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Relationship between oxygen saturation, gestational age, and level of oral feeding skills in preterm infants

A relação entre saturação de oxigênio, idade gestacional e nível de habilidade de alimentação oral de recém-nascido pré-termo

Keywords

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Descritores

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ABSTRACT

Purpose: To correlate the peripheral oxygen saturation with gestational age and the level of oral feeding skills in the introduction of oral feeding in preterm infants. **Methods:** This is a cross-sectional, quantitative study whose sample was composed of 169 clinically stable preterm infants. Peripheral oxygen saturation was assessed before and after introduction of oral feeding. The preterm infants were stratified into three groups based on their gestational age at birth: 26-29, 30-33, and 34-36 weeks. The preterm infants were classified into four levels according to their oral feeding skill and resistance. **Results:** No differences in oxygen saturation were observed between the strata of gestational age and between the levels of oral feeding skill. Differences were observed in the groups of preterm infants aged 30-33 weeks ($p=0.04$) and 34-36 weeks ($p=0.02$) and on the level I of oral feeding skills ($p=0.04$) when oxygen saturation was compared at pre- and post-first oral feeding. Significant correlations ($p<0.001$) were found between gestational age and the levels of oral feeding skills ($r=0.38$); in Group A, between gestational age and oxygen saturation before the first oral feeding ($r=0.83$); in Group B, between the level of oral feeding skill and oxygen saturation before the first oral feeding ($r=0.26$) and between level of oral feeding skill and gestational age ($r=0.26$). **Conclusion:** Correlation was found for peripheral oxygen saturation when compared with gestational age and with the level of oral feeding skills.

RESUMO

Objetivo: Correlacionar a saturação periférica de oxigênio com a idade gestacional e com o nível de habilidade de alimentação oral, na introdução da alimentação oral, de recém-nascidos pré-termo. **Método:** Estudo transversal e quantitativo no qual participaram 169 recém-nascidos pré-termo, clinicamente estáveis. A verificação da saturação de oxigênio foi realizada antes e após a introdução da primeira alimentação por via oral. Os recém-nascidos pré-termo foram estratificados em três grupos baseados na idade gestacional ao nascimento: 26-29, 30-33 e 34-36 semanas. A habilidade para alimentação oral foi classificada em quatro níveis com base na habilidade oral e resistência apresentada pelo recém-nascido pré-termo. **Resultados:** Não houve diferença na saturação periférica de oxigênio entre os estratos da idade gestacional e entre os níveis de habilidade oral. Foram observadas diferenças nos grupos com idade gestacional de 30-33 semanas ($p=0,04$) e 34-36 semanas ($p=0,02$) e no nível I de habilidade de alimentação oral ($p=0,04$) quando comparada com a saturação inicial e final. Correlações significativas ($p<0,001$) foram encontradas entre a idade gestacional e níveis de habilidade oral ($r=0,38$); no Grupo A, entre a idade gestacional e a saturação inicial ($r=0,83$); e, no Grupo B, entre o nível de habilidade oral com a saturação inicial, e com a idade gestacional. **Conclusão:** A saturação de oxigênio apresentou correlação quando comparada com a idade gestacional e com o nível de habilidade oral.

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INTRODUCTION

One of the main purposes of the speech-language pathologist in a Neonatal Intensive Care Unit (NICU) is to provide functional, safe and pleasurable oral feeding (OF) to the preterm infant (PTI). However, during the neonatal period, PTIs may present several interurrences, such as sequelae and extended hospitalization time, compromising their clinical evolution, thus hindering the feeding process⁽¹⁾.

The nutritional needs of PTIs are provided through nasogastric tubes, which may interfere with their oral feeding skills (OFS)⁽²⁾. The use of nasogastric tubes may favor the occurrence of desaturation during OF, negatively affecting OF performance in very-low birth weight PTIs⁽³⁾.

Oxygen is the most commonly used resource to prevent and avoid neonatal hypoxia in NICUs⁽⁴⁾. However, the peripheral oxygen saturation (SpO₂) of PTIs needs to be monitored so that the level of blood oxygenation at the time of feeding can be verified, observing whether an ideal supply of oxygen is being provided⁽⁵⁾, thus avoiding episodes of hypoxia⁽⁶⁾. Information on desaturation events is important in the management of transition from nasogastric tube feeding to OF⁽³⁾. Because pulse oximeter reading is a non-invasive, transcutaneous, continuous method, it is usually adopted in these situations⁽⁷⁾.

The baseline SpO₂ interval considered clinically stable for PTIs is still unknown and it may vary as corrected gestational age (CGA) advances. A standard baseline SpO₂ level of 95% during normal breathing can help prevent adverse episodes of desaturation⁽³⁾. SpO₂ indices between 91% and 95% are the safest target⁽⁸⁾. Adequate SpO₂ levels can maximize survival and minimize pulmonary complications in PTIs⁽⁹⁾.

Significant changes in SpO₂ were found during bottle suckling sessions, with a higher occurrence of hypoxia events in PTIs and very-low birth weight newborns^(10,11). Even if PTIs are able to maintain a normal baseline SpO₂ level in ambient air, the physiological demand created by the OF activity may increase their oxygen requirements beyond their capacity to maintain physiological regulation⁽¹⁰⁾. Therefore, a below-adequate baseline SpO₂ level during feeding hinders the ability of PTIs to organize and maintain their OF abilities⁽³⁾. Preterm infants who develop respiratory complications in their clinical outcomes, such as bronchopulmonary dysplasia, present increased risk of desaturation during OF, requiring constant monitoring⁽¹²⁾.

It is important that the speech-language therapist who works directly with preterm infants be knowledgeable about SpO₂ monitoring, because PTIs may require SpO₂ monitoring during OF until they are able to maintain oxygenation throughout the entire suckling process⁽¹⁰⁾. It is essential to observe this aspect, because alterations may be indicative of lack of coordination between the breathing and swallowing functions during OF. It is possible to know when preterm infants have developed the ability to begin OF by observing their feeding readiness and willingness⁽¹³⁾. Therefore, the assessment of nonnutritive suction before the introduction of oral feeding can be used as an investigative measure to promote the coordination between the functions of sucking, swallowing, and breathing (S/S/B) in order to prevent episodes of desaturation⁽³⁾. However, PTIs

must remain clinically stable and be able to be fed exclusively on OF to be discharged from hospital⁽¹⁴⁾.

The present study was based on two hypotheses: the first hypothesis predicted that the more premature the PTI, the more unstable the SpO₂; the second hypothesis was generated in the assumption that the more mature the level of OFS, the more stable the SpO₂. Based on the considerations previous presented, this study aims to determine the peripheral oxygen saturation of preterm infants before and after the introduction of oral feeding, relating it to gestational age at birth and to the level of oral feeding skill.

METHODS

This cross-sectional, quantitative study was approved by the Research Ethics Committee of the aforementioned Institution under process no. 11155312.7.0000.5346. The parents/legal guardians of the participating preterm infants signed an Informed Consent Form prior to study commencement.

The study sample was composed of 169 preterm infants (PTI) who were hospitalized in the Neonatal Intensive Care Unit (NICU) of "Hospital Universitário de Santa Maria" from April 2012 to July 2015. Newborn infants with gestational age (GA) <36.6 weeks, clinically stable, released by the medical team to begin oral feeding (OF) were included in the study. Exclusion criteria comprised infants with neurological disorders, genetic syndromes, head and/or neck malformations, neonatal asphyxia (Five-minute Apgar score ≤5), hyperbilirubinemic encephalopathy, and intraventricular hemorrhage grades III and IV, as well as PTIs who had previously undergone speech-language therapy.

SpO₂ of PTI was measured before and after the first oral feeding using a pulse oximeter (GE Medical System, model B20, China) available at the hospital NICU by placing a sensor on the newborn skin, measuring the arterial oxygen saturation⁽⁶⁾, and annotating the value stipulated on the monitor screen. Although breastfeeding is the most natural method available⁽¹⁵⁾, a nursing bottle was used for the first oral feeding because of the need to accurately measure the amount of milk ingested, thus allowing the researcher to assess the PTI's oral feeding skill in addition to controlling the milk flow.

It is also worth mentioning that these infants generally require clinical care because of their prematurity, and that any risk of respiratory complication should be avoided at this time. After the newborns' ability to suckle is verified, they are considered apt to begin OF and, consequently, are offered the maternal breast, thus not endangering the safety and efficacy of OF.

The preterm neonates were stratified into groups according to GA: Group A - from 26 weeks to 29 weeks and 6 days; Group B - from 30 weeks to 33 weeks and 6 days; and Group C - from 34 weeks to 36 weeks and 6 days.

The level of OFS, assessed during the first oral feeding, was obtained from a combination of the parameters proficiency (PRO) and rate of milk transfer (RT), as proposed in a previous study⁽¹⁶⁾. With respect to the PRO, which represents the percentage of the volume ingested in relation to the volume prescribed in the first five minutes, these first five minutes of feeding are monitored, believing that during this period the fatigue factor is minimal

and thus represents the actual OF capacity or ability of the PTI. Rate of milk transfer, which is the volume of milk accepted per minute during the entire feeding session, reflects a resistance index. Based on these data, the level of OFS of the preterm infants was classified as follows: Level I = PRO < 30% and RT < 1.5 ml/min (low OFS and low resistance - high fatigue); Level II = PRO < 30% and RT > 1.5 ml/min (low OFS and high resistance - low fatigue); Level III = PRO > 30% and RT < 1.5 ml/min (high OFS and low resistance - high fatigue); and Level IV = PRO > 30% and RT > 1.5 ml/min (high OFS and high resistance - low fatigue).

The results were classified and analyzed using the STATA 10.0 statistical software. Analysis of variance (ANOVA) and the paired Student's t test were applied for comparison between the means, the Chi-square test was used for comparison between the categorical variables, and the Spearman's correlation coefficient was applied to nonparametric correlations. Significance level of 5% ($p < 0.05$) was considered for all statistical analyses.

RESULTS

Table 1 shows the characterization of the study sample. Of the 169 PTIs investigated, 58% were male with weight at birth of 1968 (± 1736) grams and gestational age (GA) of 33.4 (± 2.0)

Table 1. General characteristics of the 169 preterm infants at birth and at first oral feeding

Variables	
Gender (%)	
Male (n=98)	58
Female (n=71)	42
At birth	
Gestational age (in weeks)*	33.4 (± 2.0)
Weight (in grams)*	1968 (± 1735)
At first oral feeding	
Corrected gestational age (in weeks)*	35 (± 1.4)
Chronological age (in days)*	11.1 (± 10.6)
Weight (in grams)*	1937 (± 381)
SpO ₂ before*	96.9 (± 2.3)
SpO ₂ after*	96.0 (± 4.3)

*Values expressed in mean and standard deviation

weeks. First oral feeding occurred at chronological age of approximately 11.1 (± 10.6) days, corrected gestational age (CGA) of 35 (± 1.4) weeks, with weight of 1937 (± 381) grams. Mean pre- and post-first oral feeding baseline SpO₂ levels were 96.9% (± 2.3) and 96.0% (± 4.3), respectively.

Table 2 shows the variables weight and GA, as well as SpO₂ before and after first oral feeding according to the groups formed by GA at birth. Significant differences were observed regarding weight ($p = 0.01$) and GA ($p < 0.001$) at birth for the three strata analyzed. At first oral feeding, the newborn infants presented the following means of CGA: 33.4 (± 1.0) weeks for Group A, 34.3 (± 1.1) weeks for Group B, and 35.9 (± 1.0) weeks for Group C ($p < 0.001$), showing difference between the groups of different GA at birth. As expected, difference was observed at first oral feeding for the variables weight and chronological age ($p < 0.001$). Regarding both pre- and post-first oral feeding SpO₂ levels, no significant difference was found between the groups, with their mean value within the normality range.

The variables weight and GA at birth and at first oral feeding, as well as pre- and post-first oral feeding SpO₂ values, according to the level of OFS observed are shown in Table 3⁽¹⁷⁾. Of the 169 PTIs analyzed, 72 (42.6%) were classified as Level I; eight (4.7%) as Level II; 34 (20.1%) as Level III; and 55 (32.6%) as Level IV. Differences were found according to level for the variables weight ($p < 0.001$), CGA ($p = 0.05$), chronological age ($p < 0.001$), and SpO₂ ($p = 0.05$) at first oral feeding. It was possible to observe that the preterm infants with high OFS and resistance (Level IV) presented greater stability in relation to the SpO₂ parameters. The PTIs classified as Levels I and III, with low resistance to OF, presented reduction of up to two points in the SpO₂ means after the first oral feeding.

Correlation with the levels of OFS was also found in PTIs (%) regarding the strata of GA at birth for Groups A, B and C. Significant correlation ($p < 0.001$) between GA and level of OFS ($r = 0.38$) was found when the Spearman's correlation coefficient was applied.

Comparison between the SpO₂ baselines before and after the first oral feeding is presented in Table 4. The results show significant differences, although still within the limit of normality, between the PTIs in the GA at birth strata of 30–33 weeks

Table 2. Comparison between the variables "at birth" and "at first oral feeding" according to the strata of gestational age of the preterm infants

Variables	Gestational age at birth			p ¹
	Group A 26w-29w (n=13)	Group B 30w-33w (n=79)	Group C 34w-36w (n=77)	
At birth				
Gestational age (in weeks) ¹	28.8 (± 0.7) ^a	32.6 (± 1.1) ^b	35 (± 0.8) ^c	<0.001
Weight (in grams) ¹	1234 (± 160) ^a	1696 (± 382) ^b	2097 (± 520) ^c	0.01
At first oral feeding				
Corrected gestational age (weeks) ¹	33.4 (± 1.0) ^a	34.3 (± 1.1) ^b	35.9 (± 1.0) ^c	<0.001
Weight (in grams) ¹	1805 (± 172) ^a	1809 (± 244) ^{a,b}	2090 (± 460) ^c	<0.001
Chronological age (in days) ¹	32.7 (± 9.1) ^a	12.3 (± 9) ^b	6.1 (± 6.6) ^c	<0.001
SpO ₂ before	97.4 (± 2.1)	96.8 (± 2.5)	97.0 (± 2.0)	0.65
SpO ₂ after	96.2 (± 3.3)	95.6 (± 5.6)	96.3 (± 2.7)	0.61

Values expressed in mean and standard deviation; ¹Oneway ANOVA; post-hoc Bonferroni correction, different superscript letters $p < 0.05$

Caption: SpO₂ before = oxygen saturation before the first oral feeding; SpO₂ after = oxygen saturation after the first oral feeding

Table 3. Comparison between the variables “at birth” and “at first oral feeding” according to the levels of oral feeding skill

Variables	Oral Feeding Skill				p ¹
	Level I n=72	Level II n=8	Level III n=34	Level IV n=55	
At birth					
GA (in weeks) ¹	32.5 (±2.2) ^a	33.9 (±1.3) ^{a,b,c}	34.1 (±1.4) ^b	34.1 (±1.8) ^{c,b}	<0.001
Weight (in grams)	1642 (±426)	1612 (±368)	2532 (±3700)	2098 (±605)	0.08
At first oral feeding					
CGA (in weeks)	34.7 (±1.5)	35.9 (±0.9)	34.9 (±1.1)	35.2 (±1.2)	0.05
Weight (in grams) ¹	1834 (±313) ^a	1844 (±296) ^{a,b,c}	1888 (±238) ^b	2114 (±478) ^{c,b}	<0.001
Chronological age (in days) ¹	15.7 (±10.8) ^a	14.4 (±7.7)	5.4 (±6.0) ^{b,c}	8.0 (±10.1) ^c	<0.001
SpO ₂ before	96.5 (±2.4)	95.6 (±3.1)	97.2 (±2.1)	97.5 (±1.9)	0.05
SpO ₂ after	95.6 (±5.2)	95.6 (±3.3)	95.7 (±5.0)	97.0 (±2.1)	0.23
Gestational age at birth (%)					
Group A (26w-29w)	77	0	0	23	
Group B (30w-33w)	55	5	20	20	
Group C (34w-36w)	25	5	23	47	

¹Oneway ANOVA; post-hoc Bonferroni correction, different superscript letters p<0.05; Values expressed in mean and standard deviation

Caption: GA = gestational age; CGA = corrected gestational age; SpO₂ before = oxygen saturation before the first oral feeding; SpO₂ after = oxygen saturation after the first oral feeding

Table 4. Oxygen saturation at pre- and post-first oral feeding in the strata of gestational age and in each level of oral feeding skill presented by the preterm infants

Variables	N (%)	SpO ₂ before	SpO ₂ after	p
Gestational age				
Group A	8	97.4 (±2.1)	96.2 (±3.3)	0.15
Group B	47	96.8 (±2.5)	95.6 (±5.6)	0.04*
Group C	45	97.0 (±2.0)	96.3 (±2.7)	0.02*
Oral feeding skill				
Level I	43	96.5 (±2.4)	95.6 (±5.2)	0.04*
Level II	5	95.6 (±3.1)	95.6 (±3.3)	1.00
Level III	20	97.2 (±2.1)	95.7 (±5.0)	0.05
Level IV	32	97.5 (±1.9)	97.0 (±2.1)	0.06

Values expressed in mean and standard deviation; *Statistically significant values (p≤0.05)

Caption: SpO₂ before = oxygen saturation before the first oral feeding; SpO₂ after = oxygen saturation after the first oral feeding

(p=0.04) and 34–36 weeks (p=0.02), as well as for PTIs classified as Level I (p=0.04) for OFS.

Application of the Spearman’s correlation coefficient showed significant correlation (p<0.05) between GA and SpO₂ before the first oral feeding (r=0.83) in Group A; significant correlation (p<0.05) between OFS and SpO₂ before the first oral feeding (r=0.26) and between OFS and GA (r=0.26) in Group B. No statistically significant correlation between the variables was observed in Group C.

DISCUSSION

SpO₂ is a measure that has been considered in speech-language pathology in the hospital environment. Nevertheless, to date and the best of our knowledge, there are no studies in the specific scientific literature addressing the correlation of SpO₂ variations, before and after first oral feeding, in groups of PTI of different gestational ages (GA) and regarding levels of OFS at first oral feeding.

Clinical stability, corrected gestational age (CGA), and weight are the medical criteria most commonly used for the introduction of oral feeding in PTIs. However, these criteria are not always sufficient to ensure efficient performance during OF for this population.

At first oral feeding, the PTIs herein investigated presented CGA of 33.4 (±1.0), 34.3 (±1.1), 35.9 (±1.0) and mean weight of 1805 (±172), 1809 (±244), 2090 (±460) for infants with GA at birth of 26-29, 30-33, and 34-36 weeks, respectively. These results are in agreement with the criteria used in the Unit evaluated, that is, CGA of approximately 34 weeks and weight over 1500 g. It is worth stressing that coordination between the functions of sucking, swallowing, and breathing (S/S/B) is initiated in this phase of infant development⁽¹⁷⁻¹⁹⁾.

The means found in the speech-language pathology assessment with regard to the chronological age of the PTIs are in compliance with their GA at birth. It should be considered that the smaller the measure of GA, the longer it takes to reach adequate CGA to begin OF and, consequently, the longer the hospitalization time.

The PTIs with smaller measures of GA and lower weight at first oral feeding were the ones that presented low OFS and low resistance during OF, corresponding to Level I. The Spearman’s coefficient showed significant correlation between GA and OFS level, denoting that the higher the GA measure of preterm infants, the higher their OFS level and, consequently, the better the baseline SpO₂ measures - positively responding to the first hypothesis of this study, which predicted that the more premature the PTI, the more unstable the SpO₂.

This result may be a consequence of the interferences that these PTIs experience due to prolonged hospitalization time, which may hinder their adequate feeding process. Likewise, neurological immaturity, altered muscle tone, weak oral reflexes, and poor alertness may also interfere with the quality of oral motor skills of these infants⁽¹⁾. A study using modified barium swallow evaluation showed that preterm infants born after 28 weeks of GA presented adequate airway protection, thus

suggesting that lower-birth weight infants are at higher risk for airway protection, in addition to showing significant desaturation during OF, even if they present similar CGA compared with that of infants with higher GA measure at the time of assessment⁽²⁰⁾.

Analysis of the SpO₂ measure showed a 1-point decrease in the comparison between the pre- and post-first oral feeding means, although still within the expected normality range. It is expected that the PTI maintain adequate ventilation support during oral feeding⁽²¹⁾, insofar as PTIs with smaller measure of CGA were more vulnerable to a reduction in the baseline SpO₂ level during OF⁽¹⁰⁾.

All preterm infants stratified according to GA at birth presented means within the normality range; however, significant difference was observed for Groups B ($p=0.04$) and C ($p=0.02$) in the comparison of SpO₂ data before and after first oral feeding. Group A, which is composed of neonates with smaller measure of GA at birth (26–29 weeks), did not present a significant result probably owing to its sample size - only 13 PTIs.

A study that compared the results of fluoroscopic examination of swallowing between preterm and full-term infants found that PTIs presented a higher chance of reduced SpO₂⁽²²⁾. This decrease may also be related to immaturity in the coordination of the S/S/B functions due to prematurity^(23,24).

Level I (low OFS and low resistance) preterm infants presented difference in the comparison between SpO₂ levels before and after first oral feeding, whereas Level III (high OFS and low resistance) premature newborns showed difference close to statistical significance for the same comparison. It is worth mentioning that both in Level I and Level III the fatigue factor is present owing to the low resistance of PTIs⁽¹⁶⁾.

Even with appropriate CGA and weight to begin OF, these PTIs were more likely to present fatigue when performing efficient nutritive sucking. This demonstrates that the PTI is not able to receive OF longer than five minutes - a time limit up to which the fatigue factor does not interfere with nutritive sucking performance. Resistance training with nutritive sucking, along with a program of oral sensory motor stimulation, could possibly help infants maintain their postprandial SpO₂. This justifies the importance of inserting speech-language therapists in the NICU, where they would be able to evaluate infant readiness, confirm or not the introduction of oral feeding, and in the latter case, plan a stimulation program, in addition to promoting riskless initiation of breastfeeding.

In contrast, all Level II and Level IV preterm infants presented satisfactory pre- and post-first oral feeding SpO₂ means and remained clinically stable during OF because of their high resistance. Risks in clinical stability are considered moderate if SpO₂ values are maintained between 85% and 80%, but they are severe if baseline SpO₂ is <80%⁽²⁵⁾.

Application of the Spearman's coefficient showed significant correlation ($p<0.05$) between the levels of OFS and SpO₂ ($r=0.20$), indicating that the higher the OFS level presented by the PTIs, the better their SpO₂ levels during OF. This information confirms the second hypothesis of this study, which was generated on the assumption that the more mature the level of OFS, the more stable the SpO₂.

Therefore, monitoring of SpO₂ before and after introduction of oral feeding aims to assess possible physiological complications that may occur owing to the efforts made by the PTI during OF.

Both the OFS and the resistance of the PTIs are of great importance to determine the success of OF⁽¹⁶⁾. Some authors have been using the classification of OFS in levels, considering that this classification has an indicator that allows quantification of the preterm infant's ability to receive OF, because this assessment is performed in the first five minutes of oral feeding and, during this period, the difficulties caused by fatigue would not yet be observed^(16,26-29).

Monitoring the PTI allows the speech-language pathologist to detect possible physiological alterations during OF and suspend the procedure, avoiding episodes of aspiration or clinical destabilization, favoring the success of the transition from the nasogastric tube to OF⁽³⁰⁾. Moreover, newborn infants will only be discharged from hospital when they are able to be fed safely, efficiently and exclusively by OF⁽¹⁴⁾, which is an important milestone in their development^(14,26), because prolonged hospitalization time, inadequate oral stimulation, and medical procedures may contribute to feeding difficulties⁽¹⁾.

Monitoring of the SpO₂ index in PTIs at first oral feeding should always be considered and observed thoroughly. Abrupt and sudden reductions in SpO₂ levels should be considered, because they may be an indication that the PTI probably is not presenting a favorable coordination between the S/S/B functions.

Therefore, it is imperative that the speech-language therapist be inserted in the neonatal team, taking conducts and being attentive to the clinical conditions of the newborn during the feeding process, observing the SpO₂ indexes presented by the PTIs to avoid possible clinical interferences, in addition to promoting safe breastfeeding without risks of respiratory complications.

Based on the data of this study, we suggest that further research on the observation of SpO₂ indices be conducted with previously stimulated preterm infants in order to evidence their conditions at first oral feeding.

CONCLUSION

We conclude that the higher the measure of gestational age of preterm infants, the better their level of oral feeding skill, and that the higher the level of oral feeding skill, the better the saturation during oral feeding.

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Author contributions

RCCY assessed the study participants, classified and analyzed the data, and participated in the writing and corrections of the manuscript; LSP evaluated the study participants and collaborated on the writing and corrections of the manuscript; GPB assessed the study participants and participated in the writing and corrections of the manuscript; ARMW was the study co-adviser, analyzed the data and collaborated on the corrections of the manuscript; MKS was the study adviser, analyzed the data and collaborated on the corrections of the manuscript.