Ischemia-Modified Albumin May be a Novel Marker for the Diagnosis and Follow-up of Necrotizing Enterocolitis

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Aim: We investigate the efficacy of serial ischemia-modified albumin (IMA) measurements in diagnosis and follow-up of necrotizing enterocolitis (NEC), and compare its effectiveness with C-reactive protein (CRP), interleukin-6 (IL-6), in NEC. **Methods:** Preterm infants, whose gestational age and weight matched each other, were grouped as control ($n = 36$) and NEC ($n = 37$). IMA, CRP, IL-6 levels were measured on the third day of life for the control group and on the day of diagnosis (first day), third, and seventh days of NEC. **Results:** IMA, CRP, and IL-6 levels were significantly increased in NEC patients compared to the control group ($P < 0.001$) on the follow-up. IMA levels were significantly higher in infants with stage-III NEC than those in in-

fants with stage-II NEC on the first, third, and seventh days ($P < 0.001$). The area under curve for IMA (0.815 at diagnosis, 0.933 at the third day, 0.935 at the seventh day) were significantly higher than CRP and IL-6 at all days for predicting perforation in infants with NEC ($P < 0.001$). Similarly, the area under curve for IMA (0.952 at diagnosis, 0.929 at the third day, 0.971 at the seventh day) was significantly higher than CRP and IL-6 at all consequent days of diagnosis for predicting mortality in infants with NEC ($P < 0.001$). **Conclusion:** Ischemia-modified albumin was found to be superior to CRP and IL-6 in both diagnosis and follow-up of NEC. J. Clin. Lab. Anal. 28:170–177, 2014. © 2014 Wiley Periodicals, Inc.

Key words: necrotizing enterocolitis; ischemia-modified albumin; newborn; preterm; diagnosis

INTRODUCTION

Necrotizing enterocolitis (NEC) is the most common life-threatening disease of the gastrointestinal (GI) tract in the newborn period. The incidence of NEC is 1–5% of infants in neonatal intensive care units. Nearly 90% cases of NEC occur in premature infants. Very small, ill preterm infants are particularly more susceptible to NEC. A rising incidence of NEC may reflect improved survival of this high-risk group of infants. As both incidence and case fatality rates increase with decreasing birthweight and gestational age (1–3). The cause of NEC remains unclear, and most likely believed to be multifactorial. Although many factors may contribute to the development of NEC, the greatest risk factor for NEC is prematurity. This devastating disorder probably results from an interaction be-

tween loss of mucosal integrity due to a variety of factors (hypoxia-ischemia, infection, inflammation) and the host's response to that injury (circulatory, immunologic, inflammatory), leading to necrosis of the affected bowels. Infants with NEC have a variety of signs and symptoms and may have an insidious or sudden catastrophic onset (1–4). The first sign of this impending disease may be nonspecific including lethargy and temperature

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instability, or related to GI pathology. Progression of NEC may be rapid resulting in a mortality rate of 10–50%, in addition to morbid sequelae including impaired growth, short bowel syndrome, prolonged neonatal hospitalization, and poor long-term neurodevelopment (5–7).

In spite of advances in neonatal medicine, diagnosis of NEC remains a major challenge. Initial clinical signs are nonspecific and the laboratory findings are not properly confidential. Hence, its late occurrence after birth, its rapid onset, highly fulminant nature, and progression to death as well as its severe morbidity require to ascertain new markers specific for diagnosis and early intervention of NEC (8). Recent studies have determined efficacy of some inflammatory mediators such as C-reactive protein (CRP), interleukin-6 (IL-6), serum amyloid A, and complement 5a measurement in diagnosis of NEC (8,9). However, none of these parameters can adequately predict NEC. Therefore, a reliable marker is awaited in clinical practice to detect high-risk infants for NEC in order to prevent perinatal complications and related mortalities in time.

Ischemia-modified albumin (IMA) is known to be a modification of human serum albumin (HSA). Normally, N-terminal amino acids of HSA transitory connect to transitional metals such as cobalt, nickel, and copper. However, this region of HSA changes due to hypoxia-ischemia or acidosis which leads to reduction in the binding capacity of HSA to those metals. This changed HSA is called IMA (10). Some current clinical and experimental studies have suggested that IMA may be used for the early diagnosis of myocardial ischemia (11), cerebrovascular accidents (12), acute mesenteric ischemia (13, 14). Moreover, a few studies have determined that IMA can be used as a marker in perinatal asphyxia (15) and severe fetal hypoxia (16) in the newborn period. However, data about the value of IMA in the diagnosis and follow-up of NEC are limited. In the present study, we therefore investigated the efficacy of serial IMA measurements in diagnosis and follow-up of NEC, and compared its effectiveness with CRP, IL-6 in NEC.

MATERIALS AND METHODS

This study was conducted from January 1, 2011 to February 1, 2012. Infants with ≤ 32 weeks of gestation, $\leq 1,500$ g of birth weight were enrolled in the study. Local Ethics Committees approved the study, and informed consents were obtained from the parents. Gestational age was determined by clinical data and a first trimester ultrasound scan obtained from the obstetrics and gynecology department records. Modes of delivery (by normally vaginal delivery (NVD) or cesarean section (C/S)), gender, birth weight, APGAR scores at first

and fifth minutes and resuscitation requirements were recorded. Mechanical ventilation therapy, exchange transfusions, neural tube defects, and congenital heart diseases were recorded before the diagnosis of NEC.

During the treatment, clinical symptoms such as prolonged episodes of apnea and desaturation, bradycardia, lethargy, irregular body temperature, feeding intolerance, vomiting; and findings such as increased gastric residual volume ($>20\%$), bilious or bloody gastric aspirate, decreased bowel sounds, bloody stools, abdominal distention and tenderness, and abdominal wall skin color changes were evaluated. For infants with two or more of these symptoms/findings (infants with NEC frequently have these early GI symptoms), laboratory and radiographic evaluations were done. Abnormal findings on abdominal radiographs including intestinal dilatation, the presence of dilated and fixed bowel loops, thickened intestinal wall, ascites, pneumatosis intestinalis, air in the portal vein, pneumoperitoneum, and free air after perforation were evaluated by a radiologist. Laboratory studies including complete blood count, blood smear, levels of IL-6 and CRP, serum electrolytes, blood urea nitrogen, creatinine, liver function tests, urinalysis, and blood cultures were evaluated. Diagnosis of NEC was determined according to clinical and radiological findings, and modified Bell criteria were used for staging of the disease as stages I, II, and III (17). According to this staging system, stage-I (suspicious) NEC was not taken into account; and only patients with true NEC at stage II (mild-to-moderate NEC) and stage III (advanced NEC) were included. A single team of specialists including pediatric surgeons and neonatologists was formed to take care of the patients. The same team made the decision for surgical intervention, if necessary. Findings of pneumoperitoneum and/or necrotic bowel segments on serial radiographs, which displayed fixed intestinal loops in addition to persistent metabolic acidosis, shock, and persistent severe thrombocytopenia, were recognized as the criteria for surgical intervention. During the surgical intervention, the macroscopic assessment of the abdomen showed various degrees of NEC signs, including necrotic changes, pneumatosis intestinalis, fragility, decreased tissue integrity, edema, and discoloration. After surgical intervention, histopathological evaluation of the excised intestinal tissues was performed for the findings of NEC.

Patients diagnosed with true NEC were defined as the study group, and blood samples were taken from these patients and collected in serum test tubes (Minicollect[®] 1cc, Grenier Bio-one, Kremsmünster, Austria) for evaluation of CRP, IL-6, and IMA serum levels at the time of diagnosis (first day), the third day, and seventh days after the diagnosis. Infants with ≤ 32 weeks of gestation and $\leq 1,500$ g of birth weight, and without signs of NEC were included as the control group, and their blood samples

were collected for the evaluation of IMA, CRP, and IL-6 serum levels at the third day of life.

Serum CRP levels were measured with the nephelometric method (sensitive value = 0.8 mg/dl; CRP kit, Roche, Germany; IMAGE device, the Beckman-Coulter). IL-6 levels were measured with solid phase enzyme labeled chemiluminescent immunometric assay (IL-6 kit, Siemens Healthcare Products, Ltd., Hanbers; sensitivity value = 2 pg/ml; Immulite 2000 device), and values were recorded. Blood IMA levels were detected by a commercially available ELISA kit (USCN Life Science, Wuhan, China), and detection was performed according to the manufacturer's instructions. The absorbance was measured at 450 nm using Bio-Tek ELX 800 absorbance microplate reader (Bio-Rad) and Bio-Tek ELx50 microplate auto strip washer (Bio-Rad). The results were expressed in picmoles per milliliter (pmol/ml).

Statistical Analysis

Statistical analysis was performed by SPSS 15.0 statistical package program (Chicago, IL). The normal distribution of variables was tested with Shapiro–Wilk test. Descriptive statistics were provided as mean and standard deviation or median interquartile range (IQR), and categorical variables were provided as values and percent. Mann–Whitney *U*-test was used for intergroup comparisons of nonparametric variables. To compare dependent groups, Friedman's test and Wilcoxon's test were used. Kruskal–Wallis test was used for comparison of the three groups. Correlation was performed by Spearman's correlation analysis. Logistic regression analysis was used to predict perforation and mortality risk. Receiver-operating characteristics (ROC) analysis was performed to display the sensitivity and specificity of CRP, IL-6, and IMA for intestinal perforation and death. Values of $P < 0.05$ were considered significant.

RESULTS

In the present study, 37 patients were diagnosed as NEC, and included as the study group. Thirty-six patients without NEC symptoms were enrolled as the control group. There were no significant differences between the study and control groups in terms of gestational age, modes of delivery gender, birth weight, gestational age, APGAR scores, need for resuscitation, antenatal steroid treatment, maternal corioamnionitis, and mechanical ventilation therapy ($P > 0.05$). Twenty patients (54%) had stage-II NEC and 17 patients (46%) had stage-III NEC. There were no significant differences between NEC stages in terms of demographic variables ($P > 0.05$).

During the follow-up, 13 patients (76.5%) with stage-III NEC had intestinal perforation and underwent sur-

gical intervention. Additionally, 11 patients (64.7%) of stage-III NEC died. No significant differences were determined between infants who survived and died related to demographic variables ($P > 0.05$). However, birth weight of infants who died [980 (260) g] was lower than the infants who survived [1,235 (195) g] ($P < 0.05$). There were no statistically significant differences between patients with and without surgical treatment in terms of demographic variables ($P > 0.05$), except gestational age. Gestational age of infants requiring surgical intervention (26.4 ± 2.1 vs. 28.2 ± 2.7 weeks) was less than other infants ($P < 0.05$). Furthermore, mortality rate of these infants was found to be significantly higher than other infants ($P < 0.05$).

The serum levels of IMA, CRP, and IL-6 determined at the first, third, and seventh days of the diagnosis in the NEC group ($P < 0.001$) were significantly higher than control group, except serum albumin levels (control: 3.1 ± 0.8 gr/dl, NEC: 3.0 ± 0.6 gr/dl; $P < 0.05$) at the diagnosis. Moreover, IMA serum levels were found to be different on each days—at diagnosis, third and seventh days of NEC. CRP and IL-6 levels were high at the diagnosis and displayed increments at third and seventh days of NEC compared to first day (Table 1).

There were no significant differences between infants with stages II and III of NEC in terms of CRP and IL-6 serum levels at the diagnosis (first day), the third day, and the seventh days of the diagnosis ($P > 0.05$). No significant differences were found for serum albumin levels between stage II (2.9 ± 0.6 gr/dl) and III (2.8 ± 0.8 gr/dl) NEC at the diagnosis ($P < 0.05$). However, significant differences were found between infants with stages II and III of NEC cases related to IMA levels at the first, third, and seventh days. Additionally, it was determined that IMA levels had increments on the consequent days in infants with stages II and III of NEC, and these increments were higher particularly in infants with stage III NEC. CRP levels showed an increase from day 0 to day 7 both in stage II and stage III. Serum IL-6 levels increased from the first, third, and seventh days and stayed higher in infants with all NEC stages, these increments of IL-6 were higher in infants with stage-III NEC (Table 2).

When compared, no significant differences were found related to IL-6 between operated and unoperated infants at diagnosis. No significant differences were found for serum albumin levels, operated and unoperated, (2.8 ± 0.5 gr/dl, 3 ± 0.3 gr/dl, respectively) at the diagnosis ($P < 0.05$). However, CRP and IMA levels were found to be significantly higher in infants needing surgical intervention at diagnosis. Additionally, it was shown that IMA and IL-6 levels were significantly higher in infants who required surgical intervention at the third day of NEC. Infants with surgical intervention had higher levels of IMA

TABLE 1. Representing Comperation Between Crp, Il-6, and Ima Serum Levels at the Diagnosis (Day 0) and the Third Day, the Seventh Days of the Diagnosis in Nec Group and Crp, Il-6, and Ima Serum Levels at the Third Day of Life in Control Group

Variables	Control mean (IQR)	NEC day 0 mean (IQR)	NEC third day mean (IQR)	NEC seventh day mean (IQR)	P
IMA (pmol/ml)	176 (43.6)	268.13 (84.55)	278.68 (68.72)	223.45 (115.85)	<0.001 ^a
IL-6 (pg/ml)	18.60 (20.4)	78.4 (136.18)	133 (123.75)	234.5 (206.55)	<0.001 ^b
CRP (mg/l)	2.68 (1.24)	25.3 (21.9)	39.4 (28.4)	52.5 (31.25)	<0.001 ^c

^aSignificant differences among all days of IMA levels between NEC and control group.

^bSignificant differences among all days of IL-6 levels between NEC and control group.

^cSignificant differences among all days of CRP levels between NEC and control group.

and CRP at the seventh day of NEC (Table 3) Furthermore, we have evaluated infants who died and survived in terms of serum CRP, IL-6, and IMA levels. Higher levels of IMA were determined in dead infants than those who survived at all consequent days, in addition to higher levels of CRP at diagnosis and higher levels of IL-6 at the third day of NEC (Table 4). No significant differences were found for serum albumin levels between died and survived infants (2.9 ± 0.4 gr/dl, 2.8 ± 0.7 gr/dl, respectively) at the diagnosis ($P < 0.05$).

Logistic regression analysis was performed for the CRP, IL-6, and IMA serum levels as a predictor of surgery re-

quirement and death. The AUC for IMA (0.815 at diagnosis, 0.933 at the third day, 0.935 at the seventh day) was significantly higher than CRP and IL-6 at all days for predicting perforation in infants with NEC ($P < 0.001$; Table 5; Fig. 1). Similarly, the AUC for IMA (0.952 at diagnosis, 0.929 at the third day, 0.971 at the seventh day) were significantly higher than CRP and IL-6 at all consequent days of diagnosis for predicting mortality in infants with NEC ($P < 0.001$; Table 6; Fig. 2). Moreover, a positive correlation was found between IMA and IL-6 ($\text{Rho} = 0.862$, $P = 0.012$), and CRP ($\text{Rho} = 0.785$, $P = 0.016$).

TABLE 2. Indicating Comparison Between Stages II and III of NEC in Terms of CRP, IL-6, and IMA Levels for Each Day

Variables	NEC stage II (n = 20)	NEC stage III (n = 17)	P
NEC 0 day	IL-6 (pg/ml)	67.5 (157.8)	0.616
	CRP (mg/l)	34 (15.1)	0.721
	IMA (pmol/ml)	224.89 (63.84)	<0.001 ^a
NEC third day	IL-6 (pg/ml)	129.8 (80.75)	0.177
	CRP (mg/l)	41.45 (33.7)	0.811
	IMA (pmol/ml)	261.66 (57.5)	<0.001 ^a
NEC seventh day	IL-6 (pg/ml)	234.75 (259.5)	0.895
	CRP (mg/l)	46.95 (33.2)	0.246
	IMA (pmol/ml)	189.72 (66.17)	<0.001 ^a

^aSignificant differences among all days of IMA levels between stage II and III NEC groups.

TABLE 3. Representing Comparison Between Operated and Unoperated Infants With NEC in Terms of CRP, IL-6, and IMA Levels for Each Day

Variables	Operated (n = 13)	Unoperated (n = 24)	P
NEC 0 day	IL-6 (pg/ml)	124 (615.3)	0.297
	CRP (mg/l)	16.2 (15.5)	0.083
	IMA (pmol/ml)	303.7 (78.1)	<0.001 ^a
NEC third day	IL-6 (pg/ml)	244 (393.1)	<0.001 ^c
	CRP (mg/l)	47 (26)	0.112
	IMA (pmol/ml)	309.65 (30.29)	<0.001 ^a
NEC seventh day	IL-6 (pg/ml)	286 (111)	0.068
	CRP (mg/l)	65.7 (41)	0.017 ^b
	IMA (pmol/ml)	298.4 (30.79)	<0.001 ^a

^aSignificant differences among all days of IMA levels between stage II and III NEC groups.

^bSignificant differences of CRP levels between operated and unoperated at seventh day of NEC.

^cSignificant differences of IL-6 levels between operated and unoperated at the first day of NEC.

TABLE 4. Representing Comparison Between Died and Survived Infants of NEC in Terms of CRP, IL-6, and IMA Levels for Each Day

Variables		Died infants (<i>n</i> = 11)	Survived infants (<i>n</i> = 26)	<i>P</i>
NEC 0 day	IL-6 (pg/ml)	101.2 (615.3)	68 (143.05)	0.351
	CRP (mg/l)	31.5 (21.55)	16.2 (11.43)	0.034^b
	IMA (pmol/ml)	297.59 (50.13)	238.09 (61.31)	<0.001^a
NEC third day	IL-6 (pg/ml)	244 (377.35)	129.6 (68)	0.006^c
	CRP (mg/l)	41.9 (28.4)	36.9 (39.7)	0.545
	IMA (pmol/ml)	289.16 (31.8)	261.66 (58.16)	<0.001^a
NEC seventh day	IL-6 (pg/ml)	234.3 (170)	234.75 (251)	0.650
	CRP (mg/l)	64.7 (46)	48 (33.2)	0.177
	IMA (pmol/ml)	301.25 (34.25)	189.72 (60.08)	<0.001^a

^aSignificant differences among all days of IMA levels between stage II and III NEC groups.

^bSignificant differences of CRP levels between died and survived infants at the first day of NEC.

^cSignificant differences of IL-6 levels between died and survived infants at the first day of NEC.

DISCUSSION

In the current study, we have evaluated the efficacy of IMA in the diagnosis and follow-up of NEC in premature infants, and compared its effectiveness with CRP, IL-6, during the course of NEC. The results showed that IMA levels were significantly higher in infants with diagnosed NEC than those in infants in the control group. And also, it was indicated that IMA levels were significantly higher

in infants with stage-III NEC than those in infants with stage-II NEC at the first, third, and seventh days. Although it was determined that IMA levels had increments on the consequent days in infants with stage II and III of NEC, these increments were higher especially in infants with stage-III NEC. During the course of NEC, some infants, particularly with stage-III NEC, underwent surgical intervention. Our results determined that IMA levels were

TABLE 5. Representing Prediction of Surgery Requirement

Variables		Cut off values	Sensitivity	Specificity	AUC	<i>P</i>	95% Confidence interval lower bound-upper bound	
NEC day 0	IMA	252.57	89.5	64.0	0.815	<0.001	0.673	0.956
	CRP	16.05	57.9	33.0	0.346	0.084	0.181	0.511
	IL-6	73.20	63.2	56.0	0.593	0.297	0.418	0.767
NEC third day	IMA	278.68	94.7	84.0	0.933	<0.001	0.848	1.017
	CRP	33.75	68.4	48.0	0.641	0.112	0.476	0.807
	IL-6	136.50	84.2	80.0	0.839	<0.001	0.707	0.971
NEC seventh day	IMA	268.00	94.7	92.0	0.935	<0.001	0.854	1.016
	CRP	61.55	68.4	76.0	0.713	0.017	0.553	0.872
	IL-6	231.00	78.9	52.0	0.662	0.068	0.499	0.825

AUC: Area under the curve.

Significant differences among all days of IMA levels between stage II and III NEC groups.

Fig. 1. ROC curves of infants required surgical intervention. Indicating the Area under the curve (AUC) for CRP, IL-6, and IMA at the diagnosis, third and seventh day of NEC.

TABLE 6. Indicating Prediction of Died Infants

Variables		Cut off values	Sensitivity	Specificity	AUC	P	Confidence interval 95(%) lower bound-Upper bound	
NEC day 0	IMA	294.91	92.9	96.7	0.952	<0.001	0.885	1.020
	CRP	16.05	57.1	36.7	0.300	0.034	0.143	0.457
	IL-6	51.80	85.7	40.0	0.588	0.351	0.407	0.769
NEC third day	IMA	293.29	92.9	83.3	0.929	<0.001	0.852	1.005
	CRP	33.75	71.4	46.7	0.557	0.545	0.381	0.734
	IL-6	162.10	78.6	73.3	0.757	0.007	0.584	0.930
NEC seventh day	IMA	277.46	92.9	93.3	0.971	<0.001	0.930	1.012
	CRP	52.50	71.4	60.0	0.627	0.178	0.446	0.809
	IL-6	171.00	85.7	40.0	0.543	0.650	0.374	0.712

AUC: Area under the curve.

Significant differences among all days of IMA levels between stage II and III NEC groups.

higher in infants with surgical intervention than those in unoperated infants at all consequent days, in addition to higher levels of IL-6 at the third day and higher levels of CRP at the seventh day. Furthermore, higher levels of IMA were determined in dead infants than those in survived infants at all consequent days with higher levels of CRP at diagnosis and higher levels of IL-6 at the third day of NEC. IMA levels were evaluated for the prediction of the infants with perforation and death. It was found that IMA levels were significantly higher than CRP and IL-6 levels at all time points of diagnosis. Additionally, IMA was found to be superior to others for predicting surgery and death in terms of sensitivity, specificity, and AUC. Therefore, IMA may be a useful marker for the prediction of patients with possible surgery requirement and death. Moreover, a positive correlation was found between IL-6, CRP, and IMA. Since IMA increased parallel to both CRP and IL-6 during the course of NEC, it can be used for predicting the severity of NEC, surgery requirement and death with a high sensitivity and specificity. We concluded that IMA might be suggested as a useful marker in the early diagnosis of NEC in combination with others.

The mechanism of IMA generation remains unexplained. It has been suggested that IMA is a modifi-

cation of serum albumin, and it results from oxidative stress and concurrently produced superoxide-free oxygen radicals that occur during ischemic events, regardless of tissue specificity (16, 18–21). Recently, some studies have reported that IMA has a relationship with various ischemia-related conditions, such as acute coronary syndrome, ischemia of liver, brain, kidney, and bowel in adults (11, 13, 21). Hypoxic-ischaemic injury to the GI tract is an important contribution and potentially inciting factor in the development of NEC (1–4). In preterm infants, GI tract key functions, like the intestinal blood supply, are immature. Since there exists an imbalance in vasoconstrictor and vasodilatory regulation in the intestinal blood supply that resulted in a predisposing risk factor to ischemic injury of the premature intestine, which is recognized to be an important contributing factor in the development of NEC (22, 23). Furthermore, immature intestinal tissues are very sensitive to ischemia, which results in cellular injury and triggers a complex series of biochemical events (24). In newborn period, few studies have determined that cord blood levels of IMA are higher in perinatal asphyxia and complicated deliveries are associated with fetal distress, hypoxia, and oxidative stress in newborns (14, 15). Moreover, some clinical and experimental studies have recently indicated elevated levels of IMA in acute

Fig. 2. ROC curves of infants with severe NEC resulted in death. Indicating the AUC for CRP, IL-6, and IMA at the diagnosis, third and seventh day of NEC.

mesenteric ischemia. These studies have concluded that serum IMA levels may be used in the early diagnosis of acute mesenteric ischemia (11–13). In the present study, we evaluated IMA levels in infants with NEC as IMA levels can be used as a diagnostic marker for NEC. Our results indicated that IMA levels were significantly higher in infants with diagnosed NEC than in infants in the control group. It would be said that IMA has a diagnostic value for NEC.

The diagnosis of NEC prior to severe disease is very important for therapeutic approaches but is difficult in clinical practice. As, mortality and morbidity in infants with severe NEC are high, which causes difficulty in early diagnosis (1–5,8). Available data suggest that IMA might be generated in a number of potentially life-threatening conditions that may cause either local or generalized hypoxic circumstances. For instance, IMA has been recently proposed for early detection of myocardial ischemia without infarction as well as having a diagnostic value in acute mesenteric ischemia (12,13,21). In the present study, our findings indicated that IMA levels were significantly higher in infants with stage-III NEC than those in infants with stage-II NEC at the first, third, and seventh days. These data suggest that IMA shows the severity of NEC.

In the current study, it has been determined that IMA rapidly increases within 5–10 min after the ischemic event such as acute coronary syndrome, and remains high for 30 min; and it returns to baseline 12 hr after the ischemia event, and it has been found that if the ischemic event persists, it continues to rise (18). Some other experimental studies on acute mesenteric ischemia have determined that IMA levels increase as the duration of ischemia prolongs (12,13). Our results showed that IMA levels had increments on the consequent days in infants with stages II and III of NEC, and these increments were higher especially in infants with stage-III NEC. It may be speculated that due to severe NEC intestinal injury persisted in addition to ischemic injury. Unfortunately, some infants particularly with stage-III NEC underwent surgical intervention and were determined with higher levels of IMA at all consequent days. Moreover, higher levels of IMA were determined in died than in survivals at all consequent days. This suggests that IMA levels increase continuously with time in infants with severe NEC, and further indicates the severe morbidity such as bowel perforation and death.

During hypoxic-ischemic states, hypoxia-ischemia induces a pro-inflammatory process that induces the migration and activation of neutrophils, cytokines, and reactive oxygen species, and these are important pathogenic mediators during intestinal hypoxia-ischemia injury affecting the structure and function of intestinal cells (1–4,23–25). IL-6 is a pro-inflammatory cytokine released from mononuclear phagocytes with various stimuli. It forms a systemic inflammatory response syndrome by

neutrophil activation and endothelial intercellular adhesion molecules upregulation (26). It is known that IL-6 and CRP are rapidly acting acute-phase proteins, and IL-6 triggers the production of CRP from the liver after the onset of inflammation. It has been found that IL-6 levels increase continuously after intestinal ischemia, and these cytokines are released from Kupffer cells into the blood stream (27). Additionally, it has been demonstrated that serum IMA levels are significantly associated with elevation of some markers such as highly sensitive CRP related to inflammation and ROS produced as a result of ischemic events (28). In an experimental acute mesenteric ischemia model, a positive correlation between the blood levels of IMA and IL-6 has been determined (13). In our study, we examined the relationships between the blood levels of IMA, IL-6, and CRP. IMA levels were also evaluated for the prediction of the infants with perforation and death. We found that IMA levels were significantly higher than CRP and IL-6 levels at all time points of diagnosis. Additionally, IMA was found to be superior to CRP and IL-6 levels for predicting surgery and death in terms of sensitivity, specificity, and AUC. Therefore, IMA may have a predictive value in the detection and prevention of infants with possibly surgery requirement and death due to this devastating disorder.

In conclusion, this study investigated the efficacy of IMA in the diagnosis and follow-up of NEC in preterm infants. We suggest that IMA blood levels may be a sensitive marker for predicting the severity of NEC, surgery requirement, and death. We may accept elevated blood IMA levels as a novel, useful marker in NEC. However, to date no single marker of NEC has shown good predictive efficacy and only a combination of various indices can help in early diagnosis of NEC. Therefore, the results of the present study are still preliminary and future prospective and controlled studies including larger series are warranted.

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