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Feeding intolerance, inflammation, and neurobehaviors in preterm infants

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Abstract

Purpose—Identifying relationships between feeding intolerance (FI), inflammation, and early measures of neurodevelopment may provide the basis for clinically relevant assessments for NICU clinicians and staff. The purpose of this secondary analysis was to examine the relationship of FI to inflammatory markers and/or neurobehaviors in the first week of life.

Methods—This was a retrospective, matched case-control design with data drawn from 114 infants born at 32 weeks gestation.

Results—Eight infants developed FI prior to full enteral feedings. These infants were more likely to have dysregulated levels of cytokines, specifically IL6, and lower neurodevelopmental scores compared to infants without FI.

Conclusions—Results suggest physiologic dysregulation and an immature nervous system may contribute to the phenomenon of FI in preterm infants. Further research to identify the role of the brain-gut-immune axis on FI and other GI complications in this population is warranted.

Keywords

Feeding intolerance; preterm infants; allostatic load; brain-gut-immune axis

INTRODUCTION

Feeding intolerance (FI), defined as a change in the feeding plan by decreasing or discontinuing feedings because of suspicion of enteral intolerance (Moore & Wilson, 2011), is a potentially serious complication in the first weeks of life for preterm infants. FI can be associated with necrotizing enterocolitis (NEC), one of the leading causes of morbidity and

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mortality in this population (Patel et al., 2015). Thus, the occurrence of FI remains a concern for care providers in the neonatal intensive care unit (NICU).

Researchers have examined FI as a stress-related disease of the gastrointestinal (GI) tract using the theoretical model of *Allostatic Load and Complications of Prematurity* (Moore et al., 2014). This model posits that complications of prematurity, such as FI, are associated with physiologic dysregulation from chronic stress. The model was tested in a study in which it was found that infants with FI were more likely to have dysregulation of stress biomarkers (cortisol and oxidative stress) in the first weeks of life (Moore et al., 2013). Specifically, infants with FI had lower levels of urinary cortisol on day 1 and higher levels of salivary cortisol on day 14. Infants with FI also had extremely high levels of oxidative stress on day 7. Results supported the theoretical model and suggested that allostatic load may be related to the development of FI in preterm infants. Results also suggested a maternal influence on physiologic dysregulation during the neonatal period and in particular, the presence of maternal inflammatory biomarkers.

FI and inflammation are related by several mechanisms. For example, intestinal mucosal inflammation associated with microbial colonization is one mechanism described in the literature that may play a role in the development of FI. In a recent review (Di Mauro et al., 2013), the interactions between microbial colonization, brain-gut-immune axis, FI, and inflammation were discussed. As the neonatal microbiota is forming, the intestinal response to bacteria is to secrete pro-inflammatory cytokines such as IL-6 and IL-4 from T-helper type 2 cells. An adaptive immune system response is the release of T-helper type 1 cells to maintain immune homeostasis; commensal bacteria such as *Bifidobacteriaceae* are believed to aid in this balanced immune response. The microbiota may also play a role in immunity and inflammation via the brain-gut-immune axis suggesting the nervous system plays a role in GI functions such as motility, secretions, and immunity. In preterm infants, the immature and underdeveloped GI tract and immature brain-gut-immune axis may exacerbate GI complications such as FI.

Another associated mechanism involving FI and inflammation is the stress response. Activation of the HPA-axis eventually leads to the release of cortisol whose mechanisms are believed to act as a GI protectant during periods of acute stress (Charmandari et al., 2005). Decreased levels of cortisol may lead to irritation and inflammation during acute periods of stress, which would lead to FI, consistent with Moore's findings reported above (Moore et al., 2013). This phenomenon has been recognized in the intensive care unit for the adult population (Marik, 2009). Critically ill adult patients show signs of FI (i.e. vomiting and lethargy) which is suspected to be the result of an underlying systemic inflammation. Specifically, the release of pro-inflammatory cytokines would suppress the HPA-axis resulting in dysregulation of cortisol. In preterm infants, similar changes in the autonomic nervous system related to allostatic load (AL) would then affect motility, inflammation, and secretions in the GI tract leading to FI (Mayer, 2000).

The occurrence of FI may adversely affect preterm infants in several realms. Although preterm infants with FI that does not progress to surgical NEC are believed to have better neurodevelopmental outcomes compared to infants with surgical NEC (Rees et al., 2007;

Tobiansky et al., 1995; Wadhawan et al., 2014), little is known about the relationship between FI in preterm infants and neurodevelopment in the first week of life. However, the *Allostatic Load and Complications of Prematurity* model (Moore et al., 2014) posits that general stress of prematurity and exposure to stress in utero negatively affect outcomes in preterm infants, including FI and neurodevelopment. Identifying relationships between FI, inflammation, and early measures of neurodevelopment may provide the basis for novel and clinically relevant assessments for NICU clinicians and staff.

The purpose of this analysis was to examine the relationship of FI in preterm infants to inflammatory markers and/or neurobehaviors in the first week of life. The specific aims were to 1) determine correlations between FI and inflammation as measured by inflammatory cytokines obtained in the first week of life; 2) determine correlations between FI and neurodevelopment in the first week of life using scores on the Neurobehavioral Assessment of the Preterm Infant (NAPI) (Constantinou et al., 2005).

METHODS

This secondary analysis used a retrospective, matched case-control design with data drawn from 114 infants born at 32 weeks gestation who participated in a larger study (Pickler et al., 2015). Infants with FI were matched on gestational age at birth (GA), birthweight, gender, race, and intrauterine growth restriction (IUGR). Participants were recruited to the study in the first day of life and exclusion criteria included no known gastrointestinal, craniofacial, cardiovascular, neuromuscular, and/or genetic defects. The study was approved by the institutional review board and parents of all infants provided written informed consent.

The theoretical framework used for the analysis was the model of *Allostatic Load and Complications of Prematurity* (Moore et al., 2014). The model, described above, suggests that general stress of prematurity leads to physiologic dysregulation and complications of prematurity from the ‘wear and tear’ of chronic dysregulation. The complication measured in this analysis was FI, which was defined as having occurred when an infant required discontinuation of feedings from enteral intolerance prior to achievement of full enteral feedings.

Inflammation was measured using serum cytokines (IL6, TNFa, CGSF, MCP1, IL8, IL10, GMCSF, and IL1ra) obtained from blood samples taken during the first week of life. The specific cytokines were chosen for their known association with neurodevelopmental outcomes in preterm infants (Dammann & O’Shea, 2008; Kaukola et al., 2006). The timing of the blood sample was made with consideration that the early presence of inflammatory substances would be associated with poorer outcomes (Kaukola et al., 2006). Analyses were completed using a Bio-Plex (Bio-Rad, Hercules, CA, USA) multiplex suspension array system capable of measuring up to 100 different analytes simultaneously in small (12-μL) amounts of fluid in 1 well of a 96-well plate. Plasma and lymphocytes were separated using standard Ficoll gradient methods, and samples were divided into aliquots, frozen, and stored at -70 °C for batch assays. The assay accurately measured cytokine values in the range of 1–

2500 pg/mL with an intra-assay coefficient of variability (CV) < 10 %, interassay CV < 15 %) and showed less than 1 % cross-reactivity among cytokines or with other molecules.

Neurobehavior was measured using the NAPI, which assesses eight domains of neurobehavior: scarf sign, motor development and vigor, popliteal angle, alertness/ orientation, irritability, cry quality, dominant behavior state, and number of state changes (Constantinou et al., 2005). It is a non-intrusive, objective assessment with construct validity; scores have been associated with neonatal morbidity. The NAPI was administered with the infant in an incubator and could be administered if the infant was receiving oxygen. Assessments took less than 30 minutes and were done before feeding using a standardized format. Most of the exam is observational and did not require handling the infant. The NAPI was administered at by an experienced NAPI examiner.

Descriptive and statistical analyses were completed using SPSS version 19 (SPSS Inc, Chicago, IL). A plot analysis was done to compare NAPI scores and inflammatory cytokines for infants with FI and the matched control group. For comparison of inflammatory cytokines between the infants with FI and the matched control group, cytokines were distributed into interquartiles (25%-50%-25%). Interquartile distributions were compared between the two groups using chi-square testing.

RESULTS

Eight infants (14%) from the larger study sample of infants included in this analysis had FI. Table 1 displays the demographic and medical variables for the complete sample, infants without FI from the larger sample (n=106 includes 8 case-controls), infants with FI (n=8), and the case-control group of infants without FI (n=8). Compared to the larger sample, infants with FI had lower GA at birth ($p=0.001$), lower birthweight ($p=0.001$), higher morbidity as measured by the Neonatal Morbidity Index (NMI) (Korner et al., 1993) ($p=0.048$), and received a smaller percentage of breastmilk (BM) ($p=0.026$). However, there were no significant differences in these demographic or medical variables between the FI group and the case-control group.

Table 2 shows the cytokine values and NAPI scores for the larger sample, infants with FI, and the case-control group of infants without FI. Infants with FI had significantly higher mean IL-6 levels (81.8 pg/ml) compared to infants without FI in the larger sample (23.4 pg/ml) ($p<0.05$). The infants with FI also had significantly lower NAPI scores for scarf sign, motor development and vigor, popliteal angle, and quality of crying compared to infants without FI in the larger sample ($p<0.05$). These differences were not seen in the comparison between infants with FI and the case-control infants. However, plot analysis of cytokine levels between the two groups of infants showed infants with FI had noticeably higher levels of TNF- α and lower levels of GCSF and MCP1 (Figures 1a and 1b). Interquartile distributions of cytokines between infants with FI and the case-control group showed a significant difference in IL-6 levels. Specifically, infants with FI were more likely to be distributed in the extreme quartiles of IL-6 compared to infants without FI in the case-control group (Figure 2). Plot analysis also showed lowered NAPI scores across all domains for infants with FI (Figure 3).

DISCUSSION

We studied the relationships between FI and measures of inflammation and neurobehaviors obtained in the first week of life in a sample of preterm infants. Comparisons were completed between infants with FI and both the larger sample from which the data were obtained, as well as a case-control group from that larger sample. Differences were found in infants with FI and both the larger and case-control sample, although differences between the latter were qualitative rather than statistically significant.

The differing levels of cytokines between infants without and with FI support the theoretical model used for this study. Dysregulation in inflammatory markers was noted for all infants in the sample. However, the dysregulation in the sample of infants with FI and in the matched case-control sample was more apparent. Specifically cytokine values were higher in both the FI and the case-controls than were the values seen in the larger sample; both the FI and case-control groups were smaller and born at earlier gestational ages than the larger sample, perhaps representing an inability to respond appropriate to infectious or antigenic substances. For infants with FI, the dysregulation was again greater, although not significantly different from the case-controls. Lower levels of pro-inflammatory cytokines (GCSF, GMCSF, and MCP1) in infants with FI compared to infants without FI in the case-control group were noted while higher values were noted for other pro-inflammatory cytokines (IL6, TNF α , IL8), although only IL-6 was significantly different when examined by quartiles. Previous reports have shown an increase IL-6 with necrotizing enterocolitis (NEC), but few studies have identified IL-6 levels of infants with FI. DiMauro and colleagues (2013) stated the immune system of the neonate is believed to show bias towards the T-Helper type 2 response related to colonization of microbiota which would lead to the secretion of IL-6. The dysregulated levels of IL-6 in infants with FI in our study compared to the case-control sample suggest an inadequate and/or over-exaggerated response to microbial colonization by the immature immune system in preterm infants. It should be noted that infants with non-surgical NEC were included in the group of infants with FI. The dysregulated relationship with both the upper and lower quartiles of IL-6 and FI may be a reflection of varying pathways resulting in the phenomenon of FI. For example, the infants with NEC may have an inadequate response to colonization of microbiota resulting in lower levels of IL-6 while infants without NEC may have an over-exaggerated response resulting in higher levels of IL-6. Essentially, the dysregulation of IL-6, either low or high, would result in symptoms of FI. Infants with FI also had higher levels of IL10 but lower levels of IL1ra, both anti-inflammatory cytokines, again suggesting dysregulation although also perhaps an attempt to regain homeostasis from various pathways.

The role of stress and inflammation may play a role in the dysregulation of cytokines in our analysis. Research findings are inconsistent regarding the relationship between IL-6 and cortisol. Some researchers suggest IL-6 and cortisol are positively correlated (Steensberg et al., 2003) while others suggest an inverse relationship exists between the two (Gawuga et al., 2012). In a review of the effects of stress on the GI tract, Mayer et al (2000) stated that the activation of the sympathetic nervous system stimulates the release of IL-6 which then activates the HPA axis. This suggests a positive relationship between IL-6 and cortisol. Moore (2013) found FI was related to lower levels of cortisol on Day 1, a finding consistent

with this analysis, suggesting a dysregulation of the sympathetic nervous system leads to decreased immune and HPA-axis function leading to an increased risk of complications. Mayer and colleagues (2000) also stated that allostatic load and dysregulation of the nervous system plays a role in secretion, motility, and inflammatory responses in the GI tract. The role of the brain-gut-immune axis in structure and function of the GI tract may explain the effects of a disruption in the sympathetic nervous systems and complications related to the GI tract, such as FI.

Our findings showing lower NAPI scores for preterm infants with FI compared to the matched control group of preterm infants without FI are consistent with our findings that suggest dysregulated immune function. Infants with FI may have an immature and underdeveloped nervous system that would affect their brain-gut-immune axis thus leading to more immature neurobehaviors. In particular, the elevation of certain cytokines are associated with abnormal development, including IL6, IL8, and TNF α (Cordeiro et al., 2015). However, much remains unclear about the role of cytokines in human development and particularly with their predictive value. The dysregulated levels of IL-6 suggest varying inflammatory mechanisms may be associated with FI and/or stress response. Further research on the role of IL-6 in FI is necessary to understand the physiologic mechanisms associated with this complication. The results of this analysis suggest linkages among feeding intolerance, inflammation, and neurodevelopment. Additional research is required however, before we can conclude the veracity of these relationship or recommend the use of one measure as a predictor of preterm infant outcomes.

This study was a secondary analysis therefore the definition of FI was not identified a priori and data about FI were obtained from the medical record after discharge. Another limitation of the study is the small number of infants with FI for comparison analyses. Differences in cytokines and NAPI scores may have been insufficiently powered to detect significance thereby making it difficult to interpret and identify conclusions.

CONCLUSION

We conducted an analysis to identify relationships between FI, inflammation, and neurodevelopment. Infants with FI were more likely to have dysregulated levels of IL-6 and lower neurodevelopmental scores compared to infants without FI. Results are consistent with theoretical model *Allostatic Load and Complications of Prematurity* and suggest physiologic dysregulation and an immature nervous system may contribute to the phenomenon of FI in preterm infants. Further research to identify the role of the brain-gut-immune axis on FI and other GI complications in this population is warranted.

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Figure 1.

Plot analysis of cytokine values (pg/ml) and the incidence of feeding intolerance. Figure 1a: cytokine levels ranging less than 500pg/ml for infants without/with feeding intolerance. Figure 1b: cytokine levels ranging greater than 500pg/ml for infants without/with feeding intolerance. Feeding intolerance defined as a feeding withheld, discontinued, or decreased at any time because the infant was not tolerating enteral feedings by full enteral feedings.

Figure 2.

Interquartile distributions of IL6 with the incidence of feeding intolerance. Feeding intolerance defined as a feeding withheld, discontinued, or decreased at any time because the infant was not tolerating enteral feedings by full enteral feedings. Infants with feeding intolerance were more likely to be distributed in the extreme quartiles of IL-6 compared to infants without feeding intolerance ($p=0.01$).

Figure 3.

Plot analysis of scores from the Neurobehavioral Assessment of the Preterm Infant (NAPI) with the incidence of feeding intolerance. SS: scarf sign; MDV: motor development and vigor; PA: popliteal angle; AO: alertness/orientation; IRR: irritability; QC: quality of cry; DS: dominate behavior state; SC: number of state changes. Feeding intolerance defined as a feeding withheld, discontinued, or decreased at any time because the infant was not tolerating enteral feedings by full enteral feedings.

Table 1

Demographic differences among the larger sample, infants without FI in the larger sample, infants with FI, and case-control infants without FI.

	Sample (n=114)	Infants without FI (n=106)	Infants with FI (n=8)	Case-Control Infants Without FI (n=8)
Gestational Age at Birth (weeks)	29.7 (2.3)	29.9 (2.2) *	27.0 (2.2)	26.9 (1.9)
Birthweight (grams)	1299.1(401.4)	1332.8 (391.0) *	852.6 (251.9)	931.9 (258.7)
APGARS				
1-minute	5.9 (2.6)	5.9 (2.5)	4.8 (3.3)	4.1 (2.9)
5-minute	7.7 (2.1)	7.8 (2.0)	6.4 (2.9)	7.9 (1.0)
Gender				
Male	58	56	2	2
Female	56	50	6	6
Ethnicity (n=111)				
Non-Hispanic	107	99	8	7
Hispanic	4	4	0	1
Race				
White	65	61	4	6
African American	43	40	3	2
More than 1 Race	6	5	1	1
IUGR				
Yes	12	11	1	1
No	102	95	7	7
Maternal Age (years)	28.4 (6.2)	28.3 (6.0)	29.1 (8.9)	32.0 (6.5)
Maternal Steroid Use (n=113)				
Yes	91	85	6	7
No	22	21	1	1
Chorioamnionitis (n=97)				
Yes	32	30	2	4
No	65	61	4	3
NMI	2.8 (0.6)	2.8 (0.6) *	3.3 (0.7)	2.8 (0.5)
Gestational Age at Full Enteral Feedings	31.1(2.2)	31.2 (2.1)	30.5 (2.6)	28.4 (2.0)
Percent of Feeding Breastmilk	57.2% (37.8%) (n=101)	59.1% (37.4%) * (n=96)	20.6% (29.4) (n=5)	52.7% (44.7%) (n=7)

	Sample (n=114)	Infants without FI (n=106)	Infants with FI (n=8)	Case-Control Infants Without FI (n=8)
NEC	2	0	2	0

*
<0.05 between infants without/with FI in larger sample

Table 2

Cytokine and NAPI[†] differences for the larger sample, infants without FI in the larger sample, infants with FI, and case-control infants without FI^{††}

	Sample (n=114)	Infants without FI (n=106)	Infants with FI (n=8)	Case-Control Infants Without FI (n=8)
Cytokines (pg/ml) (n=73) Mean (SD)				
IL-6	27.5 (46.7)	23.4 (35.8) *	81.8 (115.8)	70.4 (79.1)
TNF-a	38.8 (19.8)	37.7 (18.5)	53.5 (32.1)	51.0 (16.0)
GCSF	343.8(912.1)	329.9 (922.3)	528.3 (831.2)	1353.2 (2577.6)
MCP1	2265.5 (2599.9)	2288.9(2626.6)	3429.5 (4011.9)	4970.0 (3970.4)
IL-8	141.2 (228.9)	127.8 (186.9)	317.4 (554.7)	184.0 (134.8)
IL-10	10.7 (17.9)	9.8 (16.4)	23.3 (32.2)	22.7 (33.7)
GMCSF	7.2 (5.5)	6.9 (4.8)	10.7 (11.9)	12.2 (9.0)
IL1ra	327.9 (571.2)	310.4 (542.8)	558.6 (922.8)	790.8 (792.6)
NAPI Scores (n=112-114) Mean (SD)				
Scarf Sign	37.1 (17.1)	38.1 (17.0) *	25.0 (15.4)	33.3 (25.2)
Motor Development and Vigor	35.7 (14.1)	36.4 (14.4) *	26.5 (4.1)	30.1 (22.5)
Popliteal Angle	43.0 (20.0)	43.8 (20.5) *	33.3 (0.0)	41.7 (15.5)
Alertness and Orientation	21.7 (13.0)	21.9 (12.9)	19.6 (15.1)	23.1 (12.8)
Irritability	40.0 (28.3)	40.6 (27.8)	31.1 (34.2)	37.5 (26.7)
Quality of Crying	31.1 (32.9)	32.5 (33.1) *	12.5 (23.1)	18.8 (25.9)
Dominate State	4.3 (1.2)	4.4 (1.2)	3.8 (1.1)	3.9 (1.3)
State Change	4.4 (2.1)	4.5 (2.0)	3.6 (2.5)	4.9 (1.8)

[†] Neurobehavioral Assessment of the Preterm Infant

^{††} Case-control group of infants matched on gestational age at birth, birthweight, gender, race, intrauterine growth restriction

* p<0.05 between infants with/without FI in whole sample