et. al., (1992). This higher values for jitter and shimmer measurements in children probably may be due to the morphological differences between the larynges of them and adults or may be due to the continuous neuro-muscular maturation process which children are undergoing before puberty.

Thus the present study highlights, the need to have separate normative data for the pitch and amplitude perturbation measurements in children for appropriate diagnosis.

The jitter ratio and shimmer (dB) values for the three vowels obtained in the present study for the 10 years old normal male children were compared with the jitter ratio and shimmer (dB) values for the three vowels obtained in the 7 years old normal male children (Neelu, 1992) and 8 years old normal male children (Sai Prasanna, 1992). The data for the three vowels across 3 age groups are shown in Table 10. The data was subjected to ANOVA followed by DMRT. The results, of the ANOVA test is shown in Table 11. From Table 11, it may be inferred that there is a significant difference in jitter ratio among the three age groups. Thus it may be concluded that jitter values are different for different

Table 8.	Pitch	pertu	Pitch perturbation me	easureme	nts in 1	0 уеаг о	ld norma	measurements in 10 year old normal males and normal male	nd normal	male
-	adults.	ω.								
					Рага	Parameters				
Subjects			Jitter I	Ratio		DPQ		RAP 3	RAP 3 (point)	
		/a/	/i/	/n/	/a/	/i/	/n/	/a/	/i/	/n/
10 year old male children		9.11	14.54	13.36	62.78	68.53	68.43	0.00585	0.01282	0.00785
Adult males (Venkatesh et al., 1992)	92	9.17	7.824	8.50	58.28	55.7	56.02	0.0062	0.0054	0.0058

years old normal male children and	
10 Yea	
in	
urbation measurements	
perti	r r
Amplitude	г
Table 9.	

normal male adults.

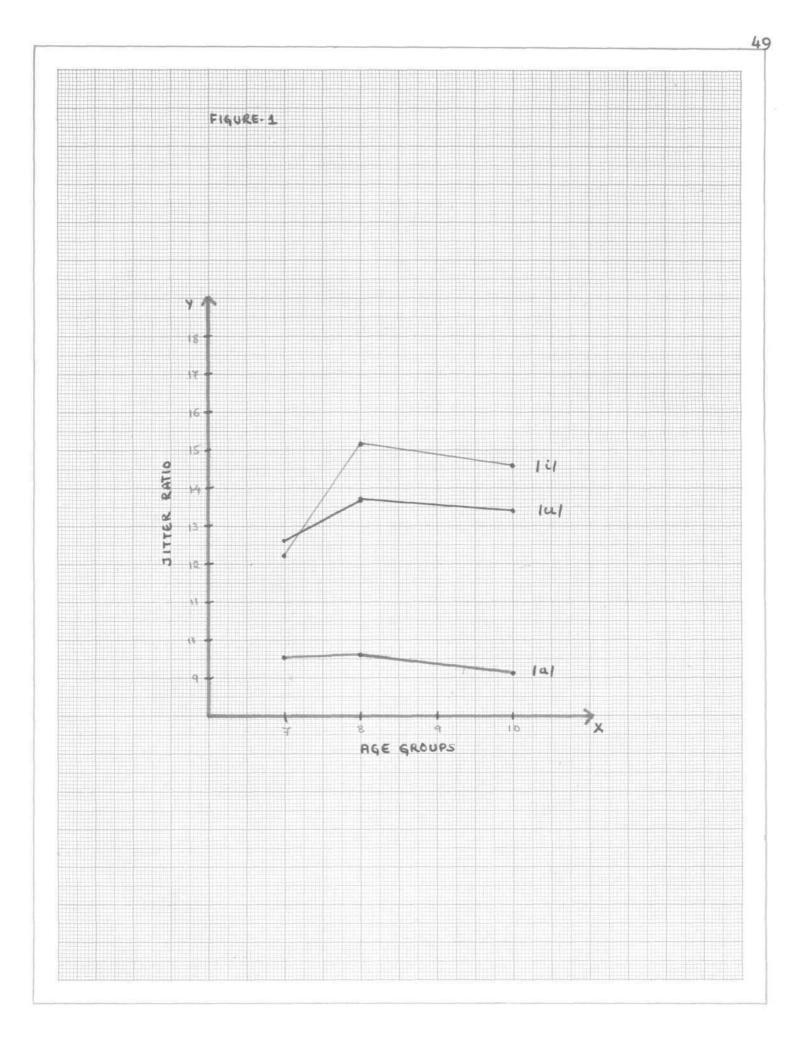
			Гал	Parameters					
Subjects		Shimmer (dB)	(dB)	С С С С С С С	DPQ for shimmer		APQ		
	/a/	/i/	/n/	/a/	/i/	/n/	/a/	/i/	/n/
10 years old male children	0.3071	0.244	0.2422	62.71	66.09 63.24	63.24	2.175 1.85	1.85	1.695
Normal adult males (Venkatesh et.al., 0.28 1992)	0.28	0.175	0.215	60.24	59.46	60.74	1.873 1.17	1.17	1.427

Table	10.	Jitter	ratio	and	shimmer	(dB)	values	for	three
		age gro	oups.						

	Jit	ter rat	io	Sh	immer (dB)
	а	i	u	а	i	u
7 yrs (Neelu, 1992)	9.54	12.23	12.53	0.338	0.239	0.217
8 yrs (Sai Prasanna, 1992)		15.08	13.71	0.331	0.241	0.246
10 yrs (Present study		14.54	13.36	0.31	0.244	0.242

Jitter Ratio	DF	F-Ratio	Significant/Non- significant
(a) age	2	8.42	S
(b) Vowel	2	106.18	S
ахb	4	3.89	S
Shimmer (dB)			
a	2	0.21	NS
b	2	25.04	NS
a x b	4	0.75	NS

Table 11. Showing ANOVA Test results for jitter ratio and shimmer (dB)



age groups. The posthoc test (DMRT) indicated that jitter value of 10 and 8 years old are higher and are significantly different from 7 years old children. This finding is contrary to the theoretical expectation. However, from this we may infer that there is need to have different jitter ratio values for different age groups. In other words it may be concluded that jitter ratio is sensitive to the age of the individual. Different vowels have different jitter ratios for different age group which is illustrated in Fig. 1. This demonstrates that while measuring jitter ratio, the type of vowel should also be considered.

From Table 11 it may be inferred that there is no significant difference in shimmer (dB)among the three age groups. Thus, it may be concluded that shimmer (dB) doesn't differ across the 3 age groups. The ANOVA test also indicates that there is no interaction effect between vowel type and age level, for shimmer (dB). Thus, it may be concluded that shimmer (dB) is not so sensitive to age unlike jitter ratio.

SUMMARY AND CONCLUSION

Variations in pitch and amplitude is an essential aspect of normal voice. This normal variations (perturbations) in the voice can be grouped into voluntary perturbations (intonational) and involuntary perturbations (pitch perturbation-jitter and amplitude perturbation - shimmer). These involuntary perturbation measurements are quantified by different parameters such as absolute jitter, jitter factor, jitter ratio, directional jitter and similarly shimmer (dB), directional shimmer and amplitude perturbation quotient etc.

Many investigators have studied these different pitch and amplitude perturbation measurements in normals and in abnormals. They have reported that these measurements can be used for screening and diagnostic purposes of laryngeal disorders. Most of these studies have established norms for jitter and shimmer measurements in adult population only. It is well known that children's voice characteristics differ from that of adults because of the continous neuromuscular maturation they undergo before puberty and the obvious morphological factors. So the adult data may not hold good for children. Therefore, this study was aimed at.

- Obtaining norms, for the following 6 pitch and amplitude perturbation measurements in thirty 10 years old normal male children
 - (i) Jitter ratio
 - (ii) Directional perturbation quotient for jitter (DPQj)
 - (iii) Relative average perturbation (RAP 3 pt)
 - (iv) Shimmer (dB)
 - (v) Directional perturbation quotient for shimmer (DPQs)
 - (vi) Amplitude perturbation quotient (APQ)
- 2) Comparing the data obtained for 10 years old normal male children with that of adult normals.
- Comparing the data obtained for 10 year old normal male children with that of 7 and 8 years old normal male children.

Thirty normal school going male children who had normal ENT findings, normal audiological findings and normal intelligence with no known history of voice problem, vocal abuse or other relevant vocal history were chosen for this study. After a practice session of 5-7 minutes their voice sample i.e. phonation of /a/, /i/ and /u/ for 5 seconds was recorded and analysed for the six parameters chosen, in the present study. The data obtained were subjected to descriptive statistics such as mean, standard deviation, ANOVA and DMRT to interpret the results and following conclusions were made.

- (i) Since pitch and amplitude perturbation measurements were obtained for 30 normal 10years old male children, the data provided may be used as norms for that age group.
- (ii) It was observed that the parameters which account for intensity variability show greater values for open vowels and parameters which reflect frequency variations tend to show greater values for tense vowels /i/ and /u/ as opposed to lax vowels /a/.
- (iii) It was found that children have higher perturbation values as compared to adults as per the theoretical expectations. This only strengthensour contention that we should have seperate normative data for children.
- (iv) It was found that jitter ratio was sensitive to age as its value changed across age groups.
- (v) It was found that shimmer (dB) was not so sensitive to age as its value was same across the three age groups unlike jitter ratio.

Recommendations:

Since the study included only male children because of nonavailability of subjects, time pressure, and nonavailability of computer time, it is recommended that the 6 parameters studied in this study may be carried out in females, and across different age groups. And such a study may provide additional data and strengthen some of the conclusions of the present study.

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