# The efficacy of oral motor interventions on feeding outcomes in newborns with hypoxic-ischemic encephalopathy who received therapeutic hypothermia

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#### **ABSTRACT**

**Background.** Feeding difficulties continue to be a serious problem in newborns with hypoxic-ischemic encephalopathy (HIE) undergoing therapeutic hypothermia (TH). The aim of this study was to investigate the efficacy of oral motor interventions (OMI) on feeding outcomes in neonates with HIE/TH.

**Methods.** This was a prospective randomised control study conducted between January 2022 and September 2022. Premature Infant Oral Motor Intervention (PIOMI) was used as OMI. Newborns with HIE/TH, who underwent PIOMI, constituted the study group, and newborns, who did not receive any feeding exercise, constituted the control group. Transition time to full oral feeding (FOF) was determined as the time between initiation of tube feeding and full oral breastfeeding or bottle feeding. The day per oral (PO) feeding was started was specified as PO first, the day the infants could take half of the volume of the feedings by mouth was PO half, and the day the infants could take all the feedings by mouth was PO full.

**Results.** There were 50 neonates in each group. Time to FOF was significantly shorter in the study group than in the control group in all stages of HIE/TH (P=0.008 for stage 1, and <0.001 for stage 2 and 3 HIE). However, times to PO first, PO half, PO full and discharge were shorter in the study group than in the control group only in the neonates with stage 3 HIE (P=0.003, 0.014, 0.013, 0.042, respectively).

**Conclusions.** The PIOMI, which could be named as "HIE-OMI" in our study, is an effective intervention in shortening the transition time to FOF in neonates with all stages of HIE undergoing TH. In addition, "HIE-OMI" shortens the length of hospital stay, and improves feeding outcomes in neonates with severe HIE/TH.

**Key words:** hypoxic ischemic encephalopathy, feeding outcomes, oral motor interventions, therapeutic hypothermia.

Hypoxic-ischemic encephalopathy (HIE) is an important cause of morbidity and mortality in newborns, and therapeutic hypothermia (TH) is the only proven treatment option that is known to decrease the rates of mortality and neurologic sequelae in neonates who are diagnosed as having moderate and severe HIE.<sup>1,2</sup>

90% of the neonates with moderate to severe basal ganglia and thalamic hypoxic-ischemic lesions had oral feeding difficulties, and almost all of those neonates subsequently needed a gastrostomy tube or long-term home gavage feedings.<sup>3</sup> After the TH era, it was found that 31% of surviving infants with HIE had persistent feeding difficulty despite TH, and it was demonstrated that persistent feeding difficulty could be predicted by brainstem involvement on post-hypothermia magnetic resonance imaging (MRI).<sup>4</sup> Although there has been a decrease in

the frequency of feeding difficulties after TH,

Before the TH era, it was found that almost 50-

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feeding difficulties continue to be a serious problem affecting one third of newborns with HIE/TH.<sup>4</sup>

It has been shown that the application of "Premature Infant Oral Motor Intervention (PIOMI)" improved oro-motor skills, decreased the transition time from gavage to full independent feeds by mouth with better weight gain rates and reduced hospital stay in preterm neonates.<sup>5,6</sup>The aim of this study was to investigate the efficacy of the PIOMI exercises in infants with different stages of HIE/TH.

#### Materials and Methods

This was a prospective randomised controlled study. Newborns, who underwent TH between January 2022 and September 2022 with the diagnosis of HIE, were included in the study. Approval for the study was obtained from the Local Clinical Research Ethics Committee under the approval number 22/02/20. The parents of the newborns were informed about the purpose of the study, and verbal and written consents were obtained. The neonates who died before discharge and whose parents did not give consent were excluded from the study.

# Neonatal intensive care unit protocol for management of hypoxic-ischemic encephalopathy

The decision to apply TH was made based on the Turkish Neonatal Society Guidelines on neonatal encephalopathy.<sup>7,8</sup> Treatment criteria were as follows: (1) newborns with a gestational age of  $\geq$  36 w and aged below  $\leq$  6 h; a pH value of  $\leq 7.00$  or BE value of  $\leq -16$  mmol/L in the blood sample collected from the cord or in the blood sample collected from the baby in the first hour of life; (3) a tenth minute APGAR score of < 5 or persisting need for resuscitation; (4) signs of moderate or severe encephalopathy on clinical evaluation. Absolute contraindications for TH used in this study were as follows: (1) babies who were aged over twelve hours; (2) babies under the gestational age of 34 w; (3) babies weighing less than 1800 g; (4) babies

with major congenital anomalies; (5) babies with very severe or diffuse parenchymal cranial hemorrhages or very severe life-threatening coagulopathy. The treatment plans for babies, who either did not fully meet the TH criteria or did not have an absolute contraindication for TH, were made according to the decision of the consultant neonatologist in line with our national guidelines. Therapeutic hypothermia was applied with either the Tecotherm TS Med 200 N device (Inspiration Healthcare Ltd., Leicester, United Kingdom) or Arctic Sun 5000 Temperature Management System (Medivance, Inc., Louisville, Colorado, United States). Cooling to a rectal temperature of 33.5 ± 0.5°C was achieved in all infants within the postnatal 6 hours. Rewarming to 36.5°C was started following 72-hour cooling by elevating the temperature with a rate of 0.5°C per hour. The patients underwent detailed neurological examination, and the Sarnat and Sarnat staging system was used to determine the stage of HIE (stage 1/ mild, stage 2/ moderate or stage 3/ severe HIE). The severity of HIE was defined daily during the first 72 hours of life, and the worse stage was recorded. Gestational age (GA) was estimated based on the last menstrual date or the Ballard score was used to estimate GA, if last menustual date was not known.9

In our hospital, MRI can be performed and NICU babies were given priority for MRI. Cranial MRI was performed as soon as possible after rewarming in all newborns who were admitted to our NICU with the diagnosis of HIE. However, cranial MRI scans of the newborns who could not be weaned from the ventilator or whose clinical findings were not stable enough to be transferred to the radiology unit were delayed until the patient was stable. Normal MRI findings were defined as follows; increased signal intensity corresponding to myelination seen in the posterior half of the posterior limb of the internal capsule, absence of increased signal intensity in the thalamus, or subtly increased signal intensity that was restricted to the posterolateral quadrant of the thalamus on T1-weighted images.<sup>10</sup> Abnormal MRI findings were defined as the presence of a HIE-specific brain injury pattern as follows; basal ganglia and thalamus dominant injury pattern, white matter/watershed dominant injury pattern or near-complete injury/global hypoxia pattern. Presence of HIE-nonspecific MRI abnormalities such as intracranial hemorrhage, venous thrombosis, etc were not included in the abnormal MRI group, and were evaluated in the normal MRI group. The postnatal day when cranial MRIs of the neonates were performed was recorded.

# Feeding policy of the neonatal intensive care unit

Fluid balance was maintained with 10% dextrose infusion in the first 24 hours of life, and later continued by daily adjusted fluids according to the patient's biochemical values, weight and urine output. During TH, the babies were not fed. Enteral feedings were initiated as < 20 mL/ kg/d after rewarming the neonates to 36.5°C with an orogastric tube, and routine residual control was performed. Enteral feeding was terminated, if abdominal distention and large (> 5 mL/kg), bilious or blood stained gastric residuals were observed. The volume of enteral feeding was increased, if the infant tolerated the feeds. Enteral feeding was increased at a rate of 10-20 mL/kg/d as tolerated. If enteral feeding could not be started just after rewarming, parenteral nutrition was started.

We routinely fed term neonates eight times a day with three-hour intervals, and trained neonatal nurses of the unit provided care and feeding. For the oral feeding trials, feeding behavior of infants with respiratory stability/ weaned from the ventilator was considered on the basis of cue-based feeding. The speech and language therapist (SLT) evaluated the neonates readiness for oral feeding by observing the presence of the rooting reflex, the pattern, rhythm, speed and strength of non-nutritive sucking, oral motor coordination, and orofacial muscle tone. A multidisciplinary team of neonatologists, pediatricians, neonatal nurses and SLT decided on neonates readiness for

oral feeding. If the team agreed that newborns were ready for the transition from tube feeding to oral feeding, neonatal nurses attempted to feed the infants orally with an injector every three hours. Mothers of the neonates who were confirmed to be able to be fed with injectors at least three times successfully were called to the unit to breastfeed, and feeding directly from the mother's breast was attempted. In cases in which the mothers did not have milk or breast milk was contraindicated, mothers were called to the unit to feed their babies with a bottle.

Transition time to full oral feeding (FOF) was determined as the time between initiation of tube feeding and full oral breast feeding or bottle feeding. The day per oral (PO) feeding was started was specified as PO first, the day the infants could take half of the volume of the feedings by mouth was PO half, and the day the infants could take all the feedings by mouth was PO full. Length of hospital stay was specified as the time between admission to the unit and discharge from the unit. The clinical findings that might influence the feeding transition process, including information on the duration of ventilation, inotropic requirements, presence of seizures, number of anticonvulsive medications used, and echocardiographic findings were recorded. The duration of mechanical ventilation was defined as the sum of invasive and noninvasive mechanical ventilation. The APGAR scores were categorized as  $\leq 7$  and > 7, and APGAR scores were compared between groups.

The number of total participants to be included in the study was specified via <a href="http://www.randomizer.org">http://www.randomizer.org</a> programme. Randomization was performed by randomly and equally assigning the babies, who constituted the sample group, to two groups using the random numbers obtained from the programme. Fifty newborns, who received TH with a diagnosis of HIE were applied PIOMI exercises and constituted the study group, while 50 newborns, who received TH with a diagnosis of HIE and were not applied PIOMI exercises, constituted the control group. Postnatal ages (PNA) and weights of

the neonates on the days PO first, PO half, and PO full, when the FOF was provided, and at discharge were recorded.

The Premature Infant Oral Motor Intervention (PIOMI) was adapted from the Beckman Oral Motor Intervention exercises such that it included eight steps, and its application lasts for five minutes.12 PIOMI exercises were applied by SLTs ten minutes before tube feeding once daily, five times weekly in babies who were initiated enteral feeding by paying attention to sterilization. Before the application, the babies were placed in incubators in supine position in the awake state. The SLTs applied the exercises by opening the incubator windows without taking the babies out of incubators. Sucking was triggered by giving sensory stimuli to the cheeks, upper and lower lips, gingivae and the lateral and middle lines of the tongue, respectively, in accordance with the PIOMI exercise protocol with the objective of increasing oral-motor skills. The babies were monitored during the procedure, if venous saturation reduced by >20% and/or apical heart beat increased by >30% the exercises were terminated. PIOMI exercises were continued in the babies in the study group until FOF was initiated. Premature infant oral motor intervention tool with illustrations is presented in Fig. 1.12

## Sample size calculation

The required sample size was determined through a power analysis using the G\*Power (v3.1.9.4) program. The study power and alpha value was set at 93% and 0.05, respectively and effect size was 0.30. Based on these values, a minimum sample of 100 participants was required, meaning 50 participants in each trial group.

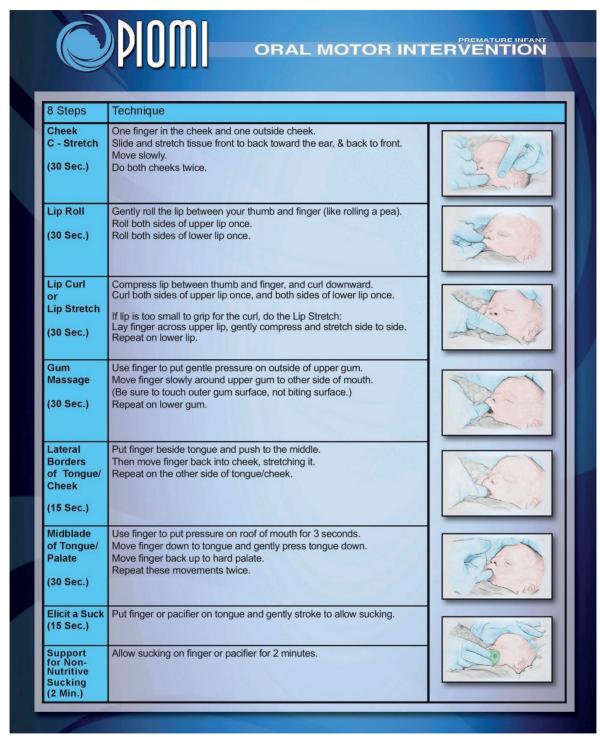
### Statistical analysis

The Statistical Package for Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The data were assessed for normality using visual and analytic methods. The data were tested for normality with the Shapiro-Wilk test and Kolmogorov

Smirnov test. The qualitative variables are expressed as percentages and frequencies, normally distributed continuous variables are expressed as means (standard deviation) and non-normally distributed variables are expressed as medians (interquartile range [IQR], p25-p75). The chi-square test was performed for categorical variables. Differences between two groups were tested using the Student's t-test or Mann-Whitney U test, as appropriate. In 2x2 comparisons between the categorical variables, chi-square test or Fisher's exact test was used, as appropriate. In >2x2 comparisons between the categorical variables, the Fisher-Freeman-Halton test was used, as the expected value was <5. A p value of <0.05 was considered statistically significant.

#### Results

During the study period, 113 newborns with the diagnosis of HIE were admitted to our NICU, of these newborns, 8 with stage 3 HIE died, and the parents of 5 did not allow for the inclusion of their baby in the study. A hundred neonates who underwent TH with the diagnosis of HIE were included in the study. PIOMI exercises were applied to 50 neonates, who constituted the study group, and no feeding exercises were applied to the other 50 patients (control group). The PIOMI was not terminated in any newborn due to changes in vital signs, and no adverse effects related to the PIOMI were observed. Gestational age, birth weight, 1st and 5 th minute APGAR scores, cord blood gas pH and base deficit, postnatal day of MRI and HIE stages were similar between the groups. The frequency of convulsions, the number of anticonvulsant drugs used, the duration of mechanical ventilation, and the frequency of cardiac anomalies did not differ between the study and control groups. No critical congenital heart disease was detected in any patient. Three patients in the study group received sildenafil treatment for mild to moderate pulmonary hypertension. The demographic, clinical and laboratory characteristics of the neonates are presented in Table I.



**Fig. 1.** Premature infant oral motor intervention tool with illustrations. Reprinted with permission. © Brenda Lessen. 12

**Table I.** Comparison of demographic, clinical and laboratory characteristics of the neonates by groups.

	Control group (n=50)	Study group (n=50)	P value
Sex, male <sup>a</sup>	37 (74)	24 (48)	0.008
GA, w <sup>b</sup>	$37.3 \pm 2.0$	$37.3 \pm 2.4$	0.978
BW, g <sup>b</sup>	3011± 737	$3011 \pm 555$	0.743
APGAR score, 1 min <sup>c</sup>	4 (1-8)	4 (1-6)	0.094
APGAR 1 min ≤ 7 a	4 (8)	0 (0)	0.041
APGAR score, 5 min <sup>c</sup>	6 (3-9)	6 (3-9)	0.050
APGAR 5 min ≤ 7 <sup>a</sup>	15 (30)	8 (16)	0.096
рН ь	$6.9 \pm 0.3$	$6.9 \pm 0.1$	0.442
Base excess, mmol/L <sup>b</sup>	$-18.9 \pm 20.9$	$-17.5 \pm 5.1$	0.396
MRI, postnatal day <sup>b</sup>	4.8±3.3	4.7±3.5	0.060
Ventilator, day <sup>b</sup>	8.6±8.7	7.6±7.0	0.538
Convulsion <sup>a</sup>	29 (58)	27 (54)	0.687
Anticonvulsive, number b	$0.9 \pm 0.8$	$0.8\pm0.8$	0.830
Normal ECHO <sup>a</sup>	44 (88)	44 (88)	1.000
HIE stage <sup>d</sup> 1	13 (26)	13 (26)	
2	22 (44)	18 (36)	0.647
3	15 (30)	19 (38)	

BW: birth weight, ECHO: echocardiography, GA: gestational age, HIE: hypoxic-ischemic encephalopathy, MRI: magnetic resonance imaging.

The mean postnatal day of starting PIOMI was 7.6±7 days. Times to PO first, PO half and FOF were found to be shorter in the study group compared to the control group in terms of postnatal day (P= 0.028, 0.011, 0.003, respectively), but the time to start gavage feeding, time to PO full and time to discharge did not differ between the groups (Table II). The weights of the neonates on the days PO first, PO half, PO full and FOF and at discharge did not differ between the groups (Table III).

When the groups were compared according to the HIE stages, times to PO first, PO half, and discharge were similar between the groups in neonates with stage 1 and stage 2 HIE/TH in terms of postnatal day. However, the time to FOF was significantly shorter in the study group compared to the control group in neonates with all stages of HIE/TH in terms of postnatal day (P= 0.008 for stage 1 HIE, <0.001 for stage 2 and 3 HIE). In addition, the times to PO first, PO half, PO full and discharge were shorter in the study group compared to the control group in neonates with stage 3 HIE/TH in terms of postnatal day (P= 0.003, 0.014, 0.013,

0.042, respectively (Table III). All newborns included in the study were discharged by full oral breast feeding or bottle feeding and no tube or gastrostomy feeding was required.

When the groups were compared according to the HIE stages, the frequency of abnormal brain MRI was similar between the groups in all stages of HIE/TH (Table III).

#### Discussion

In our study, we found that oral motor intervention exercises were effective in shortening the time for FOF in all stages of HIE in neonates with HIE undergoing TH. In addition, we showed that these exercises improved feeding outcomes and shortened the length of hospital stay in neonates with severe HIE/TH. In neonates with HIE, prolonged and poorly coordinated peristaltic responses may result in dysfunction of aerodigestive regulation, as it has been shown that esophageal motility is abnormal in neonates with HIE, and TH can ameliorate these dysmotility issues.<sup>13</sup>

<sup>&</sup>lt;sup>a</sup>Fisher's Exact test (number and percentage), <sup>b</sup>Student's t-test (mean and standard deviation), <sup>c</sup>Mann-Whitney U test (median and interquartile range), <sup>d</sup>Fisher-Freeman-Halton test (number and percentage).

Table II. Comparison of postnatal age and weight of neonates on the specified days by group.

	Control group (n=50)	Study group (n=50)	D l à
	Mean ± SD	Mean ± SD	P value <sup>a</sup>
Gavage feeding start, day	4.3±0.7	4.6±1.1	0.060
PO first, day	11.9± 8.2	$9.2 \pm 7.2$	0.028
PO half, day	12.2±8.2	$9.8 \pm 7.4$	0.011
PO full, day	$12.4 \pm 8.2$	$10.4 \pm 7.7$	0.213
Time to FOF, day	$10.3 \pm 6.8$	$5.3 \pm 5.2$	0.003
Discharge PNA, day	$13.6 \pm 8.6$	$12.5 \pm 8.5$	0.948
PO first weight, g	$2965 \pm 533$	$2965 \pm 504$	0.997
PO half weight, g	2961± 532	$2969 \pm 506$	0.941
PO full weight, g	$2961 \pm 528$	2993± 496	0.757
Discharge weight, g	$2980 \pm 524$	$3063 \pm 515$	0.429

FOF: full oral feding, PNA: posnatal age, PO: Per oral, SD: standard deviation

**Table III.** Comparison of postnatal age of newborns on specified days and MRI findings according to HIE stages by group.

HIE Stage		Control group (n=50)	Study group (n=50)	P value
1	PO first, day	8.5±2.6	9.1±8.1	0.253ª
	PO half, day	8.6±2.5	9.5±8.0	$0.379^{a}$
	PO full, day	8.9±2.2	10.0±8.0	$0.437^{a}$
	Time to FOF, day	7.2±1.8	6.2±7.1	$0.008^{a}$
	Discharge PNA, day	10.4±2.9	11.5±7.6	$0.814^{a}$
	Abnormal Brain MRI	6 (12)	4 (8)	$0.420^{b}$
2	PO first, day	8.9±3.6	8.3±6.2	0.184ª
	PO half, day	9.2±3.6	8.6±6.3	$0.139^{a}$
	PO full, day	9.4±3.7	9.4±6.5	0.293ª
	Time to FOF, day	8.0±3.3	3.9±4.6	<0.001a
	Discharge PNA, day	10.1±3.6	11.7±7.2	0.946ª
	Abnormal Brain MRI	16 (32)	10 (20)	$0.260^{b}$
3	PO first, day	19.3±11.2	10.1±7.6	0.003a
	PO half, day	19.7±11.1	11.2±8.1	$0.014^{a}$
	PO full, day	19.9±11.2	11.6±8.5	$0.013^{a}$
	Time to FOF, day	16.4±9.4	6.0±4.1	<0.001a
	Discharge PNA, day	21.5±11.7	14.1±10.1	0.042 a
	Abnormal Brain MRI	11 (22)	18 (36)	0.070 b

FOF: full oral feding, HIE: hypoxic-ischemic encephalopathy, MRI: magnetic resonance imaging, PNA: posnatal age, PO: Per oral

Also, newborns with HIE/TH are at high risk of having oropharyngeal dysphagia, and in this case, early action should be taken to prevent feeding difficulties. <sup>14,15</sup> Relatedly, a considerably high number of neonates with HIE develop

long-term feeding problems, such as failure to thrive, impaired growth and development, and increased morbidity and mortality, that are difficult to manage. <sup>16</sup> In a study conducted with 100 NICU infants, the structure and physiology

<sup>&</sup>lt;sup>a</sup>Student's t-test

<sup>&</sup>lt;sup>a</sup>Student's t-test (mean and standard deviation), <sup>b</sup> chi-square test (number and percentage)

of the aerodigestive system in at-risk neonates were evaluated with manometric studies of pharynx and esophagus, and swallow studies, and individualized feeding strategies were applied to these neonates. They found that the rate of successfully fed newborns increased approximately fivefold at discharge and nearly twofold at one year of age.17 Therefore, it is advisable that a team of healthcare providers in NICUs, including neonatologists, SLTs, lactational nurses, and physiotherapists, should always be on hand to prepare at-risk newborns for discharge. It also seems necessary to provide an individualized feeding strategy as early as possible based on pathophysiology to achieve better feeding and long-term outcomes.<sup>16,18</sup>

The PIOMI is known to increase feeding success and efficiency, improve oral-motor skills, sucking capacity and anthropometry, and reduce hospital stay in preterm infants. 12,19-22 Especially, when the PIOMI is applied with breast milk, it has been shown to be more effective compared to PIOMI alone in improving sucking and oral feeding.21 No study has been found that evaluates the effectiveness of oral motor interventions in neonates with HIE/TH, and a limited number of case-based interventions have been tried to improve feeding in these high-risk neonates. In a case study, nonnutritive sucking, which we apply routinely in all hemodynamically stabile neonates in our NICU, was found to facilitate oral feeding in an infant with stage 2 HIE.23 In another case with stage 1 HIE, oromotor stimulation was shown to improve effective breastfeeding and led to a steady increase in the baby's weight.24 Therefore, this subject matter deserves further studies.

In our study, the PIOMI had no effect on the weights of the neonates with HIE/TH, as the weights of the newborns did not differ between the groups at any of the specified time points. The effect of PIOMI on infant weights is unclear in studies conducted with premature infants. Similar to our findings, there was no difference between the weights of premature babies, who did and did not receive PIOMI, in one study<sup>12</sup>, whereas the weights of the babies, who received

PIOMI, were found to be higher than those who did not in another study, and it was concluded that PIOMI improved anthropometric measurements.<sup>22</sup>

To our knowledge, this is the first study in the literature evaluating the efficacy of oral motor intervention exercises in a large group of neonates with HIE/TH, which was the main strength of the study. On the other hand, the accuracy and appropriateness of applying PIOMI exercises, which is an oral motor intervention with proven effectiveness in preterm infants, to term infants with HIE/TH is debatable. However, feeding problems in these at-risk neonates with HIE/TH should be promptly resolved with every effort. Based on this idea, we showed that PIOMI, which could be named as "hypoxic-ischemic encephalopathy- oral motor intervention (HIE-OMI)" in our study, was an effective intervention in shortening the transition time to FOF in HIE/TH neonates, and also improved feeding outcomes and shortened the length of hospital stay in neonates with severe HIE/ TH. The other strength of our study was that the groups were homogeneous in terms of demographic, clinical and laboratory findings that may influence the feeding transition process, such as gestational age, birth weight, APGAR scores, blood gas pH and base deficit, HIE stages, the frequency of abnormal MRI, frequency of convulsions and cardiac anomalies, and duration of mechanical ventilation. Finally, the groups were also homogenous in terms of feeding protocols, although we did not perform minimal enteral feeding (MEN) during TH, which is recommended, as it is safe and has many beneficial effects such as shortening the time to full enteral feeding and increasing the rate of breastfeeding at discharge. 25,26 Furthermore, we changed our feeding protocol in neonates with HIE/TH after the study period, and started to apply MEN during TH to benefit from the advantages of MEN.

Our study has some limitations. The first limitation was that an oral motor intervention developed for premature babies was applied to term newborns for the first time, making

the method of study questionable. The second limitation is that there might be other factors that we did not consider that affected the time to FOF, such as a background abnormality in the electroencephalogram in the first 24 hours of life.27 The third limitation was that except for the time to PO feeding, FOF and discharge, and the weight of the neonates on those specified days, no other parameters that might be related to oropharyngeal dysphagia based feeding problems were evaluated in this study. Clinicianobserved scales that evaluated variables such as problems in feeding behavior, negative effects of feeding incoordination on respiration, and sucking-swallowing-respiratory incoordination could be included in the study. In addition, it would be useful to evaluate newborns with follow-up sessions and to address the negative effects of feeding problems (transition to solid food, breastfeeding at home, etc.) in the first year of life.

PIOMI, which can be named as "HIE-OMI" in our study, is an effective intervention in shortening the transition time to FOF in neonates with all stages of HIE undergoing TH. This intervention improves feeding success and shortens the length of hospital stay in neonates with severe HIE/TH. In the light of our findings, the importance of working as a team with SLTs is obvious in NICUs. Prospective longitudinal studies with larger numbers of neonates with HIE/TH should be conducted to evaluate the effectiveness of "HIE-OMI" exercises.

# Ethical approval

Ethics Committee approval was obtained for the study from Harran University Clinical Research Ethics Committee (date: 24.01.2022, number: 22/02/20). Verbal and written informed consent were obtained.

## **Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: AOG,

AB; data collection: AB, HBC, SD; analysis and interpretation of results: AOG, AB; draft manuscript preparation: AOG. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

#### Conflict of interest

The authors declare that there is no conflict of interest.

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