Does Positioning Affect Tracheal Aspiration of Gastric Content in Ventilated Infants?

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ABSTRACT

Objectives: Gastroesophageal reflux and aspiration can occur in premature infants who are supported with mechanical ventilation. The relation between physical positioning and gastric aspiration in ventilated infants has not been studied. Pepsin measured in tracheal aspirate (TA) emerged as a specific marker for aspiration. The objective of our study was to assess pepsin in TA of ventilated infants at 2 different positions: supine and right lateral.

Methods: We conducted a randomized controlled trial on premature infants who were enterally fed and supported with mechanical ventilation. Patients were randomized into intervention and control groups. In the intervention group, infants were placed supine for 6 hours before a sample of TA was obtained. A second sample was collected 6 hours later while lying in the right lateral position. In the control group, the 2 samples of TA were obtained while infants remained in the supine position during the entire study time. Pepsin in TA was measured while blinded to the group assignment.

Results: A total of 34 patients were enrolled and randomized to intervention ($n = 17$) and control ($n = 17$) groups. Gestational age was 32.7 ± 2.7 weeks, and birth weight was 1617 ± 526 g; both groups had similar demographic and clinical characteristics. Pepsin concentration did not differ between groups at baseline. In the intervention group, pepsin concentration significantly declined from 13 ng/mL (interquartile range [IQR] 11.9–38.7) to 10 ng/mL (IQR 7–12; $P < 0.001$), whereas it did not change in the control group ($P = 0.42$).

Conclusions: The right lateral positioning is associated with decreased TA pepsin. The implications of the present study on hospital practice and clinical outcomes need further investigations.

Key Words: gastroesophageal reflux, GERD, pepsin, premature

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Premature infants with surfactant deficiency are frequently managed with tracheal intubation and mechanical ventilation. They are at increased risk for gastroesophageal reflux (GER) and microaspiration, especially because the used endotracheal tubes are not cuffed (1). Factors that may increase the risk of aspiration in ventilated infants include the use of sedation, supine positioning, the presence of an indwelling gastroesophageal tube, and intermittent feeding delivery methods (1). Aspiration carries the risk of causing ventilation-associated pneumonia or lung injury, and plausibly may predispose to chronic lung diseases (2–6).

In the absence of a reliable and applicable marker for aspiration, confirmation of such cause-and-effect relation is difficult. Pepsin is a proteolytic enzyme derived from pepsinogen, which is secreted by the gastric chief cells and the mucus neck cells in the stomach. Its presence in endotracheal secretions is considered a marker of aspiration of gastric content (3,7–9).

Right-side positioning has been associated with improved gastric emptying and may be recommended for premature infants after enteral feeds (10,11); however, the role of positioning in decreasing reflux, and subsequent aspiration, is generally controversial (12). The aim of the present prospective randomized trial was to examine the relation between positioning and gastric aspiration in ventilated premature infants. We hypothesized that when compared with supine positioning, the right lateral position would decrease the risk of gastric aspiration as evidenced by a lower concentration of pepsin retrieved from tracheal aspirate (TA).

METHODS

A prospective randomized controlled trial was conducted in the neonatal intensive care unit (NICU) at Cairo University Children's Hospital (Cairo, Egypt). This unit is the primary referral center for the majority of hospitals in the city. Infants admitted to this unit are usually critically ill and mostly supported by mechanical ventilation. The study was approved by the ethics committee and was conducted in accordance with the Cairo University bylaws for human research. Parental consents were obtained for all of the infants.

Patients

Infants were included in the study if all of the following criteria were fulfilled: born prematurely at a gestational age between 28 0/7 and 32 6/7 weeks, postnatal age >72 hours, tracheally intubated and receiving conventional mechanical ventilation, and feeding enterally >20 mL/day via nasogastric tube. Infants were excluded from the study if they experienced neurological insult in the form of perinatal asphyxia (pH < 7, base deficit >12 mEq/dL, and Apgar score <3 at 5 minutes of life), intracranial hemorrhage grade 3 or 4 diagnosed at 72 hours of age, or periventricular leukomalacia; had major congenital anomalies, or gastrointestinal anomalies such as tracheoesophageal fistula, or necrotizing enterocolitis; or were receiving xanthine derivatives, H_2 blockers, prokinetics, proton pump inhibitors, or sedatives.

Infants managed with mechanical ventilation in the NICU are orally intubated using an uncuffed endotracheal tube. The size of the endotracheal tube is determined based on the weight of the infant in accordance with the American Academy of Pediatrics neonatal

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resuscitation standards, and the position is confirmed with a portable chest radiograph. When enteral feeds are initiated, an indwelling nasogastric tube is inserted in accordance with the American Academy of Pediatrics standards; its position is confirmed by auscultation (13). The primary outcome of the study was to compare the differences in TA pepsin measurements between supine and right lateral positions in preterm infants receiving mechanical ventilation.

Randomization and Data Collection

Enrolled patients were assigned to 1 of 2 groups according to a predetermined randomization sequence using the opaque envelopes technique. Randomly generated treatment allocations were kept within sealed opaque envelopes. Following consent, an envelope was opened and the patient was assigned to the allocated group. The bedside nurse was not blinded to the procedure because she was the one performing the intervention, but those performing the assays for pepsin were masked to patient allocation. Data regarding gestational age, birth weight, sex, mode of delivery, weight, postnatal age, amount of feeds, and ventilator settings were collected.

Infant Positioning

Infants were suctioned to ensure the removal of any tracheal secretions at enrollment; these aspirates were discarded. Infants were then placed supine with the head of the bed tilted 15° upward for 6 hours. While in this position, all of the infants were fed twice; the first feed was administered at the start of the study, and the second feed was given 3 hours later. All of the feeds were delivered via an indwelling feeding tube using gravity. TA was obtained for pepsin assays at the end of 6 hours (sample 1).

After 6 hours in the supine position, infants were then randomly assigned to 1 of 2 groups. In the intervention group, infants were turned on their side, with the right side down for another 6 hours. The infant's back was supported with rolled towels to maintain an exact 90° angle on the bed. Infants in the control group remained in the supine position throughout the study. During this phase of the study, infants in both groups received 2 more feeds and TA was obtained again for pepsin assays (sample 2) (Fig. 1).

Tracheal Aspirate Sample Collection

Two TA samples were collected from each infant. The first sample was obtained after the infant was lying in the supine position for 6 hours. The second sample of TA was collected after another 6 hours, while lying in the right lateral position (intervention group) or in the supine position (control group). Samples were collected 3 hours after the last feed.

The total length of the endotracheal tube was measured. A 5-F suction catheter connected to a suction trap was used. The depth of the suction catheter was determined to reach 0.5 to 1 cm beyond the tip of the endotracheal tube. A total of 0.5 mL of sterile normal saline was installed into the endotracheal tube before the suction catheter was introduced to collect TA in the suction trap. This procedure was repeated 3 times using 0.5 mL of normal saline each time; the suction catheter was then flushed with 0.5 mL saline to collect the residue. Thus, the total volume of normal saline used for each collection was always 2 mL. TA samples were immediately transported to the laboratory on ice and processed within 30 minutes. They were centrifuged at 4°C for 10 minutes at 3000*g*. The supernatant was then collected and stored at −70°C for subsequent analysis for pepsin.

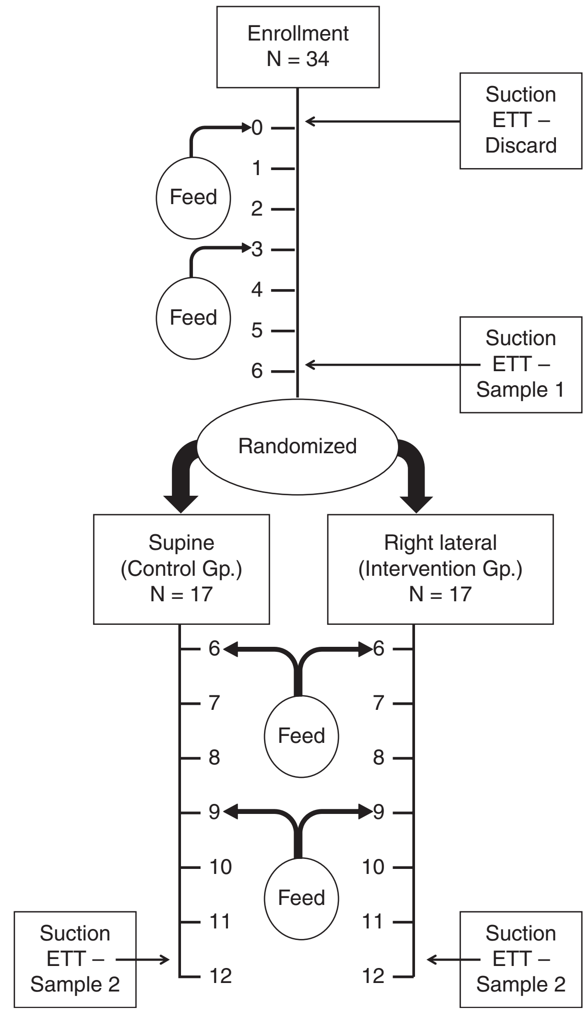

FIGURE 1. Illustration of the study design.

Pepsin Analysis

Pepsin was measured using enzyme-linked immunosorbent assay technique (kit E90632Hu; USCN Life Science Inc, Wuhan, Hubei, PRC). This kit allows quantitative measurement of pepsin in human serum, plasma, and other biological fluids. Samples were added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to pepsin. Next, avidin conjugated to horseradish peroxidase was added to each microplate well and incubated. After tetramethylbenzidine substrate solution was added, only those wells that contained pepsin, biotin-conjugated antibody, and enzyme-conjugated avidin exhibited a change in color. The enzyme–substrate reaction was terminated by the addition of sulfuric acid solution, and the color change was measured spectrophotometrically. The concentration of pepsin in the samples was then determined by comparing the optical density of the samples with a standard curve. The detection range for the kit was 1.56 to 100 ng/mL. All of the assays were conducted while blinded to the group assignment.

Statistical Analysis and Sample Size

Descriptive statistics were presented as proportions for categorical variables, mean test, Mann-Whitney test, and Fisher exact test for continuous parametric, continuous nonparametric, and categorical variables, respectively. The related-samples Wilcoxon signed-rank test was used to compare the initial and repeat pepsin concentrations in both intervention and control groups. Statistical analysis was performed by PASW Statistics (version 18; SPSS Inc, Chicago, IL). To detect a difference between the 2 treatment groups in pepsin concentration that equals 1 SD, a sample size of 17 infants in each group was required (power 80%, $\beta = 20\%$, and $\alpha = 0.05$).

RESULTS

A total of 34 patients were enrolled between January 2012 and July 2013 with gestational age 32.7 ± 2.7 weeks and birth weight 1617 ± 526 g. The study was conducted at postnatal age 11.9 ± 7.9 days and infants' weight at the time of study was 1573 ± 475 g. The intervention (n = 17) and control (n = 17) groups did not differ in gestational age, birth weight, or sex. All of the enrolled infants were fed cow-based premature milk formula (80 cal/100 mL). The volume of feed in both groups (median [IQR]) was comparable: 15 (10–18) mL/feed in the control group versus 12 (8–18) mL/feed in the intervention group ($P = 0.27$). The primary diagnoses of enrolled infants included sepsis, hyaline membrane disease, and pneumonia. The 2 groups did not differ in diagnoses. The mode of ventilation in all of the neonates was synchronized intermittent mandatory ventilation. Demographic and clinical characteristics of both groups are presented in Table 1.

Pepsin was detectable in TA of all of the infants both at baseline and in the second sample. Baseline pepsin that was measured in sample 1 did not differ between the 2 groups: 13 ng/mL (IQR 11.9–38.7) versus 21 ng/mL (IQR 10–141; $P = 0.47$). Within the intervention group, the pepsin concentration declined significantly with positioning ($P < 0.001$), whereas the concentration did not change over time in the control group ($P = 0.42$). Figure 2 illustrates the changes in pepsin concentrations over time in both groups. Pepsin concentration in sample 2 was significantly decreased in the intervention group when compared with the control group (10 ng/mL [IQR 7–12] vs 23 ng/mL [IQR 12–140.5]; $P = 0.002$).

DISCUSSION

The present study demonstrates that aspiration of gastric contents is frequently encountered in mechanically ventilated premature infants. It also provides substantial evidence that right lateral positioning can ameliorate the risk of aspiration in these infants. GER is commonly seen in premature infants secondary to immature tone of the lower esophageal sphincter and small stomach capacity (14,15). In addition, gastrointestinal motility is commonly compromised in this population because of immaturity or as a consequence of using multiple medications such as xanthine derivatives and opioids (16). This problem can be exacerbated by the presence of indwelling nasogastric or orogastric tubes (17). Unlike older children and adults who use cuffed endotracheal tubes, the use of uncuffed tubes in ventilating premature infants increases the propensity to develop pulmonary aspiration (9). The undeveloped swallowing reflex is another factor that may contribute to aspiration in premature infants. In the present study, infants in both groups were comparable in regard to being intubated with an uncuffed endotracheal tube, having an indwelling feeding tube, and not receiving any xanthine derivative or sedative agents.

Proper positioning of infants has been the focus of many studies. The supine position is considered the standard of care for healthy term infants because it is presumed to decrease the risk of sudden infant death syndrome. Ventilated premature infants in the NICU represent a different setting; positioning of these infants can be manipulated to achieve best physiological stability. For example, the prone position can improve oxygenation, whereas lateral positioning of intubated infants decreases microbial colonization in the trachea (18,19); however, the role of positioning in managing GER in neonates has been controversial. Although some studies have shown prone and left lateral positions to improve reflux, with no benefit of bed elevation, other studies did not find any difference in clinically important GER or obstructive apnea episodes (20–23). It is important in this context to differentiate between GER and gastric aspiration. In the present study, we aimed to ameliorate gastric aspiration; it was not in our objectives to assess GER. We chose the right lateral position because it plausibly avoids gastric compression that may occur if stomach is dependent. The right lateral position is known to affect gastric motility and is equivocally considered to be the best position in the first postprandial hour (10,11).

One of the strengths of the present study is that it specifically addressed aspiration and could quantify its magnitude. Aspiration of gastric contents carries the risk for mechanical obstruction of the endotracheal tube, lung injury, and bacterial infection, and can predispose to bronchopulmonary dysplasia (BPD) (2–6,24–26). Without a reliable measure able to detect and quantify gastric fluid aspiration, it is difficult to assess the effect of different interventions. Available tools such as dye studies, lipid-laden macrophages, glucose, and lactose assays in TA lack the sensitivity and specificity (3,27–29). TA pepsin has emerged as a promising marker for

TABLE 1. Demographic and clinical characteristics of the intervention and control groups

	Control group (N = 17)	Intervention group (N = 17)	*P*
Gestational age, wk	32.8 ± 2.5	32.7 ± 2.9	0.85
Birth weight, g	1587 ± 539	1646 ± 529	0.75
Boys	8 (47)	7 (41)	0.99
Cesarean section	13 (77)	15 (71)	1
Days of life	9 (6–15)	9 (6.5–19.5)	0.51
Weight at time of study, g	1609 ± 504	1537 ± 458	0.67
Amount of feed, mL	15 (10–18)	12 (8–18)	0.27
Peak inspiratory pressure, cm H_2O	18 (16–20)	19 (17–20.5)	0.65
Positive end-expiratory pressure, cm H_2O	4 (4–5)	4 (4–5)	0.99
Ventilation rate, bpm	26 (22–32)	25 (22–32.5)	0.55
Days of ventilation	14 (6.5–18.5)	12 (10–18.5)	0.43
Death	4 (24)	5 (29)	0.99

Results are presented as median [IQR], mean ± SD, and number (%). IQR = interquartile range; SD = standard deviation.

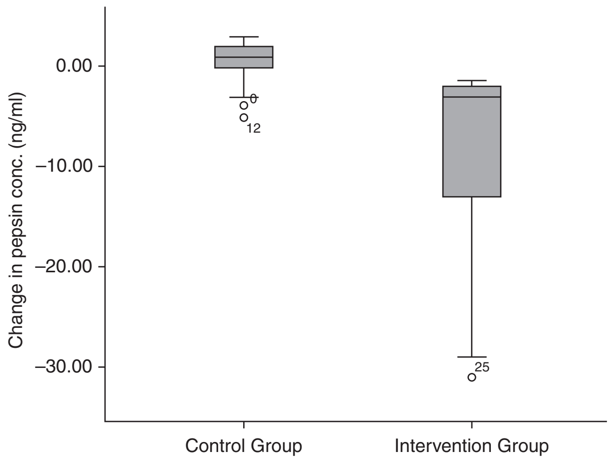

FIGURE 2. Change in pepsin concentrations in intervention and control groups. Two outliers in the intervention group are not presented.

assessment of gastric fluid aspiration. Pepsin, a proteolytic enzyme secreted by the gastric chief cells and the mucus neck cells of the stomach as inactive pepsinogen, was found to be a specific and sensitive marker of aspiration in both adults and children (3,7–9,30). Pepsin, which is only present within gastric secretions, remains biologically active and detectable after aspiration and is physically retrievable from the lungs using minimally invasive procedures (31). In older children, pepsin was associated with the presence of GER disease and respiratory symptoms (31). In premature infants, it was commonly detected in the trachea, increased with feeds and with the use of xanthines (33), and was associated with the development of BPD (5).

The present study is the first to correlate right lateral positioning with a decrease in TA pepsin concentration as a marker of decreased aspiration of gastric content. Of note, a feasibility study could not associate lateral positioning of adults with a decrease in TA pepsin (32). This could be attributed to 2 factors: first, that study did not specifically use the right lateral that we strongly advocate to prevent compression of the stomach, and second, adults are ventilated via cuffed endotracheal tubes that could modify the mechanics of aspiration when compared with premature infants (9).

A limitation of the present study is the short duration of positioning. It is not clear how long and how often neonates should be repositioned to maintain that effect. Also, because of the small sample size, apparent variation in pepsin concentration was noted, but it was not statistically significant. In future studies, the design can be altered so that each infant can act as its own control, and patients can be randomly assigned to start on 1 position and then switch to the other. Although physiologically plausible, it is not known whether the proposed right lateral positioning will have a significant change in clinical outcomes such as duration of mechanical ventilation or BPD. Another limitation of the study is the relatively increased gestational age and birth weight, which typically are not associated with a need for prolonged invasive support; however, the present study was completed in a tertiary center that gets referral for sicker neonates. Further studies are needed to test and compare other positioning techniques, for longer duration in a wider range of gestational ages and modes of ventilatory support. Another limitation of the present study is the use of normal saline to obtain TAs. There is no agreement on whether to correct measurements to account for the dilution caused by saline (33). Because we used the exact volume of saline for all of the infants in both groups, the comparison between groups should be accurate. Moreover, suctioning TA without saline would be inefficient and can lead to inaccurate findings.

CONCLUSIONS

Right lateral positioning of premature infants receiving mechanical ventilation was associated with decreased TA pepsin levels, which is a marker of decreased aspiration. Further studies are needed to examine the impact of these findings on clinical outcomes.

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