TITLE: Effects of Birth Asphyxia on the Modulation of Pharyngeal Provocation Induced Adaptive Reflexes

SHORT TITLE: Perinatal Asphyxia and Aerodigestive Sphincters

Authors:

1. Ish K. Gulati, MD  
2. Theresa R. Shubert, BA  
3. Swetha Sitaram, MS  
4. Lai Wei, PhD  
5. Sudarshan R. Jadcherla, MD *

1. Sections of Neonatology, Pediatric Gastroenterology and Nutrition, Department of Pediatrics, The Ohio State University College of Medicine, The Research Institute at Nationwide Children’s Hospital, Columbus, OH.

2. The Neonatal and Infant Feeding Disorders Program, The Research Institute at Nationwide Children’s Hospital, Columbus, OH.

3. Center for Biostatistics, The Ohio State University College of Medicine, Columbus, OH.

ADDRESS FOR CORRESPONDENCE*:

Sudarshan R. Jadcherla, MD, FRCPI, DCH, AGAF  
Professor of Pediatrics, The Ohio State University College of Medicine  
Associate Division Chief, Neonatology  
Divisions of Neonatology, Pediatric Gastroenterology and Nutrition,  
The Research Institute at Nationwide Children’s Hospital  
700 Children’s Drive, Columbus, OH 43205, USA.
Phone: 614-355-6643
Facsimile: 614-355-5899
Email: sudarshan.jadcherla@nationwidechildrens.org

KEYWORDS: neonate; pharyngo-esophageal motility; aerodigestive sphincters; hypoxic ischemic encephalopathy

STATEMENT OF FINANCIAL SUPPORT: This study has been supported in part by National Institute of Health grant 2RO1 DK 068158 (Jadcherla).

FINANCIAL DISCLOSURE: No authors have financial relationships relevant to this article to disclose.

CONFLICT OF INTEREST: No authors have any conflicts of interest to disclose.
CONTRIBUTOR’S STATEMENT

Ish K. Gulati: Design of the study, performing studies, data analysis and interpretation, drafting the initial manuscript, reviewing and revising the manuscript, and approval of the final manuscript as submitted.

Theresa R. Shubert: Study and data compliance, data analysis and interpretation, data verification, drafting of the initial manuscript, reviewing and revising the manuscript, and approval of the final manuscript as submitted.

Swetha Sitaram: Data and statistical analysis, data verification, reviewing and revising the manuscript, and approval of the final manuscript as submitted.

Lai Wei: Statistical design, verification of statistical codes, performance and independent verification of data, reviewing and revising the manuscript, and approval of the final manuscript as submitted.

Sudarshan R. Jadcherla: Development of conceptual design, developing proof of principle, developed study design, performing studies, data analysis, statistical design and interpretation, drafting the initial manuscript, reviewing and revising the manuscript, approval of the final manuscript as submitted.
**ABSTRACT**

**Background and Objectives:** Perinatal asphyxia and aerodigestive symptoms are troublesome. We tested the hypothesis that pharyngeal provocation alters proximal and distal aerodigestive reflex coordination and kinetics in infants with hypoxic ischemic encephalopathy (HIE), compared with healthy controls. Specifically, we characterized the sensory-motor properties of pharyngeal provocation induced effects on upper esophageal sphincter (UES) and lower esophageal sphincter (LES) reflexes.

**Methods:** Ten orally-fed controls (32.0 ± 1.5 weeks gestation) and 25 infants with HIE (38.1 ± 0.4 weeks gestation), were evaluated at 39.7 ± 0.9 weeks and 41.9 ± 0.6 weeks postmenstrual age respectively. Pharyngo-esophageal reflexes evoked upon graded water stimuli were tested using water-perfusion micromanometry methods. Analysis included sensory-motor characteristics of pharyngeal reflexive swallow (PRS), pharyngo-UES- contractile reflex (PUCR), esophageal body-waveform kinetics, and pharyngo-LES-relaxation reflex (PLESRR).

**Results:** For controls vs. infants with HIE, median APGAR scores were 6 vs. 1 at 1 min (P< 0.001), and 8 vs. 3 at 5 min (P< 0.001). Upon pharyngeal- stimulation, HIE infants (vs. controls) had frequent PUCR (P= 0.01); increased UES basal tone (P= 0.03); decreased LES basal tone (P=0.002); increased pharyngeal-waveforms per stimulus (P=0.03); decreased frequency of LES relaxation (P=0.003); and decreased proximal esophageal contractile amplitude (P=0.002), with prolonged proximal esophageal contractile duration (P=0.008).

**Conclusions:** Increased tonicity and reactivity of the UES and dysregulation of LES may provide the pathophysiological basis for pooling of secretions, improper bolus clearance, and aspiration-risk. Deficits in function at the nuclear or supranuclear level involving
glossopharyngeal and vagal neural networks and respiratory regulatory pathways involved with aerodigestive protection may be contributory.

**ABBREVIATIONS:**

HIE-hypoxic ischemic encephalopathy

LES-lower esophageal sphincter

PLESRR- pharyngo-lower esophageal sphincter relaxation reflex

PMW- polymorphic waveform

PRS- pharyngeal reflexive swallow

PUCR- pharyngo-upper esophageal sphincter contractile reflex

UES- upper esophageal sphincter
Introduction

Birth asphyxia occurs in 1-4 per 1000 live births in the United States and other developed countries, and in 1-9 per 1,000 live births in under-resourced countries, often manifesting as hypoxic ischemic encephalopathy (HIE) (2, 28, 31). HIE presents with a multitude of airway and digestive symptoms; as such, aerodigestive management of these patients can be challenging. Specifically, swallowing reflexes are needed to handle oral secretions, gastroesophageal reflux (GER), as well as during post-emetic throat clearance or post-tussive states; such troublesome symptoms pose a constant challenge to parents and health care providers. As survival with morbidity is increasing among these prototypes of HIE, the consequences of dysphagia, gastroesophageal reflux disease and aspiration syndromes are also increasing (6, 21, 27).

Gastrostomy tube feeding, a common outcome in infants with severe HIE, is associated with an increased risk of aspiration (23). While some neonatal centers in developed and developing countries may discharge infants with feeding tubes, many countries, including the United States continue to require competent feeding by breast or bottle as a prerequisite for hospital discharge (1). As such, delays in the ability to achieve the oral feeding milestone can significantly prolong the length of stay for affected infants.

Pharyngeal provocation can happen during oral bolus transit, expectoration, or proximal ascent of refluxate or emesis. In the absence of timely activation of aerodigestive reflexes, infants are at risk for airway compromise including recurrence of asphyxiating events, aspiration pneumonia, and persistent feeding difficulties. In addition, potential recurring asphyxiating events can occur that require interventions such as suctioning of aerodigestive tract, supplemental oxygen, airway resuscitation, or sepsis work-up (12). The neurosensory and neuromotor mechanisms behind infant adaptation to provocative events are not well understood in this developmentally vulnerable cohort. Recently, we demonstrated that healthy neonates
respond to pharyngeal stimulus more frequently with pharyngeal reflexive swallows (PRS) so as
to clear the bolus, as opposed to pharyngo-upper esophageal sphincter contractile reflexes
(PUCR) (12). Additionally, healthy neonates have intact function of the lower esophageal
sphincter (LES), including relaxation during spontaneous swallowing as well as during
pharyngeal provocation-induced adaptive swallowing, thereby facilitating bolus transit (24-26).
However, the relationship between pharyngeal stimulation and adaptive aerodigestive function in
infants with HIE is unclear. Infants with HIE have feeding difficulties manifesting as
oropharyngeal inertia, pooling of secretions, delayed clearance of feeds and inadequate airway
protection. Whether this is due to sensory or motor malfunctions (or both) is unclear. Therefore,
our aims were to characterize pharyngeal stimulation induced adaptive responses in infants with
HIE, and compare these responses with those of healthy controls. The rationale for our
hypothesis was infant hypoxic ischemic injury may affect both afferent and efferent nerve
impulse transmission, leading to both skeletal and smooth muscle dysfunction, which may result
in pooling of oro-pharyngeal secretions. Specifically, we tested the hypothesis that infants with
HIE have altered response sensitivity and magnitude of aerodigestive reflexes and coordination
manifesting as altered initial response, variation in response duration, terminal swallow
characteristics and respiratory adaptation. Specifically, we examined PRS, PUCR and pharyngo-
LES relaxation reflex (PLESRR).

Materials and Methods

A total of 35 pharyngo-esophageal manometry studies were performed. No adverse
cardio-respiratory events were noted during any of these studies. Infants were included in the
HIE group if they were > 35 weeks gestational age (GA), experienced acute perinatal asphyxia
defined by an acute perinatal event (placental abruption, cord prolapse, abnormal fetal heart
rate), and presented with signs of encephalopathy as per Sarnat staging (32). The control group
included infants > 31.0 weeks GA with safe oral feeding skills at study, who did not have any congenital, genetic, or chromosomal abnormalities. Practical and ethical obstacles impede recruitment of healthy age-matched full-term controls. Since the control infants were studied at term equivalent postmenstrual age (PMA), they are maturationally appropriate for comparison, as they had acquired oral feeding skills comparable to term infants by the time of study (16). Protocol approval was obtained from the Institutional Research Review Board at the Nationwide Children’s Hospital Research Institute prior to initiation of this project. The study protocol conforms to the guidelines of the IRB policy and the health insurance portability and accountability acts (HIPAA). Informed consents and HIPAA authorization were obtained from parents prior to study. Enrollment and studies were performed consecutively, based on the inclusion and exclusion criteria, as well as scientific appropriateness, and parental consenting. Parents were not compensated for allowing infants to participate in the study. As infants with HIE receive an MRI as standard of care, this information was obtained before hospital discharge. Feeding method at one year of age was obtained from electronic medical record.

**Manometry Methods**

Multimodal pharyngeal provocations, using graded volumes of sterile water, were given and manometric recordings were analyzed as previously described (10-15). Briefly, the catheter assembly (Dentsleeve International, Mui Scientific, Ontario, Canada) was connected to the pneumohydraulic micromanometric water perfusion system via resistors, pressure transducers (TNF-R disposable pressure transducers) and amplifiers (Solar modules, Solar 2, MMS medical instruments, Dover, NH). A 6-French manometry catheter assembly with dual sleeves (positioned at the UES and LES), 4 side-ports recording from the pharynx, the proximal-, middle-, and distal esophageal loci, in addition to a terminal gastric recording port was used.
The micromanometric water perfusion rates were 0.02 mL/min/port for esophageal ports, 0.01 mL/min/port for the pharyngeal port, and 0.04 mL/min/port for the sleeves. The catheter was zeroed at the level of the subject’s esophagus, and then passed nasally while the infant lay supine and unsedated. The manometry catheter was properly positioned using the pull through technique to identify high-pressure zones, and was well secured.

**Manometric Experimental Protocol**

After about fifteen minutes of adaptation upon catheter placement, upstream and downstream responses to increasing graded volumes of pharyngeal infusions (sterile water stimuli, 0.1 mL, 0.3 mL, 0.5 mL) were evaluated. The rationale for using graded volumes of water was to determine the threshold sensitivity and response latency to abrupt liquid stimuli (34). The rationale for giving infusions in this manner was to simulate an abrupt pharyngeal bolus provocation and to test volume dose-response relationships. Abrupt provocations happen naturally such as in the event of gastroesophageal reflux, during the post-tussive state or during bolus entry into the pharynx. The rationale for giving infusions in this manner was to simulate a natural pharyngeal bolus and test volume dose-response relationships. The specific responses of interest PRS, PUCR, esophageal body contraction amplitude, duration, and velocity, and PLESRR. Proximal and distal sphincter resting tone and presence of polymorphic waveforms (PMWs) were also evaluated. Respiratory waveform characteristics were monitored using respiratory inductance plethysmography methods. Subject safety was also monitored by direct nursing observation.

**Manometry Data Analysis**

Analytical methods for defining the manometry characteristics have been described before (5, 9-15, 26), and are briefly defined as follows: a) *Pharyngeal Reflexive Swallow (PRS)*
was defined as a deglutition response to pharyngeal stimulation; onset is indicated by the
presence of a pharyngeal waveform in association with UES relaxation, followed by propagation
into the proximal, middle, and distal esophageal segments and further accompanied by LES
relaxation; b) Pharyngo-Upper Esophageal Sphincter Contractile Reflex (PUCR) was defined as
an increase of ≥ 4 mmHg above UES resting tone in response to a pharyngeal stimulus; c) Pharyngo-Lower Esophageal Sphincter Relaxation Reflex (PLESRR) was defined as LES
relaxation of at least 5 mmHg below LES resting pressure after stimulus onset; d) Response
latency to PRS was defined as the duration from the onset of the stimulus to the onset of the
pharyngeal contraction; e) Response latency to PUCR was defined as the duration from the onset
of stimulus to an increase in the UES of at least 4 mmHg; f) LES Nadir Duration was defined as
the onset time from which the LES relaxes to 5.0 mmHg, across the lowest LES pressure, to the
offset time until it recovers to 5.0 mmHg; g) Duration of Respiratory Change was defined as
modification of normal respiration in the thoracic plethysmography channel from the onset of
the infusion until normal respiration patterns were restored; h) Polymorphic Waveforms were
defined as multiple esophageal body waveforms noted in the proximal, middle, or distal
esophageal body. UES and LES resting pressures were evaluated prior to infusion onset, relative
to atmospheric or gastric pressure respectively, at end expiration. All manometric patterns and
reflexes were visually identified at the time of study, and co-investigators were trained to
recognize them during subsequent waveform analysis. Our analysis was conducted based on
previously validated and published methods and definitions, to which we strictly adhered. Inter
rater/intra rater variability was avoided by agreement of at least 2 investigators on recorded
analysis. Training and re-training of personnel has been completed during periodic training
sessions as conducted by the principal investigator (SRJ). Because of strict adherence to a priori
definitions and agreement by at least 2, if not 3, co-investigators, our agreement rates are close to perfection.

**Statistical Analysis**

Comparisons of demographic characteristics between HIE and control groups were performed using unpaired-t-tests for continuous variables or chi-square tests for categorical variables. Wilcoxon rank-sum tests were used if the continuous variable was not normally distributed. Due to the presence of repeated measures within each subject, manometric data were analyzed using linear mixed models. Generalized Estimating Equation (GEE) models, which are an extension of Generalized Linear Models, were chosen for categorical outcomes. Linear mixed and GEE models were performed with and without controlling for the effects of GA and CA, so as to mitigate the discrepancies in GA and chronological age (CA) between HIE and control groups. Gestational and chronological age-matched healthy controls would have been ideal, but ethical reasons preclude us from such clinical study designs. Since both groups were similar with respect to PMA, we did not control for its effects. Pearson’s correlation was used to test the correlation between GA, CA and PMA. GA was found to be significantly correlated with PMA \( (r = 0.6, p=0.0003) \) and CA \( (r = -0.7 <.0001) \). Data are presented as mean ± SEM, estimated mean ± SEM, percentage, median (IQR), or as otherwise indicated. P-values <0.05 were considered significant. Analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC).

**Results**

Responses to a total of 240 sterile water infusions administered into the pharynx were analyzed (N=69 infusions in controls; N=171 infusions in HIE infants) from 35 patients (N=10 control; N=25 HIE). Clinical characteristics for 10 control patients (4 males; 32.0 ± 1.5 weeks GA) and 25 HIE patients (11 males; 38.0 ± 0.4 weeks GA; P=0.003 for GA comparison; 7.8 ±
1.5 vs. 3.8 ± 0.4 weeks CA; P=0.03) are outlined (Table 1). In control infants, 2 of 10 infants (20%) were discharged with gastrostomy tubes. These infants had oral skills at the time of study and discharge. In infants with HIE, 13 of 24 (54%) had gastrostomy tubes placed at discharge (P= 0.1 vs. control infants). Infants were discharged with G-tube placement for supplemental nutritional intake to avoid failure to thrive. This was a clinical decision made by the health care providers. One infant with HIE did not have discharge feeding method documented.

In infants with HIE, per MRI during hospitalization, white matter changes were evident in n= 10 (40%), grey matter changes were evident in n= 6 (24%), and cerebellar injury was evident in n= 17 (68%). At one year of age, one control infant was lost to follow-up; the remaining 9 control infants had documented survival; 8 out of these 9 (89%) control infants had additional growth and neurodevelopmental follow up outcomes recorded. Of these infants, 6 of 8 (75%) were exclusively oral feeding at one year of age. For infants with HIE, 100% had documented survival at one year age, and 12 of 21 (57%) infants were exclusively oral feeding at one year of age (P= 0.009 vs. control infants). Of these infants, 23 had additional neurodevelopmental outcomes recorded. No control infants required respiratory support at one year, however 5 infants with HIE required respiratory support (n= 4 tracheostomy; n=1 nasal cannula). No control infants were given a diagnosis of cerebral palsy, hearing loss, or vision problems at one year of age; however cerebral palsy was diagnosed in 52% (12 out of 23), hearing loss in 26% (6 out of 23), visual problems in 17.4% (4 out of 23) infants with HIE.

An example of pharyngeal infusion induced esophageal manometry changes in both control infants and infants with HIE are shown in Figure 1 a & b. We analyzed UES and LES resting tone, cumulative aerodigestive response duration, number of pharyngeal peaks to the terminal swallow that resulted in restoration of aerodigestive quiescence, presence of PUCR, and
response latency to PUCR or PRS, duration of LES nadir and respiratory perturbations, and 
esophageal body characteristics of the terminal swallow.

**Neurosensorial and Neuromotor Characteristics of Proximal Aerodigestive Interactions**

Resting UES pressure in control infants was 11.0 ± 2.2 mmHg vs. 16.8 ± 1.5 mmHg in 
infants with HIE (P= 0.03). In response to pharyngeal stimulation, the proportions of PUCR:
PRS were compared between the groups (Figure 2a). The frequency of recruitment of 
pharyngeal peaks with each stimulus were also compared (Figure 2b). Sensory-motor 
characteristics of esophageal and respiratory adaptive responses to pharyngeal stimulation were 
compared, with reference to PUCR and PRS response latency, duration of pharyngo-esophageal 
motor activity and respiratory change (Figure 2c).

Stimulus-volume response relationships were compared for number of pharyngeal peaks 
with each infusion per volume for control vs. HIE infants until restoration of aerodigestive 
quiescence (Figure 3a). The relationship between duration of respiratory change and latency until 
the terminal swallow for control vs. HIE infants is shown (Figure 3b). The Spearman correlation 
coefficient for the data set is indicated in the figure; with the indicated outliers removed, the 
correlation remains positive (R = 0.95 and R= 0.86 for control infants and HIE infants 
respectively).

**Characteristics of Esophageal Body Propulsion**

Esophageal body contraction amplitude and duration with regard to terminal swallowing 
is shown in Table 2. With each pharyngeal stimulus, the number of polymorphic esophageal 
body waveforms in controls vs. HIE was 1.0 ± 0.1 vs. 1.2 ± 0.04 (P= 0.01). The duration (sec) of 
polymorphic waveform activity in controls vs. HIE was 3.2 ± 0.8 vs. 5.5 ± 0.4 (P= 0.02).

Peristaltic velocity (cm/s) in controls vs. HIE from the proximal to distal esophagus was 4.2 ±
1.0 vs. 2.1 ± 0.7 (P= 0.09). The rate of esophageal body contraction (mmHg/sec) was for the proximal esophagus 52.4 ± 7.9 vs. 24.8 ± 5.1 (P= 0.005); for the mid esophagus 48.2 ± 4.5 vs. 43.0 ± 2.8 (P= 0.3); and for the distal esophagus 29.6 ± 2.2 vs. 31.9 ± 3.1 (P= 0.6) for controls vs. HIE infants respectively.

**Effects of Pharyngeal Provocation on the Lower Esophageal Sphincter**

Resting LES tone was 26.0 ± 2.5 mmHg in control infants vs. 16.4 ± 1.6 mmHg in infants with HIE (P= 0.002). The frequency of PLESRR in control vs. HIE infants was 79% vs. 57% (P= 0.003). The effect of pharyngeal stimulus on LES nadir pressure and duration are shown (Figure 4a). Stimulus-volume response relationships were compared for LES nadir duration (Figure 4b).

**Discussion**

Using an experimental design and provocative interrogation of pharyngo-esophageal motility reflexes, we investigated the mechanism of pharyngeal adaptation in infants with HIE compared to neurologically intact controls. As infants with HIE have frequent aerodigestive problems and difficulty achieving feeding milestones, our working hypothesis was that HIE modifies central swallowing pattern generation and adaptive pharyngo-esophageal reflexes. In the current study, we demonstrated the neurosensory and neuromotor characteristics of significant pharyngeal maladaptation in infants with HIE. Importantly, central swallowing generation, swallowing frequency and duration, in addition to UES and LES tone and reactivity during pharyngeal provocation were all noted to be aberrant. Specifically the cardinal findings of our study indicate that compared with control infants, infants with HIE have: 1) increased resting UES tone; 2) increased frequency of PUCR in response to pharyngeal stimulation; 3) increased recruitment of pharyngeal peaks per stimulus; 4) Increased duration to restoration of aerodigestive quiescence (until terminal swallow); 5) increased presence and duration of
polymorphic waveforms; 6) decreased contraction amplitude and increased duration from the peak to offset of contraction in the proximal esophagus; 7) increased contraction duration in the mid-esophagus; 8) decreased resting LES tone; 9) decreased frequency of PLESRR; and 10) increased LES nadir duration.

Infants with HIE clearly had more difficulty with feeding, clearance of oral secretions, maintenance of airway and aerodigestive coordination as evidenced by the significant number needing gastrostomy, tracheostomy, and supportive oxygen therapy. Prolonged and infrequent adaptive responses to pharyngeal stimuli are suggestive of delays in conductivity of afferent and/or efferent neural communications between the point of stimulation and point of motor responsiveness. Aerodigestive symptoms in HIE, such as pooling of oral secretions, may be related to altered neurosensory mechanisms that fail to evoke swift clearance of secretions away from the oral cavity. It may be noted that although the HIE group did maintain sensitivity of upper aerodigestive tract reflex recruitment, the motor responses were uncoordinated and prolonged; however sensitivity of reflex recruitment in the lower aerodigestive tract was decreased.

Pharyngo-upper esophageal sphincter interactions upon pharyngeal provocation in healthy preterm infants have been characterized by us as well as others (12, 15, 18, 29) . Specifically, in healthy neonates, PRS occurs more frequently than PUCR in response to pharyngeal provocation (12). In addition, PLESRR is activated during pharyngeal reflexive swallowing (22) and becomes robust with maturation (15, 26). On the other hand, in infants with HIE, esophageal mechano-distension results in up-regulation of central vagal (cholinergic) effects manifesting as increased excitation demonstrated by exaggerated magnitude of UES contractile reflex, or increased inhibition as evidenced by exaggerated LESRR pressure and
Similarly, our current study data demonstrate modified and possibly increased cholinergic neural activity as the basis for increased UES resting pressure and more frequent PUCR responses. Decreased contraction amplitude coupled with decreased rate of muscle contraction and prolonged duration of waveforms or polymorphic waveforms in the skeletal muscle part of the proximal esophagus may be due to phenomenon of summation of contractions akin to tetanic contractions (3). Alternatively, animal experimental data reveal that antecedent hypoxic exposure can result in a decrease in end-plate potential to below firing threshold, which could contribute to modified muscle function in infants with HIE, as the release of acetylcholine at the neuromuscular junction would be blocked (7).

Infants with HIE also experienced an increased number of adaptive pharyngeal waveforms, prolonged contraction and relaxation in the mid esophagus, and prolonged duration to reach terminal swallow restoring respiratory quiescence vs. control infants. Heightened responses to graded infusion volumes in HIE compared to controls suggests preserved volume sensitivity in HIE infants. HIE infants experience prolonged respiratory changes which are proportional to the time taken for bolus clearance. Altered sensory-motor characteristics of pharyngo-UES interactions and prolonged respiratory changes in the HIE group, when compared with control infants, suggest dysfunctional interactions between neural pathways and circuitry related to swallowing and breathing. These observations are supported by animal experiments demonstrating that exposure to acute intermittent hypoxia leads to synaptic inhibition when norepinephrine secretion is increased within the prebotzinger complex, which is an area critical for breathing (37).

Supranuclear or nuclear level lesions in HIE infants may modify functioning of the vagus nerve and respiratory neural pathways involved with swallowing, respiration, and aerodigestive
protection. HIE infants are noted to have decreased basal LES tone, decreased frequency recruitment of PLESRR and volume dependent prolonged nadir duration. Decreased LES resting tone is likely be due to an imbalance between excitatory and inhibitory vagal tone. Prolonged PLESR nadir duration in infants with HIE may be due to sustained inhibitory input to the LES, implicating dysregulation in restoration of LES tone, while decreased frequency of PLESRR may be due to impaired sensitivity. Impaired sensory-motor responses may indicate neurosensory and neuromotor malfunctions of cranial nerves IX and X, which regulate and modulate pharyngeal swallowing reflexes and LES kinetics. This can be explained by sustained release of inducible nitric oxide during the reperfusion phase of HIE (4, 8, 36). Additionally, the effect of hypoxia on respiratory neural output may be linked with modulation of LES tone (17). Recent research has demonstrated feeding and communication impairment are noted among HIE survivors with grey matter lesions (20, 21). Additionally, severity of white matter injury is correlated with poor neurodevelopmental outcomes at age two (19). In the current study, the instance of white matter changes were more frequent in the studied population (40%) than grey matter changes (24%), and cerebellar abnormalities were noted most frequently (68%) in infants with HIE. The cerebellum is an important center for regulation and coordination of motor tone (30). Abnormal motor function, such as in the current study, may be due to such lesions. It is also interesting to note that about half of HIE infants were on exclusive oral feeds at discharge and remained to be on exclusive oral feeds at one year of age. This indicates that despite neurological injury, neuroplasticity may allow for the fast developing brain of an infant to adapt to injury to allow for organism survival. Additionally, radiological markers of neurological injury do not necessarily correlate with functional feeding milestones.
As is the case with any clinical study, the current study has limitations. The oral pharyngeal phase of swallowing has not been included as part of this investigation. Further work in this area is necessary. Additionally, information regarding maturation of oral feeding skills in infants with HIE is lacking. Further studies are necessary to address intervention for such infants during interval follow-up evaluation.

The implications of this study are several. It is commonly speculated that HIE infants have varying degrees of upper and lower aerodigestive maladaptation which may lead to aspiration syndromes (35). The current study supports such concerns, as compared with control infants, infants with HIE had aberrant UES function, prolonged adaptive responses to pharyngeal stimulation, and decreased LES basal tone. Implementation of various medical and surgical therapies intended to address these issues is delayed due to lack of information on pathophysiological changes in a growing and developing infant with HIE. Our findings may lend support to personalization of therapies based on specific mechanistic diagnosis. In conclusion, infants with HIE exhibited increased tonicity and reactivity of the UES in addition to dysregulation at LES. These findings may explain the pathophysiological basis for pooling of secretions, improper bolus clearance, and aspiration-risk. These conclusions implicate the presence of lesions at nuclear or supranuclear level involving glossopharyngeal and vagal neural networks and respiratory regulatory pathways which are involved with swallowing and airway protection as the basis for dysfunction.
References


Figure Legends:

**Figure 1:** Effect of pharyngeal stimulation on aerodigestive sensory motor characteristics; a) control infant response to 0.3 mL pharyngeal water infusion; b) HIE infant response to 0.3 mL pharyngeal water infusion. Manometry data were analyzed as labeled. Note the prolonged response in HIE infants.

**Figure 2:** Initial Response Characteristics and Subsequent Response Duration; ■ = Control; □ = HIE; *P= 0.01; †P = 0.02; ‡P < 0.05 a) Initial Response Distribution; b) PRS Recruitment to Terminal Swallow; c) Initial Response Latency (s) and Duration of Response (s) defined as; Response latency to PUCR - the duration from the onset of stimulus to an increase in the UES of at least 4 mmHg; Response latency to PRS - the duration from the onset of the stimulus to the onset of the pharyngeal contraction; Duration to Terminal Swallow - the time from the onset of pharyngeal stimulus to the onset of the pharyngeal waveform of the terminal swallow; Duration of Respiratory Change – time from the initial modification of normal respiration in the thoracic plethysmography channel from the onset of the infusion until normal respiration patterns were restored; Note the prolonged duration until the terminal swallow in infants with HIE. Also note the prolonged duration of respiratory change in infants with HIE, although not statistically significant (P= 0.1).

**Figure 3:** Total Response Duration and Characteristics; a) ■ = Control; □ = HIE; △ = HIE; a) *P< 0.05 for volumetric effect compared with 0.5 mL; †P<0.05 for volumetric effect compared with 0.3 mL and 0.5 mL infusions; Volumetric PRS Recruitment to Aerodigestive Quiescence. Note HIE patients have a significant volumetric response for 0.1 mL infusions compared with 0.3 mL and 0.5 mL infusions. Control infants have a significant volumetric response between 0.1 mL infusions compared with 0.5 mL infusions. b) *P<0.0001; Respiratory change duration is highly correlated with terminal swallow latency in HIE infants.

**Figure 4:** Lower Esophageal Sphincter (LES) Characteristics; ■ = Control; □ = HIE; *P< 0.05; †P< 0.05 for volumetric effect compared with 0.5 mL; ‖< 0.05 for volumetric effect compared with 0.3 mL and 0.5 mL infusions; a) LES Nadir Pressure (left) and LES Nadir Duration (right); P= 0.3 for comparison of LES nadir pressure; b) Volumetric LESRR Recruitment Relationships.
**Table 1. Demographic Characteristics**

<table>
<thead>
<tr>
<th></th>
<th><strong>Control (N=10)</strong></th>
<th><strong>HIE (N=25)</strong></th>
<th><strong>P-value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min APGAR</td>
<td>6.0 (4.0-8.0)</td>
<td>1.0 (0.0-2.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>5 min APGAR</td>
<td>8.0 (8.0-9.0)</td>
<td>3.0 (0.0-5.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PMA* at study, wks</td>
<td>39.7 ± 0.9</td>
<td>41.9 ± 0.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Weight at study, kg</td>
<td>3.2 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>FOC† at study, cm</td>
<td>35 (32-37)</td>
<td>35 (33.5-36)</td>
<td>0.8</td>
</tr>
<tr>
<td>Exclusive Oral Feeds at study, No. (%)</td>
<td>7 (70)</td>
<td>2 (8)</td>
<td>0.002</td>
</tr>
<tr>
<td>Exclusive Oral Feeds at discharge ‡, No. (%)</td>
<td>8 (80)</td>
<td>11 (44)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Postmenstrual Age; † Fronto-Occipital Circumference; ‡ n= 24 total infants with HIE had discharge feeding outcomes available. Data presented mean ± SEM, median (range) and as %.
### Table 2. Characteristics of Esophageal Body Propagation Kinetics

<table>
<thead>
<tr>
<th></th>
<th>Control (N=10)</th>
<th>HIE (N=25)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximal Esophagus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contraction Amplitude, mmHg</td>
<td>50.1 ± 3.8</td>
<td>35.3 ± 2.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Contraction Onset to Peak Amplitude, sec*,†</td>
<td>1.5 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Peak Amplitude to Contraction Offset, sec*,†</td>
<td>1.5 ± 0.2</td>
<td>2.2 ± 0.1</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Mid Esophagus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contraction Amplitude, mmHg</td>
<td>67.6 ± 7.0</td>
<td>74.1 ± 4.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Contraction Onset to Peak Amplitude, sec*</td>
<td>1.5 ± 0.2</td>
<td>2.0 ± 0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Peak Amplitude to Contraction Offset, sec</td>
<td>1.9 ± 0.2</td>
<td>2.4 ± 0.1</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Distal Esophagus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contraction Amplitude, mmHg</td>
<td>55.6 ± 5.4</td>
<td>56.3 ± 3.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Contraction Onset to Peak Amplitude, sec*</td>
<td>2.1 ± 0.2</td>
<td>2.2 ± 0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Peak Amplitude to Contraction Offset, sec*</td>
<td>1.9 ± 0.3</td>
<td>2.4 ± 0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Data presented as estimated mean ± SEM; P< 0.05 is considered significant. *P< 0.05 when controlling for gestational age; †P< 0.05 when controlling for chronological age.