ACOUSTIC CHARACTERISTICS OF PAIN CRY IN THE FIRST 4 MONTHS OF LIFE: A LONGITUDINAL STUDY

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Register No: 17SLP038

A Dissertation Submitted in Part Fulfillment of Degree of Master of Science

(Speech-Language Pathology)

University of Mysore

Mysuru

ALL INDIA INSTITUTE OF SPEECH AND HEARING

MANASAGANGOTHRI, MYSURU—570 006

May 2019

CERTIFICATE

This is to certify that this dissertation entitled **“Acoustic Characteristics of Pain Cry in the first 4 months of life: A Longitudinal study”** is a bonafide work submitted in part fulfillment for the degree of Master of Science (Speech-Language Pathology) by the student holding Registration Number: 17SLP038. This has been carried out under the guidance of a faculty member of this institute and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

Mysuru

May 2019

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CERTIFICATE

This is to certify that this dissertation entitled **"Acoustic Characteristics of Pain Cry in the first 4 months of life: A Longitudinal study"** has been carried out under my supervision and guidance. It is also certified that this dissertation has not been submitted earlier to any other University for the award of any other Diploma or Degree.

Mysuru

May 2019

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DECLARATION

This is to certify that this dissertation entitled **"Acoustic Characteristics of Pain Cry in the first 4 months of life: A Longitudinal study"** is the result of my own study under the guidance of Dr K Yeshoda, Associate Professor in Speech Sciences, Department of Speech-Language Sciences, All India Institute of Speech and Hearing, Mysuru, and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

Mysuru ***Registration Number: 17SLP038***

May 2019

PRAISE THE LORD

I dedicate my dissertation to ALMIGHTY and my family.

“God will make a way where there seems to be no way”

Acknowledgement

Thank you ***Amma, Appa, Narmadha Akka & Ranj akka*** *a lot for all the support, love and care. All that I am today or hope to be; I owe to all of u….*

I express my huge respect and thanks to ***Dr K Yeshoda ma'am****, my guide….. You planted the spark for research in Infant cry analysis, in me ma'am. You are the Best ma'am, because YOU brought out the Best in me. You've always believed in me. You stood on my side and lead me throughout the times of good and bad. Your words of encouragement and care, gave me hopes and made me to hold on to it throughout.*

Mr Arun Raj sir*, am very grateful to you. Your words of assurance and advices boosted me up and helped me to manage the tough times during data collection. Thank you so much for making it happen sir.*

I also thank ***Swapna ma'am****, for helping me to get permission for my data collection and made it possible. It was so nice of you ma'am, to help out when I needed it.*

I thank ***Dr M Pushpavathi ma'am****, the Director of AIISH, for permitting me to take up and complete this research.*

I also thank ***Dr S Radhamani****, the Dean of Mysore Medical College and Research Institute,* ***Dr Sudha Rudrappa****, Professor and HOD of paediatrics for allowing me to collect the cry samples for my research. I extend my heartfelt thanks to* ***Ms Leelavathy sister****, Staff Nurse and* ***Ms Hamsa****, nurse assistant who helped to me record the cry samples throughout the data collection.*

My sincere thanks to ***Dr C D Santhosha****, for his valuable inputs and guidance, for helping me with the statistical analysis.*

I would like to convey my huge respect and thanks to all my staff of ***Madras Medical College****, Chennai-* ***Jaya ma'am, Vignesh sir, Suma ma'am, Kamala ma'am, Johnsi ma'am, Thenmozhi ma'am*** *and* ***Karthick sir****. They moulded and prepared me for my journey at AIISH, without whom this would have not been possible.*

A good teacher can inspire hope, ignite the imagination and implant a love of learning. One of the hardest jobs is to be a good teacher. But I have got so many good teachers to count on. I take immense pleasure in thanking all my faculties of ***AIISH*** *for shaping me a better professional to be in the near future.*

I thank ***Jayashree ma'am*** *for her endless moments of motivation and appreciation throughout my academic as well as clinical experiences. Ma'am, I thank you so much for the love you had for all of us (B-ing SLP), for taking efforts and expressing them to us. It was a memorable day of my PG life (25.04.2019). Also thank you so much ma'am for guiding our Journal Club and helped us to achieve the title-BEST JOURNAL PRESENTATION for the year 2017-18.*

*I thank **Jayakumar sir** and **Rajasudhakar sir**, good teachers from my place (Tamilnadu), who made their lectures more interesting, motivating and comfortable. Also I have to tell this; many times I wished to be a cool person like you both. I thank both of you for giving me tension-free hours of lectures at AIISH.*

*I would like to thank **Goswami sir, Gopi Kishore sir,** and **Freddy Antony sir**, for their interesting and attention-grabbing lectures. To be frank, I never felt tired in any of your classes. Thank you all for providing me with more practical knowledge and clinical skills.*

*I extend my sincere gratitude and respect to **Sreedevi ma'am, Sangeetha ma'am, Y V Geetha ma'am, Brajesh sir, Santhosh sir, Venkatesan sir, Shijith sir, Yashomathi ma'am**, and all the other faculties of AIISH for making my learning very interesting throughout my PG days.*

*I thank my clinical staff- **Gopi Shankar sir, Preethi ma'am, Geetha ma'am** and other staff of DCS for assisting me in every aspects of clinical learning.*

*At this moment, I also thank **Ravi sir**, for his kindness and help, at times of technical issues.*

*Next come my **seniors**….nop…they were my non-biological sisters…I take pleasure in thanking **Priyadharshni akka, Seshasri akka, Vasupradaa akka** and **Prithvi akka** so much. You all were the anytime care, help and guidance for me. I cherish all the moments that I had with you all. Thank you for being with me throughout.*

*Also I thank my bunch of spiritual girls- **Jasper, Roja Rani, Sundu, Gracia, Sarah, Shiva, Sashi, Siri, Krupa, Binu,** and **Rishisha**. More than my sweet juniors, you all were supportive, motivating and praying souls for me. Thank you for those Thursday night prayers and moral support.*

*Here comes the most important part of this. My dearest ones……To begin with, I thank **SKYLS**, my squad **who were, are and will** continue to be with me. My UG days in **MMC** with you all taught me so much in life. Thank you all for those good olden days….!!*

*Special mention to **LP (Lakshmipriya)** and **Naana (Sridhar)**….without them I could have not done this. You were the ones who know me completely and tried to handle me at any situation. Thank you both for all the little efforts you put to keep me happy, your endless love, care and support, and for being an important part of my life. Also my **Betiya (Shalu)** for always giving me the extra push I needed. I admire the ways u listen, think and suggest me things. You have been with me during the times; I need u the most… You know what I mean!! Thank u so much de for making hostel, a better place for me to live in.*

SlothB (Renita) *and* ***DQueen (Krishnendu),*** *my all-time buddies. Thank you for making so many ordinary moments, extra-ordinary, for creating memories to be treasured. I enjoyed every little chats, fights, care and love with u gals….*

Roja(Rose), Suganya(Dr MBBS), Sush(vibration) *and* ***Dhinesh (all time irritator)-*** *my long distance besties…. You guys made my school days so memorable….thank you all for those motivation and boost…*

And I should thank my ***DPs*** *(Dissertation Partners); My* ***Grammarly (Krithika)*** *and* ***KOB (Keren),*** *who travelled with me in the same boat and helped me to accomplish this. Thank you girls for making this journey possible. Also I thank* ***Susan*** *and* ***Malavi*** *for their timely deeds.*

B-ing Family, S-peechies, *and* ***Masters United****….I would like to thank and wish you all, the very best for your future. Thank u all for making my Master's life (2017-2019) unforgettable.*

How can I forget them??? My deepest thanks to each and every ***cute little babies****, their* ***parents*** *who accepted and participated in my study.*

*And finally I thank you….yes…****you****, who is reading this right now…for taking time and reading my little contribution to* ***INFANT CRY ANALYSIS****…. I hope u will also add on your attempts to it…all the best!!!!*

I once again thank each and every good soul who helped me in all the possible ways to make it a success….

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Introduction

"Crying is a biological siren, alerting the care giving environment about the needs and wants of the infant and motivating the listener to respond" (Zeskind and Lester, 2001).

Crying is one among the primary modes of expression of their emotions, hunger, pain and pleasure for the young babies and it attracts care from the parents by serving as a sign, signal and symptom. It attracts the attention of the parents when it is altered. When a child is sick, his/her cry changes from normal to deviant; it could be due to disturbances in the larynx or in the vocal tract. It can also reflect the function of brain. Parmelee (1962) stated that crying is an indicator of the nervous system's ability to be activated and also the capacity to inhibit or modulate this activation. Cry has two aspects; (1) the cry itself, being innervated by the cranial nerves which control the autonomic nervous system (2) salience of the cry to the caretakers in the child's immediate environment. This activates the sympathetic nervous system in the caregivers and makes them to show either "fight or flight" response to the cry. Cry is thus an infant behaviour that echoes the state of a helpless baby by provoking the caregiver to meet their needs. Infant cry comprises of copious levels of information about the reason for cry, integration, maturation and functioning of central nervous system, vocal production system, language acquisition, and respiratory system. It is investigated to trace out the essential acoustic features, to realize their causes, and effects of causes which are obvious in their cry behaviour (Chittora & Hemant, 2016).

Developmental course of crying

Infant cries represent two types of responses to a stimulus; either volitional or reflexive and thereby lead to variations in acoustical and perceptual characteristics. Newborn cries are least variable and highly reflexive, thereby giving way to consistent values for comparison (Gilbert and Robb, 1996). The extent of crying increases gradually and peaks during 6 weeks of age followed by a decrease in its amount by around 4 months and remains stable till the first year of life (Zeifman, 2001). Their cry slowly begins to exhibit various psycho-physiological states like pain, hunger, distress, etc. Therefore, the older cries are assumed to indicate the combination of child's physiological growth as well as environmental influences that makes the cry, a result of volitional expression. The volitional type of cry with increasing age also reflects corresponding changes in the acoustic measures (Gilbert and Robb, 1996). This highlights the notion of slow transition from neural circuits controlling the involuntary expression of cry to voluntary expression by the limbic system and cortical systems respectively through the first year of life (Myers, 1968 and Murray 1979). Moreover, the maturational trend that can be observed in various measures of infant cries might vary with different acoustic parameters.

Despite the individual variations seen in terms of perceptual and acoustic measures, there are also predictable changes that can be seen during the course of the development that are influenced by the environment. These changes can be associated with the other features of biological and psychological maturation and growth like the extension of vocal apparatus and their memory skills. Apart from developmental changes, even individual and cultural differences could be seen at the completion of first year of life suggesting the environmental influences (Zeifman, 2001).

Maturation of anatomical & physiological systems

According to Fuamenya, Robb & Wermke (2015), the most crucial period in every infant's vocal development is their first 3 months of life. Healthy and appropriate vocal fold vibratory behaviour remains a primary requisite for an infant's oral communication. Vocalizations are shaped by their supra-glottal articulations just before the completion of the third month which is an effect of remodelling of the vocal tract of the infants and maturing neurophysiological control systems. Dynamic variations in these anatomical and neurological systems as they mature are reflected in the acoustic parameters of the crying behaviour which can be used to expand infant monitoring in the earlier days of life.

Moreover, various brain functions are found to mature during the initial 3 months of life that may be factors which lead to increase in vocal control of crying. Current outcomes propose that precise brain functional processes associated to vocal expansion occur during this principal period of 3 months, specifically during the second month. In addition, research findings have highlighted the development of neural structures like Broca's area and synaptogenesis continuing upto first three months of life in the auditory cortex (primary sensory area of the brain) and prefrontal cortex (expression of emotions), leading to improved vocal control mechanisms (Fuamenya, Robb & Wermke, 2015).

Types and causes of crying

Often the occurrence of cry is unexplained and unprovoked. The proximate causes of crying can be either exogenous or endogenous. External causes might include pain, birth, hunger, and attention. The function of the birth cry is completely physiological, having to do with the establishment of normal respiration and the oxygenation of blood. Pain or discomfort cries are evoked after the presentation of any kind of painful stimuli like vaccination, cold exposure, discomfort etc. Cries are also thought of as attachment behaviour, when it is observed many a times of physical separation. On the other side, endogenous cries are assumed as a means of physiological regulations by releasing their tension and excess energy as reported by Brazelton (1962, 1985) or any unexplained maturational changes in Central Nervous system organization as explained by Emde, Gaensbauer and Harmon in 1976. Initially the cries are endogenous and gradually turn into exogenous in nature during the later infancy period (>2.5 months or so) as they are highly related to any particular extrinsic incidents.

Pain cries

Newborns undergo several painful and stressful procedures in hospitals. “Each hospitalized newborn in intensive care, receives from nearly 50 to 150 painful procedures a day, and those newborns weighing under 1000g suffer nearly 500 or more painful interventions during the hospitalization”, (Guinsburg, 1999). “Pain is an unpleasant emotional and sensorial experience, associated with a real, potential or described tissular injury” as defined by the International Association for the Study of Pain (IASP, 1979). Researchers have observed that the anatomical, functional and neurochemical systems that allow for the pain perception are completely developed by the 20th week of gestation. Newborns respond to any kind of painful stimulation only through their behavioural and physiological reactions that includes their body movements, facial expressions, and crying, providing information regarding their levels of discomfort and stress experienced throughout the procedure.

Stimulation for Pain cries

Dealing with newborn human participants is a very sensitive task which includes recording of the infant cries. One major type of exogenous cries is Pain cries using which many studies are performed. To record the pain cry, the mode of stimulation presented to the babies has been an ethical concern for a long time. There are several ways to stimulate their pain cries during which none of the infants receive any additional behavioural and/ or pharmacological approach to lessen or control their pain like giving them sweet solutions, pain killers, comforting or holding in order to control for the possible variability in the intensity of pain that may occur during the procedure.

Few researchers in the medical domain record the pain cries by the application of a rubber snap to the infant's foot. Very few parents do not hinder them as long as it is done by the medical professionals; however, others mostly do not allow that process. On the other hand, hunger cries are even more difficult to record because the only method available to collect hunger cry is to wait patiently until the baby spontaneously begins to cry out of hunger. However, this has an important issue of convincing the parents to consent for the data collection. Recording of pain cries are possible merely during vaccination procedure or during any treatment as in immunization programs wherein injections are given (Chittora & Hemant, 2016).

The research project carried by Branco, Fekete, Rugolo and Rehder in 2007 recorded the pain cries from 111 healthy newborns during the venepuncture during 24-72 hours of life. In another study by Robb (2003), pain elicited cries were recorded from the babies through the application of painful stimulus to the right sole of the infant's foot. Runefors and Arnbjornsson in 2005 recorded the pain cries for a longitudinal study during vaccination procedures and also when the heel was pricked to draw blood sample for Phenylketonuria (PKU) screening test.

Hence to consider and resolve these sensitive issues, researches are carried out in this field only when the procedure of data collection is within the acceptable and allowable conditions as given by the Ethical Board Committee of their respective Research Institutes.

Importance of Infant cry analysis

Investigation in infant cry analysis by means of signal processing methods such as spectrographic analysis was initially done by a group of Scandinavian researchers in 1960. Most of the work done in this field was related to medical science, behavioural science, and linguistics. Nevertheless, there is little work accomplished in this direction (Chittora & Hemant, 2016). An infant's health can be estimated by perception of his/her cries. Truby and Lind (1965) indicated that the perceptual analyses of cry stimuli are used by clinicians "as supplementary criteria for the evaluation of neurological function in general and specifically of respiratory function". However, these perceptual approaches are not valid because the descriptions used are ambiguous and subjective. Acoustic analysis of the cry would seem to be a valuable adjunct tool to utilize for the evaluation of the physiological integrity and development of the infants.

Cry analysis is reported to be a substantially non-invasive tool that can be used for the timely diagnosis of wide-ranging infant medical conditions. Though such studies have considerably heightened the inclusive information of several traits of the infants' cry, they merely gave snapshots, as their focus was on the clinical implication of deviant parameters of cry in infants. Appreciating the cry phenomena remains an experiment for investigators up to the present (Heidi & Marcio, 2007).

Acoustic features of cry

High-pitched shrill cries have often been associated with diseases involving the brain. With advances in cry research, it has been demonstrated that pitch pattern is not the only indicator of pathology in infants with brain damage. Other features, such as variations in the latency in pain stimulated cries, duration of the phonation, an abnormal melody type, or a more frequent occurrence of gliding and bi-phonation, are also involved.

Investigation of these features may serve as valuable early risk markers regarding their language outcome as they are related to the development of vocal control. With the purpose of arriving at a more clear and complete picture of acoustic features embedded in the infant cries, it is highly essential to obtain and analyse longitudinal data during their early period focusing on the above-mentioned characteristics seen in the cries of healthy newborns. This will also help us in the subsequent identification and differentiation of an abnormal cry phonation from an intact one based on these acoustic properties.

Need for the study

Analyses of pain cries of newborns have revealed that newborns modify the supra-laryngeal tract extensively after a painful stimulation than during spontaneous cries (Chittora & Hemant, 2016). There are evidences showing abnormal acoustic parameters in the cries of infants having medical conditions. To date, there are only few longitudinal studies that have evaluated the growth of various acoustic parameters in the pain cries of healthy newborns.

Thus, it is crucial to realize the necessity of carrying out a longitudinal study to get an insight into the developmental trend of pain cries in healthy young infants from birth to 3-4 months. This will help in identifying and ascertaining the acoustic characteristics of the cry and the developmental trend could be speculated as an outcome measure. Further, the characteristics of the cry that are primitive and mature with age could be delineated. Hence the present study was planned to investigate the acoustic characteristics of pain cries in newborn infants and check for developmental trends as revealed by the acoustic characteristics.

Chapter II

Review of literature

Crying can be a sign, symptom and a signal. Crying as a sign indicates the state of mind like being hungry, fatigued, or in pain. Crying as a symptom refers to as a clinical concern as disabled child or temper tantrums. Signals imply the way crying functions in different contexts but without any possible clinical significance, (Barr, Hopkins, & Green, 2000).

Crying is a compelling signal which draws care and nurturance from the caregivers in the form of lifting them up, feeding them or calming them down. At the same time, information about their health, physiological state and identity is also conveyed through crying. Sometimes it may provoke even escape or abusive responses like neglect when the crying persists for a longer time or when it cannot be easily ceased (Zeifman, 2001).

Importance of Infant cry

Neonatal cry results from either intrinsic or extrinsic stimulation and involves coordination among numerous brain centers like brainstem, mid-brain and limbic system (Linda, Rebecca and Lester, 2005). Cries are used diagnostically for the identification of neurological impairment in young infants at an early stage. A healthy infant is first identified by their birth cry which is a sign of the newborn's viability (Zeifman, 2001). Crying has also contributed to the parental decisions about responding to the cry (Furlow, 1997). Previous literature has shown dissimilarities in the acoustic measures of normal infant cries from the cries of premature and atypical infants, including the infants with Down syndrome, neonatal asphyxia, neurological damage and hyperbilirubinemia. Differences are noted in terms of higher or lower fundamental frequency than the healthy babies, increased variability, and dissimilar durational patterns (Wasz-Hockert, Michelsson & Lind, 1985).

Measurement and analysis of Infant cries

Neonatal cries are mostly studied as opposed to cries in older infants as they reveal about an infant's initial neurological state before the possibility of influential changes by experiences. Moreover, pain cries are targeted frequently as they reflect an infant's ability to indicate emergency. Studies done using the cries of infants with medical conditions support the notion of association of neurological status and the cry. Over the years, several methods have been developed to carry out the analyses of infant cry like auditory analysis, time domain analysis, frequency domain analysis, and spectrographic analysis.

Table 1 shows the parameters currently being measured in infant cry assessment (Corwin, Lester, Golub, 1996; Zeskind and Lester, 2001; Lester, Tronick, LaGasse, 2002).

Table1: *Parameters measured in infant cry by different researchers*

Characteristics	**Definition**	**Biological mechanism**
Cry latency	Time from known stimulus to onset of the first utterance	Arousal from limbic-hypothalamic system
Threshold	No. of applications of stimuli to elicit a cry	Arousal from limbic-hypothalamic system
Utterances	No. of cry sounds across cry	Neural control of respiratory system
Short utterances	No. of unvoiced sounds across cry	Unstable respiratory control
Phonation	Cry mode resulting from vocal cord vibration between 350-750Hz	Neural control of muscular tension in vocal cord & airflow through the glottis
Hyperphonation	Cry mode caused by a sudden upward shift in f0 to 1000Hz	Neural constriction of vocal tract
Dysphonation	Cry mode caused by noisy or inharmonic vibration of vocal cord	Unstable respiratory control
Cry mode changes	No of times cry mode changes during an utterance	Inability in neural control of vocal tract
F0	Base frequency during vocal cord vibration, heard as the pitch of the cry	Vagal input to larynx and vocal tract
First formant	Frequencies centered at first resonance of f0, approximately 1000Hz	Neural control of size and shape of upper vocal tract
Second formant	Frequencies centered at second resonance of f0, approximately 3300Hz	Neural control of size and shape of upper vocal tract
Duration	Time from onset to offset of cry utterance (ms)	Neural control of respiratory system
Duration of inspiration (inter utterance interval)	Time between cry utterances, which is a measure of breath holding; evaluated by the second inspiratory period (ms)	Neural control of respiratory system
Amplitude	Intensity of the cry; heard as loudness (dB)	Neural control of respiratory system and capacity
Variability in f0	Changes in f0	Instability in the neural control of the larynx and lower vocal tract
Variability in F1 and F2	Changes in formant	Instability in neural control of upper vocal tract
Variability in amplitude	Changes in intensity within an utterance or averaged across utterances	Instability in neural control of respiratory system

Characteristics of pain cries

Branco et al in 2007 aimed at describing the pain cries of young infants through acoustic analysis and its relation with vocal emissions to obtain information about the laryngeal structures and its neural control. In this study, pain cries were recorded from 111 healthy newborns during the venepuncture during 24-72 hours of life. Simultaneous perceptive auditory analysis and acoustic analysis using GRAM program (version 5.7) was carried out to comment on the vocal quality of the infants. It was revealed from the pain emissions that they all contained the vowels and posterior consonants associated with or without tense strangled voice quality relating to the vocal tract constriction, configuration and dynamics. All the cries also contained hard vocal attack. Considering frequency parameters, there was homogeneity across the infants i.e. frequency variations, breaks, double harmonic breaks were present in all their cries. The fundamental frequency variation was consistently seen in all the infant pain cries. Complex modulation of melody patterns, breaks and double harmonic breaks, higher fundamental frequency, higher accretion of spectral energy; and other few characteristics of pain cries were also observed. Inspiratory phonation was recorded in all the infant cries which occurred during the inspiratory segment. There was a significantly higher value of fundamental frequency relating to their weight.

Bifurcations are the abrupt qualitative modifications in laryngeal behaviour. They occur when there is a non-linear change in the levels of either sub-glottic pressures or vocal fold tension. They are of different types with respect to onset of voicing (Biphonation-BP), period doubling (HD) or sudden shift in periodicity (FS). Past studies have reported the increased occurrences of these phenomena among the disordered infants including the preterm babies as they are assumed to have neurological immaturities. With this notion, it is predicted that preterm infants would produce these features more in numbers than the full-term infants (Robb, 2003). Hence, it is clear that bifurcations could be considered a parameter of normal healthy infant cries along with certain other markers like high pitched (high F0) cries, dysphonation or noisy, turbulent cries, increased number of cry mode changes and variability of F0 and F1 have the potentiality to indicate for further neurological evaluation to be done for the infants.

To track the developmental course of the types of bifurcations and chaotic segments, longitudinal investigation of pain cries from both the groups are essential. This would further help in the determination of neural maturation and influence of these characteristics aiding as an indicator of neurological dysfunction.

Acoustic characteristics of pain cries using longitudinal studies

Thoden and Koivisto (1980) conducted a prospective investigation of pain cries of 38 healthy infants from 0-6 months of age and analysed the 1^{st}, 2^{nd} and 3^{rd} phonation following the pain stimulus. They reported the following results: range of the mean maximum pitch of the fundamental frequency without shift was between 550 and 660Hz and the range of mean minimum pitch was between 330 and 410Hz. The predominant melody types observed in these cries were falling and rising-falling. The first cry signal had duration between 4.1 and 5.2 sec, second- 1.6 and 2.0 sec, and third- 1.2 and 1.6 sec. Two-thirds of the cry signals were voiced; voiceless cries were less abundant in second and third signals than in the first one. The continuity of the first signal was less than the other two.

A study on pain cries of healthy newborn infants of age ranging from birth to 6 months (Wasz-Hockert, Lind, Vuorenkoski, Partanen & Valanne, 1968) revealed that the mean maximum pitch of the fundamental frequency without shift was about 650 Hz, and the mean minimum pitch was 400 Hz. The mean fundamental frequency varied between 350-650 Hz and shifts with higher pitch occurred roughly in every 3rd cry after the pain stimulus (immunization). Falling or rising melody pattern was observed in 80% of cry samples. Gliding, biphonation, furcation, glottal roll and vibrato were some of the common acoustic features seen only in the crying of sick infants.

Runefors and Arnbjornsson in 2005 explored the pain cries of 10 healthy infants through the first year of life using spectrograms at specific schedules- between 3 and 5 days old, 3 months, 6 months and 12 months of age. Results revealed that Cry Latency decreased and Cry Duration increased with increasing age. Fundamental frequency increased till 3 months of age and showed a slight reduction till 12 months of age owing to the maturation of vocal tract.

Certain behaviours like sub-harmonic peaks/ Double Harmonic Peaks (DHP) and noise-like (N) phenomena that are notable in the spectrograms of infant cries are present due to the irregularities in vocal fold vibration. These features are an indication of chaotic vocal fold oscillation wherein the vocal cords are acting like a desynchronized coupled oscillator generating non-periodic irregular vibrations. They are observed both in the cries of healthy newborns and also in infants with certain neurologic disorders (Truby and Lind, 1965).

"Inspiratory Phonation (IP) is the audible phonation that occurs in the inspiratory phase of the breath cycle" (Wermke, Haschemi, Hesse and Robb, 2018). IP is one of the common acoustic features of pain cries during neonatal period (Grau, Robb and Cacace, 1995). Reports of (Wermke et al, 2018) have documented that the IPs were not a characteristic feature of comfort vocal emissions in infants with normal language acquisition such as cooing or babbling as it represents the improper control of the respiratory rhythm. Therefore, presence of IPs beyond 6 months of age could be a sign of neurological disorder. However, the exact time up to which IP can be produced in an infant's vocal emission is yet to be determined.

In Indian context, Venugopal in 1995 studied the acoustic measures of pain cries in 5 healthy newborns from first day to three months of life. Cry samples were recorded at 7 different intervals, i.e. within 24 hours, after 1st week, 2nd week, 3rd week, 4th week, 2nd month and 3rd month, and analysed using Multi-Dimensional Voice Program (MDVP). Twenty nine acoustic characteristics related to frequency, intensity and other parameters were measured. When these were subjected to statistical measures, they revealed no significant changes across the study period.

While there are number of studies available on infant cry analysis, only snapshots of developmental works could be found in the literature. Earlier studies were highly revolving about the infants with several types of medical conditions especially during the newborn period. In order to have a good temporal resolution and high focus on several essential parameters of cry acoustics simultaneously, further developmental works are warranted. Hence, to track the developmental markers as well to broaden the diagnostic window; cry features have to be studied in healthy infants longitudinally including their late infancy period as well.

Advantages of longitudinal studies in Infant cries

Certain perceptual and acoustic variables may sound abnormal that attracts listeners and medical attention. Initially the child may be symptomless in their physiological signs whereas their discomfort and pain levels may become high either prior to or simultaneously with the onset of the disease. When the initial suspicion is detected and regularly followed up on with the other medical and neurological investigations, this may lead to early identification and management of the particular disorder.

Developmental studies on infant cries are always advantageous as they may help in establishing characteristics that may signal maturation of the rudimentary physiology of the newborns to adult-like maturation as chronicled by cries. Hence, the present study was planned to attempt chronicle the acoustic signs of pain cries in healthy infant from birth to first 4 months of life. The aim and objectives are as follows:

Aim

To explore the maturational pattern of the pain cries characteristics in infants in the first 4 months of life.

Objectives

1. To make quantitative estimation of cry signals in young infants with no significant natal history ranging in age from birth to 14^{th} week i.e. day 1 to day 98 at regular intervals using acoustic analysis.
2. To document the various acoustic features of pain cries in young infants with no significant natal history ranging in age from birth to 14^{th} week i.e. day 1 to day 98 at regular intervals after acoustic analysis.
3. To highlight the developmental markers in these measured features across the first four months.
4. To check the presence of any gender differences in the features recorded.

Chapter III

Method

Design

Longitudinal study design was adopted in order to track the maturational pattern of infant**s** pain cries.

Participants

Pain cries were recorded from a total of 77 healthy newborns during their first immunization procedures after birth. Pain cry samples of newborns were collected for the proposed research from the Cheluvamba Government hospital, Mysore. During the scheduled second vaccination, only 16 babies returned for the procedure. The number further reduced to 12 for the third vaccination and the total number of babies who could be successfully followed up for the fourth vaccination procedure was only nine. All the 9 newborn infants, 6 females and 3 males fulfilled the inclusionary criteria of the study and were regularly followed for a period of 4 months at the specified intervals- 1st day, 6th week, 10th week and 14th week.

Ethical considerations

The elicitation of cry, stimulation for elicitation of cry and recording of those cries for data collection followed the Institute's Ethical practices throughout the research. Written consent was obtained from the infants' mothers and their spouses and was included only after voluntary willingness/acceptance. Then, they were informed about the aim and the protocol of the research carried out. Participant Information form was filled prior to the recording to confirm the fulfilment of inclusionary criteria of the study.

Inclusionary criteria

The revised position statement of Joint Committee on Infant Hearing (JCIH) 2007 (Appendix I) was considered as a screening register of High Risk (HR) factors. The pain cries of the infants who presented with none of the High-Risk Factors were recorded at specific regular periods. Prior to the inclusion for the study, the expected date of deliveries was found out from the medical records of the pregnant mothers. All the infants had a full-term delivery with no complications and normal birth weights. During their routine postpartum examination, it was reported by the Paediatrician that all the babies were healthy. Also they were not under any other operative or medical intervention throughout the study period.

Exclusionary criteria

The infants who failed in the High Risk Register i.e. presence of one or more High Risk factors, were not included in the study and proper referrals were made to the Institute for a detailed evaluation.

Stimuli

Pain cries of the targeted infants were recorded soon after the immunization procedures at specific periods as it had the following advantages:

- Amount of stimulation and reason for the cry was same for all the infants
- It is fully controlled by the experienced medical practitioners
- Pain stimulation was acceptable to the parents/care givers
- Cries were collected at specific age

Procedure

The first recording of the pain cry was done during the first vaccination given to the new born on their first day of life. The subsequent pain cry samples of the chosen infants were recorded on the scheduled vaccination periods during 6^{th} week, 10^{th} week and 14^{th} week. This resulted in a total of 36 cry samples for the acoustic analysis. However, in spite of the best efforts, equal number of male and female participants could not be maintained.

A portable digital audio-recorder (Olympus digital voice recorder LS-100) with facility for external unidirectional microphone (Sennheiser) was used for recording the samples. The infant was held in the supine position and the microphone of the recorder placed at a constant distance of 12 cms from the infant's mouth during the recording and the same procedure was followed for all the babies. All the cries were recorded for a continuous duration of at least 30 seconds in the respective immunization units within the hospital premises. The record button was switched on 10 seconds prior to the injection of the needle during the immunization procedures. Care was taken that at the time of recording, all the newborns were awake, comfortable at the time of injection and no attempts were made by mothers/care takers/medical professionals to calm or soothe their babies. At the time of recording, the parents and the medical professional were in the procedure room throughout the data collection period.

Analysis

The recorded data was then transferred to a 64-bit Windows-10 operating system containing AMD processor for the analysis of the recorded cries. All the 36 cry recordings were analysed using PRAAT software version 6.0.30. Only the first cry segments which were longer than 500ms were considered for analysis. For each of the cry samples, the following list of frequency-related, intensity-related, duration-related and other measures was extracted using narrow band spectrograms (45Hz).

I. Frequency-related parameters-

- Mean fundamental frequency (MF0): Mean F0 measures consider average frequency over time.
- Mean maximum fundamental frequency (MinF0): Lowest quantifiable point in the fundamental frequency seen on the spectrogram.
- Mean minimum fundamental frequency (MaxF0): Highest quantifiable point in the fundamental frequency seen on the spectrogram.
- Formants frequencies 1, 2 and 3 the formant frequencies 1, 2 and 3 were denoted as F1, F2 and F3 respectively. Frequencies that had maximum energy concentration at specified frequencies are characterized as formant frequencies. They are represented as a function of time.
- Melody pattern: Melody type was classified as rising, falling, flat, rising-falling, and falling-rising. The crying pattern was considered as falling or rising when there was at least a 10% change in pitch level during more than 10% of the duration of the cry (Wasz-Hockert et al., 1968). Melody pattern was identified from the final portion of either first or second crying segments in the sample.

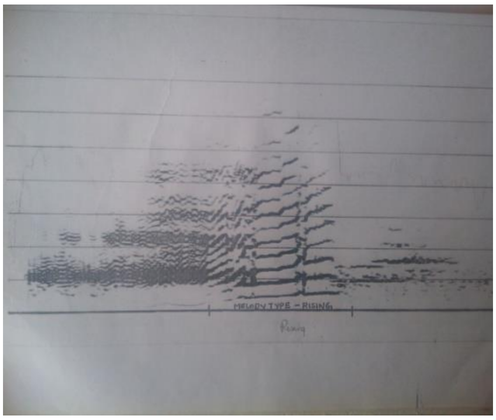

Fig. 1: Narrowband spectrogram depicting the melody type (Rising pattern)

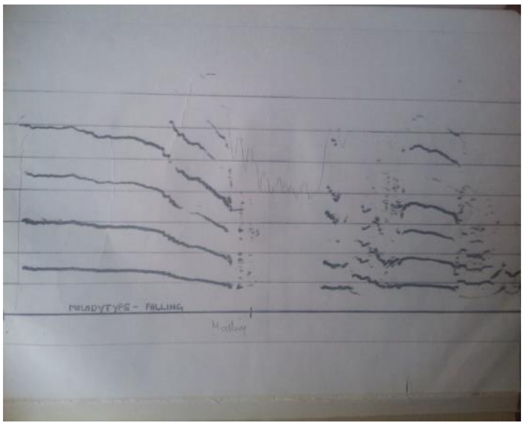

Fig. 2: Narrowband spectrogram depicting the melody type (Falling pattern)

II. Intensity-related parameters-

- Mean intensity (MI): considers average intensity over time.
- Mean minimum intensity (MinI): Lowest quantifiable point in the intensity seen on the spectrogram.
- Mean maximum intensity (MaxI): Highest quantifiable point in the intensity seen on the spectrogram.

III. Duration-related parameters-

- Cry duration (CD): Time from onset to offset of cry utterance (ms).

- Cry latency (CL): Time from known stimulus to beginning of the first cry segment. In this study, latency measures were done from the time of needle injection to the initiation of the first cry segment.

IV. Other parameters:

- Cry mode changes (CM): Number of times cry mode changes during an utterance
- Shift (F0S): an abrupt upward or downward change in F0.

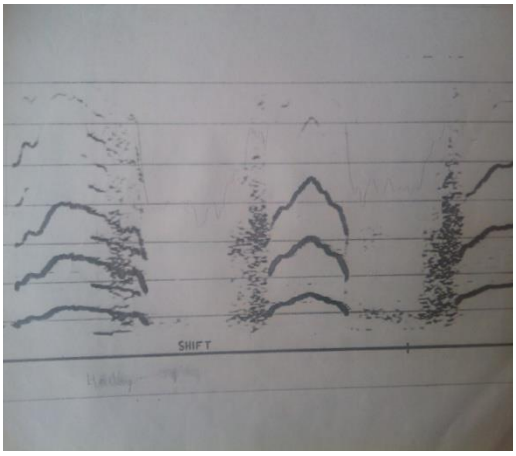

Fig. 3: Narrowband spectrogram depicting the shift

- Noise concentration (NC): denoted as a clearly audible, high energy peak of 2000-2500Hz found in both voiced and voiceless parts of the signal. It was extracted from the first cry segment for all the babies.
- Double harmonic peak (DHP): a parallel series of harmonics having the same melody pattern as the F0 and occur simultaneously with F0. It indicates roughness of the voice.

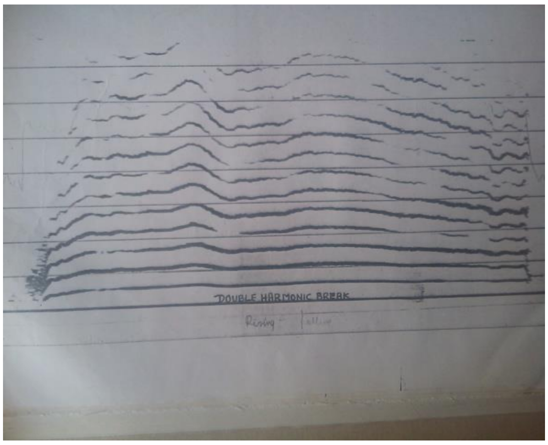

Fig. 4: Narrowband spectrogram depicting the Double –Harmonic Peak

- Inspiratory phonation (IP): "IP is the audible phonation that occurs during the inspiratory phase of the breath cycle"- Wermke et al, 2018

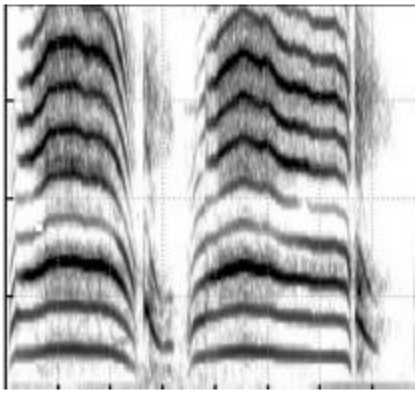

Fig. 5: Narrowband spectrogram depicting the Inspiratory Phonation

- Unvoicing ratio (VUV): VUV ratio- ratio of number of unvoiced frames to total number of frames in a cry.

Statistical analyses

SPSS version 20 was used to perform the statistical analysis of the data extracted from recorded cries of the chosen infants. The data was subjected to Shapiro Wilk's test to determine the presence of normality and it was revealed that the parameters like – CD, MF0, F1, F2, F3, MI, MaxI, F0S, VUV followed normal distribution (i.e. $p>0.05$). For these parameters, Repeated Measures ANOVA was carried out to check for the significant difference. And the parameters that were not normally distributed (i.e. $p<0.05$) like – CL, MinF0, MaxF0, MinI, CM, DHP, IP, NC, a Non-Parametric Friedman's test was done to find out the significant difference between the sampled intervals. As there was no statistically significant difference in the acoustic measures among the sampled intervals, Bonferoni Pair-wise test & Wilcoxon Signed Rank test was not carried out for further pair-wise comparisons. Descriptive statistics was performed to calculate the mean, standard deviation (SD) for all the extracted measures.

CHAPTER IV

Results

The main objectives of the current study were as follows:

1. To make quantitative estimation of cry signals in young infants with no significant natal history ranging in age from birth to 14^{th} week i.e. day 1 to day 98 at regular intervals using acoustic analysis.
2. To document the various acoustic features of pain cries in young infants with no significant natal history ranging in age from birth to 14^{th} week i.e. day 1 to day 98 at regular intervals after acoustic analysis.
3. To highlight the developmental markers in these measured features across the first four months.
4. To check the presence of any gender differences in the features recorded.

Attempts were made to include equal number of male and female infants in the study but could not be maintained due to participant attrition, and hence the fourth objective could not be studied.

A total of 9 healthy newborn infants (6 females and 3 males) were followed up and their pain cries were audio recorded during the immunization procedures at specified intervals in the first 4 months of their life. Acoustic measures were extracted from all the audio-recorded pain cries and the following frequency-related: MF0, MinF, MaxF, F1, F2, F3 and Melody, intensity-related: MeanI, MinI, MaxI, duration-related: CL, CD and other features: DHP, F0S, NC, CM, IP and VUV were noted. The results of the same are discussed in the following paragraphs.

Descriptive statistics, that is, the mean, standard deviation (SD) and the tests of significance were carried out and the results are summarized under following four sub-headings:

I. Frequency related parameters

The results for the main frequency related parameters, MF0, MinF, MaxF, F1, F2, F3, and Melody are shown in Table 2. From table 2, it is noticed that the mean MF0 and mean MinF were highest at the birth but decreased on subsequent recordings but at fourth month of age the values increased but not to the level of the first day of age. However, the mean MaxF gradually increased from first to fourth month of age. Formant frequencies, F1, F2 and F3 gradually decreased across the recordings of first day, 6^{th} week and 10^{th} week. This was followed by an increase during the 14^{th} week and was highest when compared to the pain cry recorded on all the other 3 schedules. Statistical significance was not seen for any of these parameters ($p>0.05$). The Melody patterns noticed were falling patterns at the end of cries across all the four recordings at specified intervals in all the participants.

Table2: *Mean, standard deviation (SD), F and p values for frequency related parameters across all the four recordings*

Frequency parameters	First day		6^{th} week		10^{th} week		14^{th} week		Test of significance	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F value	p value
MF0	342	31.99	328	44.58	319	49.46	326	37.86	0.52	0.67
MinF0	150	51.72	109	41.08	111	47.59	134	62.25	3.53	0.31
MaxF0	490	44.43	506	37.82	510	17.80	504	26.50	4.6	0.2
F1	1067	219.11	946	123.10	993	176.66	1148	258.25	1.74	0.18
F2	1938	234.50	1760	235.84	1834	196.80	2089	287.34	3.27	0.3
F3	2885	115.48	2899	230.15	2825	143.81	3083	317.60	2.36	0.09

II. Intensity related parameters

The Intensity related measures extracted are as follows, MI, MinI and MaxI and specified in Table 3. The mean intensity values ranged from 58.70-61.16 dB across the four recordings. The mean MI values showed an increase from first day to the 6th week and gradually decreased in the other two recordings i.e. 10th and 14th week. While the mean MinI values revealed a decreasing trend in their mean values, mean MaxI values had an increasing trend across the four recordings and these values did not reach (p>0.05) statistical significance (Table 3).

Table 3: *Mean, standard deviation (SD), F and p values for intensity related parameters across all the four recordings.*

Intensity Parameters	First day		6th week		10th week		14th week		Test of significance	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F value	p value
MI	58	7.16	61	5.80	60	5.81	58	6.43	0.5	0.68
MinI	36	7.08	36	6.64	34	5.42	33	6.43	0.86	0.83
MaxI	66	6.64	71	6.74	71	4.61	70	6.74	1.74	0.18

III. Durational parameters

CL and CD were the durational measures obtained and are indicated in Table 4. From Table 4, it is observed that the mean CL values decreased across the first 3 recordings i.e. from birth-2.5 months, and then increased in the 4threcording- 3.5 months which was lesser than that of the first recording. The mean CD values showed a gradual increase across the time from birth to 2.5 months of age for all the babies and decreased at the 3.5 months and still remained longer than the first recording (first day). However these results were not statistically significant (p>0.05).

Table 4: *Mean, standard deviation (SD), F and p values for duration related parameters across all the four recordings.*

Durational parameters	First day		6th week		10th week		14th week		Test of significance	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F value	p value
CL	2.78	3.15	0.92	0.78	0.85	0.62	1.46	1.79	1.13	0.76
CD	6.08	5.65	7.25	2.91	8.65	2.92	8.07	2.66	1.36	0.27

IV. Other parameters

Table 5 shows the other parameters, CM, F0S, DHP, IP, NC, and VUV. From table 5, it is spotted that CM values were rising from first day to the 10th week gradually and then a slight reduction in the 14th week (lesser than the mean value of first day). F0S showed a decreasing pattern across the 4 recordings. DHP was consistently seen across all the pain cries of the infants and their values increased from first day to 2.5 months and reduced in the fourth recording which was still higher than the value recorded on their first day. All the babies had IP consistently at the beginning of their second cry segment and followed by a decreasing trend across the initial three recordings followed by a sudden increase in the fourth recording. NC was decreased in the fourth recording when compared with the first recording but they did not follow any trend across the 4 months. VUV values increased at the 3.5 months pain cry than the first recording during the study period. However none of these parameters reached any significant difference statistically (p>0.05).

Table 5: *Mean, standard deviation (SD), F and p values for other parameters across all the four recordings*

Other Parameters	First day		6th week		10th week		14th week		Test of significance	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F value	p value
CM	4.78	1.92	5.22	4.26	5.56	2.12	4.44	1.23	1.55	0.67
F0S	6.00	3.64	5.78	2.63	4.44	2.96	3.44	2.18	1.5	0.23
DHP	1.67	1.11	1.89	0.92	2.00	1.32	1.78	1.30	0.13	0.98
IP	0.78	0.44	0.67	0.50	0.67	0.70	0.89	0.33	1.35	0.71
NC	1.22	1.71	0.33	0.70	1.44	1.66	0.44	1.01	7.82	0.06
VUV	32.84	22.22	50.73	14.21	47.56	19.68	52.88	20.49	2.39	0.09

To summarize, under Frequency-related parameters, it could be seen that the range of frequency is increasing with age and falling pattern of melody was noticed at the end of all the cries. All the three formant frequencies showed an increasing trend across the four recordings. Intensity range was also observed to be increasing across the four recordings. Cry latency decreased and cry duration increased with increasing age. Frequency shift showed a decreasing pattern throughout the investigation period. In general, it was observed that developmental trend was noticed for a few frequency related and temporal parameters.

CHAPTER V

Discussion

Results are discussed under the following parameters: Frequency-related, Intensity-related, Duration-related and other parameters.

I. Frequency related parameters

The current results showed that the mean MF0 of the cries gradually decreased with increasing age, and is similar to the results reported by Runefors & Arnbjornsson (2005) wherein, the authors found decreasing values of MF0 in pain cries across first three months of life. This could be explained as an outcome of total growth physically as well as in laryngeal structures (like thickening and lengthening). Due to the developing neurological and psychological maturation over the laryngeal structures, MF0 values may vary. Neurological perspectives are the increased control over laryngeal musculature and vocal fold stress. Psychological maturation allows them to understand the environment and handle them accordingly (Wolf, 1969). The contrary findings could be related to the methodological variations of each investigation like mode of cries, sampling intervals between the infant cries and so on.

The mean MaxF0 values were higher in the 14th week recording than during the first day. MinF0 showed a slight rise from 10th week to 14th week and did not follow any particular trend throughout the study period. Venugopal (1995) analysed pain cries from 1st day – 3 months of life and reported a similar trend with no significant differences in the MinF0 and MaxF0 values.

The mean F1, F2 and F3 frequencies values showed an increasing trend across the 3.5 months of life. The probable explanation could be that the developmental trends of increasing F1, F2, and F3 reflect the development of vocal structures during the newborn period.

Falling type of melody was noted at the end of the cries in all the infants of the study. Analogous results are recognized in the investigation carried by Wasz-Hockert et al (1968) as well as by Thoden & Koivisto (1980). These authors in their studies have reported falling melody pattern in the pain cries of infants from first day to six months of life. The reflexive nature of cry with the spinal control of the basic biological needs necessitates babies to follow a structured pattern in intonation patterns. It is postulated that production of complex melody increases with increasing age, in line with developing respiratory-laryngeal activity (Wasz-Hockert et al., 1968 and Thoden & Koivisto, 1980).

II. Intensity-related parameters

Current study revealed that MI decreased gradually from 6^{th} week - 14^{th} week, MinI reduced from 1st day- 14^{th} week and MaxI increased from 1st day- 14^{th} week of life. Intensity values reflect the neural control and capacity of respiratory system. Bellieni in 2004 established that the intensity of painful stimulation determines the characteristics of the pain cries, i.e. the intensity of the cry is high when the intensity of the pain stimulus is high and vice versa. Cries differ not only based on the causal event; they vary in intensity levels also based upon the discomfort levels (Wasz-Hockert et al., 1968). Hence, with the increasing age, maturation of neural control of respiratory and laryngeal system leads to variation of intensity values across the study period.

III. Durational parameters

The mean CD increased steadily from first day to 10^{th} week and decreased marginally during the 14^{th} week when compared to 10^{th} week. The mean CL was highest in the first day and decreased steadily till 10th week but again increased in the 14th week. Runefors and Arnbjornsson (2005) reported that the CD of the first cry segment was longer during the 3rd month vaccination when compared to the pain cry recorded between 5-7 days of life. CL decreased in the 3rd month vaccination than the values recorded between 5-7 days of life. Increasing values of CD can be related to the possible justification that as age increases, inspiratory phase duration might reduce and expiratory phase might increase, leading to longer durations of crying episode that may parallel the pattern of speech breathing.

Increase in the CD and decrease in the CL could be explained by the lengthened time taken by the central nervous system to interpret and respond to the painful stimulation and the improved fine-tuning and coordination of the sensory-motor skills with advancement in age resulting in decreased latency (Runefors and Arnbjornsson, 2005).

IV. Other parameters

The mean values of DHP and NC increased from first day to 2.5 months and slightly reduced at 3.5 months, whereas F0S showed a decreasing trend across the four recordings. Several studies have documented the presence of DHP, NC and F0S in adults as a characteristic of disordered voice. On the other hand, these phenomena are noted in healthy infants due to the underlying immature vocal folds and their neural control mechanisms. However, the trend in the developmental variations of these features are yet unknown (Robb, 2003).

CM represents the unsteadiness in the neural control of vocal tract during the crying. To date, there are no specific or longitudinal studies done considering CM in the infant cries. In the current outcomes they increased from 1st day- 10th week, followed by a decrease in the 14th week. This could probably reflect that the neural maturation has begun and might continue thereafter. Since the study period was short, i.e. birth-3.5 months, the significant changes in the CM could not be highlighted.

Current study shows that all the babies had IP consistently at the beginning of their second cry segment. They followed a decreasing trend across the initial three recordings, followed by an increase in the fourth recording. This is in parallel with the statements given by Grau et al in 1995 where they reported IP as one among the common acoustic features of neonatal pain cries. IP reflects the disruption in the activity of posterior cricoarytenoid muscle and diaphragmatic muscle, and is also due to the configuration of the vocal tract of young infants obstructing the upper airway (Wermke et al, 2018). This supports the notion of IP as a regular phenomenon occurring during the cries of healthy infants during early infancy. Therefore, IP can be related to the development of respiratory system along with vocal control. Consequently with descending larynx, there could be developmental decrease in the occurrence of IP. This is contrasting to the current results in which no such decrease was seen. Continued inspection of the IP beyond 4 months might be necessary to comment on the changes that could be measured.

VUV was increased in 3.5 months when compared to the first day with no significant pattern in the development. Similar results were obtained by Venugopal in 1995 wherein he found no significant differences in the VUV across initial three months of life. This feature is generally studied to track the development of the laryngeal system, as when the voluntary control develops, voicing content increases with increasing age.

Thus it can be concluded that the measured frequency, intensity, temporal and other parameters in the pain cries of newborns did not show any significant changes throughout the study period. This suggests that although there are anatomical variations taking place at the levels of phonatory, respiratory, articulatory and neural systems controlling them, the physiological changes may not be brought out completely in their acoustics of cries. This could be due to the reduced homogeneity of cries across the recordings, lack of homogeneity in participants and various other factors, such as, health status of the mothers and infants, environmental factors, factors causing methodological variations like amount of pain stimulation given to the infants, medical professionals involved in the procedure and so on.

CHAPTER VI

Summary and Conclusion

The current work was initiated with an intention to explore any trends in the development of acoustic measures of the pain cries through the first 4 months of life. All the 9 infants were regularly followed at the particular intervals like 1st day, 6th week, 10th week and 14th week of life. Using PRAAT software, infant cries were analysed for the following parameters,

I. Frequency parameters- Mean F0, Minimum F0, Maximum F0, Format frequency 1, Formant frequency 2, Formant frequency 3, Melody

II. Intensity parameters- Mean Intensity, Minimum Intensity, Maximum Intensity

III. Duration parameters- Cry latency, Cry duration

IV. Other parameters- Double Harmonic Peak, F0 Shift, Noise concentration, Cry mode, Inspiratory Phonation, Unvoicing ratio.

Results revealed that there were developmental increasing patterns of range of frequency, range of intensity, three formant frequencies, and cry duration across the four recordings. Falling type of melody was found in all the cries and decreasing pattern of frequency shift was obtained across the four recordings. Overall, the current outcomes revealed developmental patterns for a few frequency related and temporal parameters. Yet the parameters measured did not reach any significance throughout the 4 months. As the current study was performed with smaller sample size (9 newborns) and followed up for first 4 months only, this might have influenced the results. The anatomical growth and physiological maturation during the early infancy stage is vast and varied across infants and hence may not be explicitly reflected in the acoustic dimensions. Future works are warranted to include studies on larger samples, in infants followed up for longer age span, and including other types of cries in homogenous environments.

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Appendix 1

Revised position statement of Joint Committee on Infant Hearing (JCIH), 2007 of Risk Indicators associated with permanent congenital, delayed-onset, or progressive hearing loss in childhood:

1. Caregiver concern* regarding hearing, speech, language, or developmental delay (Roizen, 1999).

2. Family history* of permanent childhood hearing loss (Cone-Wesson et al., 2000; Morton & Nance, 2006).

3. Neonatal intensive care of >5 days, or any of the following regardless of length of stay: ECMO,* assisted ventilation, exposure to ototoxic medications (gentamycin and tobramycin) or loop diuretics (furosemide/lasix), and hyperbilirubinemia requiring exchange transfusion (Fligor et al., 2005; Roizen, 2003).

4. In-utero infections, such as CMV,* herpes, rubella, syphilis, and toxoplasmosis (Fligor et al., 2005; Fowler et al.,1992; Madden et al., 2005; Nance et al., 2006; Pass et al., 2006; Rivera et al., 2002).

5. Craniofacial anomalies, including those involving the pinna, ear canal, ear tags, ear pits, and temporal bone anomalies (Cone-Wesson et al., 2000).

6. Physical findings, such as white forelock, associated with a syndrome known to include a sensorineural or permanent conductive hearing loss (Cone-Wesson et al., 2000).

7. Syndromes associated with hearing loss or progressive or late-onset hearing loss,* such as neurofibromatosis, osteopetrosis, and Usher syndrome (Roizen, 2003). Other frequently identified syndromes include Waardenburg, Alport, Pendred, and Jervell and Lange-Nielson (Nance, 2003).

8. Neurodegenerative disorders,* such as Hunter syndrome, or sensory motor neuropathies, such as Friedreich ataxia and Charcot-Marie-Tooth syndrome (Roizen, 2003).

9. Culture-positive postnatal infections associated with sensorineural hearing loss,* including confirmed bacterial and viral (especially herpes viruses and varicella) meningitis (Arditi et al., 1998; Bess, 1982; Biernath et al., 2006; Roizen, 2003).

10. Head trauma, especially basal skull/temporal bone fracture* requiring hospitalization (Lew et al., 2004; Vartialnen et al., 1985; Zimmerman et al., 1993).

11. Chemotherapy* (Bertolini et al., 2004).

* Risk indicators that are marked with an asterisk are of greater concern for delayed-onset hearing loss.