Original Article

The relationship between the first episode of wheezing and matrix metalloproteinases-9 and MMP-2 and tissue inhibitors of MMP-1 levels in preterm infants

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Abstract:

AIMS: Matrix metalloproteinases (MMP) have been associated with neonatal lung morbidity and MMP dysregulation contributes to the pathology of chronic and acute lung disorders. Most of the previous studies were performed in the 1st weeks of life of the preterm newborns. There are no data on the serum levels of MMP-2, MMP-9 or tissue inhibitors of matrix metalloproteinases (TIMP-1) from preterm infants recovering from lung morbidities. We aimed to compare MMP-2, MMP-9 and TIMP-1 levels in preterm and term infants hospitalized with their first episode of wheezing.

METHODS: We prospectively evaluated 18 preterm infants with a history of chronic lung disease, respiratory distress syndrome or oxygen therapy and 14 age- and sex-matched term infants who were admitted for a first episode of wheezing. We quantified total serum concentrations of MMP-2, MMP-9 and TIMP-1 to assess whether these serum markers levels were associated with the first episode of wheezing in infants with a history of oxygen therapy during the neonatal period.

RESULTS: Upon hospitalization, MMP-2 and TIMP-1 levels were higher in preterm infants than in term infants. In contrast, there was no significant relationship between MMP-9 levels or the MMP-9/TIMP-1 ratio between preterm and term infants. The area under the receiver operating characteristic curve for MMP-2 was 0.70 (95% confidence interval [CI] 0.51-0.89). The area under the curve for TIMP-1 was 0.78 (95% CI 0.61-0.94). MMP-9, MMP-2 and TIMP-1 levels did not correlate with gestational age, gender or severity of wheezing.

CONCLUSION: The negative proportion of MMP-9 to TIMP-1 that we detected in term infants was not present in preterm infants. The balance of MMP-9 to TIMP-1 may have been disrupted by lung damage in the premature infants. Overproduction of MMP-2 and TIMP-1 in the serum may be associated with the pathogenesis of wheezing in preterm infants.

Key words:

Matrix metalloproteinases, matrix metalloproteinases-2, matrix metalloproteinases-9, preterm, tissue inhibitors of matrix metalloproteinases-1, wheezing

Advances in neonatal medicine have resulted in the survival of extremely preterm, very low-birth weight newborns. An increasing number of preterm survivors are developing chronic lung disease (CLD).[1] The features of CLD of early infancy include alveolar hypoplasia, peribronchial fibrosis, alveolar septal fibrosis, hypertensive vascular changes, airway muscle thickening and abnormalities of elastic fiber architecture.[1,2] The early abnormal lung development in patients with CLD may prevent subsequent lung growth and development.[1] The long-term respiratory consequences of CLD can extend beyond childhood.[3] Preterm infants have a tendency to wheeze throughout early childhood and these children experience viral bronchiolitis, which necessitates frequent hospital readmissions.[4]

Matrix metalloproteinases (MMP) are enzymes that are able to degrade collagen, fibronectin, elastin and laminin, which are all components of the extracellular matrix. The proteolytic activity of MMPs is counteracted by tissue inhibitors of matrix metalloproteinases (TIMPs). MMPs function in growth and vascular remodeling during wound healing and inflammation.[5,6] MMPs disrupt the integrity of the basement membranes of alveolar epithelial cells.[5,6] MMPs can function in both destruction and the reorganization of alveolar epithelia. In animal models, MMP-2 and MMP-9 appear to be involved in early stages of rabbit lung development and a dramatic increase in MMP-2 occurs during the late prenatal lung development and in postnatal lungs.[7]

MMP dysregulation contributes to the pathology of chronic and acute lung disorders, including bronchopulmonary dysplasia,[8] respiratory distress syndrome (RDS) of newborn,[9] asthma[10] and emphysema.[8-10] In addition, the impact of oxygen toxicity, barotrauma and volutrauma caused by mechanical ventilation disrupts the structure of the lungs and initiates an inflammatory cascade.[5] Inflammation is the physiologic hallmark of respiratory morbidity and mortality in preterm infants, with neutrophils and macrophages releasing proteolytic enzymes, such as MMPs.[11] The increased number of neutrophils, proteinases (like MMP-9) and anti-proteinases increases the likelihood of developing CLD and postnatal infections and represents the primary trigger for initiating the inflammatory process.[11] It is known that MMP-9 is increased in the lungs of preterm babies who develop CLD and that in these children, there is an imbalance between MMP-9 and its inhibitor, TIMP-1.[12]

This study examines the relationship between metalloproteinases and their inhibitors in preterm infants who are experiencing their first attack of wheezing after discharge from the neonatal intensive care unit. We hypothesized that a unique MMP and TIMP profile exists in the serum of preterm infants who have recovered from RDS and/or CLD. Most of the previous studies were performed in the 1st weeks of life of the preterm newborns. There are no data on the serum levels of MMP-2, MMP-9, or TIMP-1 from preterm infants recovering from lung morbidities. The aims of this study were (1) to serially measure plasma levels of MMP-2, MMP-9 and TIMP-1 in premature infants who have recovered from RDS and/or CLD and have been re-hospitalized for a first episode of wheezing; (2) to examine MMP-2, MMP-9 and TIMP-1 levels and their relationships to C-reactive protein (CRP), complete blood count, blood gases and postnatal history; and (3) to compare MMP-2, MMP-9 and TIMP-1 levels in preterm infants with those of term infants.

Methods

This hospital-based, prospective observational study was conducted from September 2010 to October 2011 in Istanbul, Turkey. Informed consent was obtained from the parents of all subjects and the study was approved by the local Ethics Committee.

Preterm group

Patients who were admitted with a first episode of wheezing were included if they met the inclusion criteria: A preceding viral upper respiratory infection that was followed by wheezing and crackles on auscultation; a viral respiratory infection was diagnosed on clinical grounds; the patient was a preterm infant (<37 weeks gestation at birth) who had developed and recovered from CLD of prematurity or neonatal RDS or had received oxygen treatment. The patients were symptom free and breathing room air upon discharge from the neonatal intensive care unit. All patients were treated and discharged from the same neonatal intensive care unit. We collected data on the use of mechanical ventilation, gestation, birth weight, gender and the number of doses of surfactant administered.

Patients were excluded if they experienced lung morbidities other than wheezing, were born at ≥37 weeks, were younger than 1 month or older than 6 months, had a history of recurrent wheezing episodes, had consolidation or atelectasis on a chest roentgenogram or had proven immune deficiency or severe neurological disease. In addition, infants were excluded if their parents did not provide informed consent. None of the study participants received steroids or other immunosuppressive medications during the study.

On admission, blood was drawn to check the complete blood count, CRP and blood gases. In addition, blood was obtained within 24 h of admission and separated and frozen at −70°C for analysis of MMP-2, MMP-9 and TIMP-1. On admission and discharge, serum samples were obtained and aliquoted.

Supportive care, including oxygen supplementation, aspiration and hydration, was provided to all patients when necessary. Pulses, respiratory rates and oxygen saturation were measured using a bedside monitor (SC 6002XL Multiparameter Monitor, Siemens, Germany). The severity of the wheezing was assessed using respiratory rate, pulse, oxygen saturation and length of hospitalization.

Term group

The second group was comprised of term infants with no known lung disease who were hospitalized for their first episode of wheezing. Patients were matched for age, gender and severity of wheezing. As with the preterm infants, blood was drawn for complete blood count, CRP and blood gases on admission. In addition, blood was obtained within 24 h of admission and separated and frozen at −70°C for analysis of MMP-2, MMP-9 and TIMP-1 concentrations.

Measurement of MMP-2, MMP-9 and TIMP-1 concentrations

Plasma levels of MMPs were assessed using the enzyme-linked immunosorbent assay (ELISA) kits (RayBio® Human MMP-2, MMP-9, TIMP-1 ELISA Kit; Ray-Bio Catalog#: ELH-MMP2-001, ELH-MMP9-001, ELH-TIMP1-001). Protein concentrations were measured by ELISA (CA-2000, CIOM Medical Co., Ltd., Jilin, China) according to the manufacturer's instructions. The manufacturer's minimum detectable concentration for individual kits was <3.5 ng/ml for MMP-2, 10 pg/ml for MMP-9 and 40 pg/ml for TIMP-1.

Serum CRP concentrations were measured using a nephelometric method (IMMAGE 800, Beckman Coulter, Inc., Brea, USA) and values >1 mg/dl were considered abnormal.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (version 17.0; SPSS Inc., Chicago, IL, USA). MMP-9, MMP-2 and TIMP-1 levels were not normally distributed so non-parametric tests were used to assess for differences between the two groups. Differences between groups were analyzed with the Mann-Whitney *U* test. The two consecutive measurements in the preterm group were analyzed with paired *t*-test. Correlations between groups were performed using Spearman correlation coefficients. Receiver operating characteristic curves (ROC) were used for MMP and TIMP levels. A $P < 0.05$ was considered to be significant.

Results

Characteristics of the preterm and term groups

A total of 18 infants who were born at ≤33 weeks (24-33 weeks) were recruited to the preterm group. Of those, 13 had recovered from RDS and 4 of those infants had also recovered from CLD.

The five remaining infants had received oxygen treatment during the neonatal period. Fifty blood samples were collected: 36 from the preterm group and 14 from the term group. Both groups had similar severity of wheezing as assessed by respiratory rate, oxygen saturation and length of stay. None of the patients required mechanical ventilation.

Arterial blood gases were tested in every patient and there was no difference between the term and preterm infants ($P > 0.05$). The laboratory results of the preterm and term groups are given in Table 1.

MMP-2, MMP-9 and TIMP-1 concentrations

Serum MMP-2 and TIMP-1 levels were higher in preterm patients than in term patients ($P = 0.04$ and $P = 0.007$, respectively) [Figure 1].

In the preterm group, the two consecutive measurements of MMP-9 levels were significantly different ($P = 0.03$). The median MMP-9, MMP-2 and TIMP-1 concentrations of two consecutive measurements in the preterm group and the results of term group are given in Table 1.

There was a negative correlation between MMP-9 and TIMP-1 levels in term infants ($r = -0.81$, $P < 0.001$), whereas there was no correlation in these levels in preterm infants. CRP levels were correlated with MMP-9 and TIMP-1 levels in the term group ($r = -0.80$, $P < 0.001$; $r = 0.76$, $P = 0.001$, respectively).

Upon admission to the hospital, MMP-2 levels were inversely correlated with respiratory rate ($r = -0.68$, $P = 0.002$) and percentage of neutrophils ($r = -0.47$, $P = 0.04$) in preterm infants. At the time of discharge, MMP-2 levels correlated with CRP levels ($r = -0.61$, $P = 0.007$).

The area under the ROC curve for MMP-2 was 0.70 (95% confidence interval [CI] 0.51-0.89, $P = 0.04$). The area under the curve for TIMP-1 was 0.78 (95% CI 0.61-0.94, $P = 0.007$) [Figure 2].

MMP-9, MMP-2 and TIMP-1 levels were not correlated with gestational age. There was no difference in the concentration levels of boys versus girls. There was no relationship between proteinases and gestational age; birth weight; postnatal history of intubation; continuous positive airway pressure, hood or oxygen therapy; number of surfactant replacement therapies or history of CLD. Likewise, there was no association

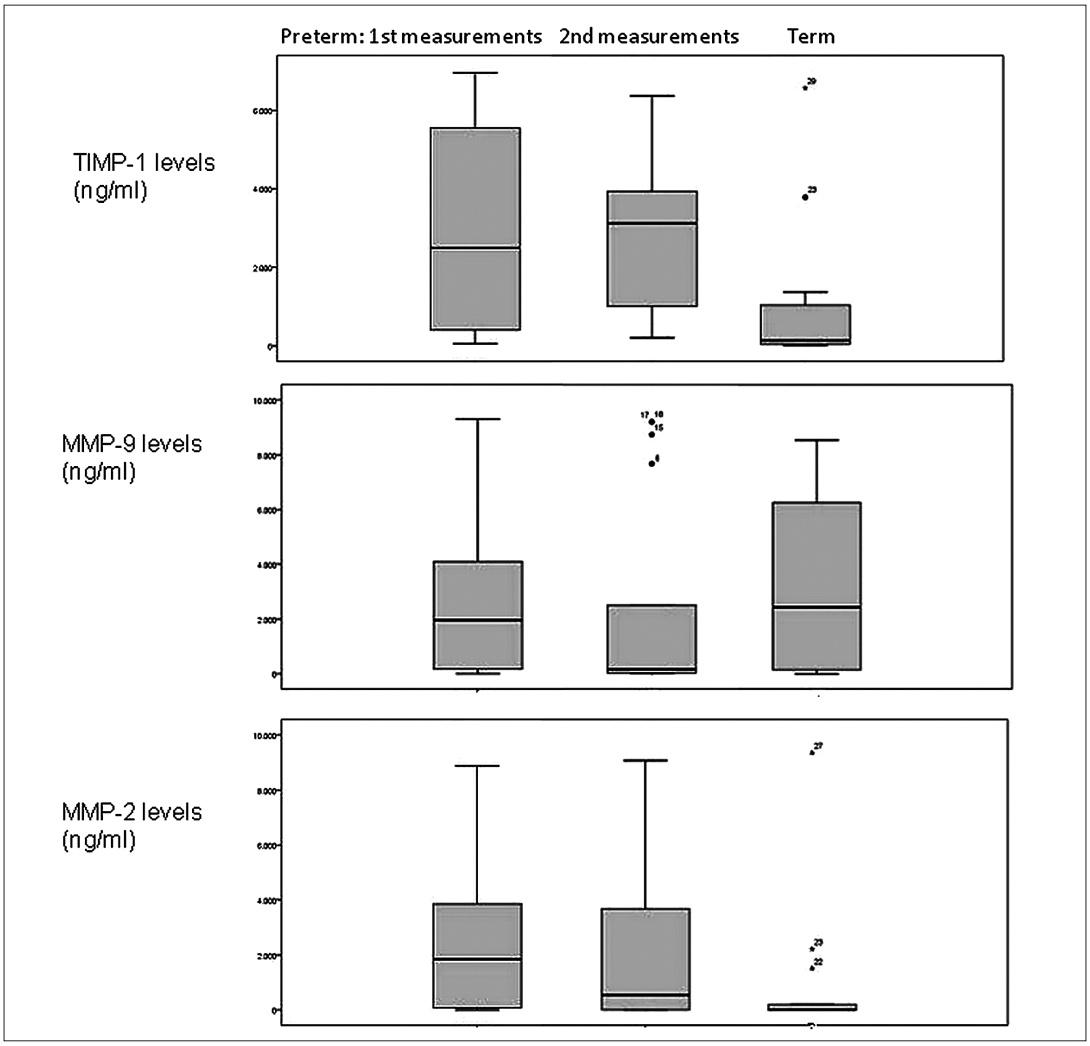

Figure 1: Comparison of matrix metalloproteinases (MMP)-2, MMP-9 and tissue inhibitors of MMP-1 levels (ng/ml) in preterm and term infants

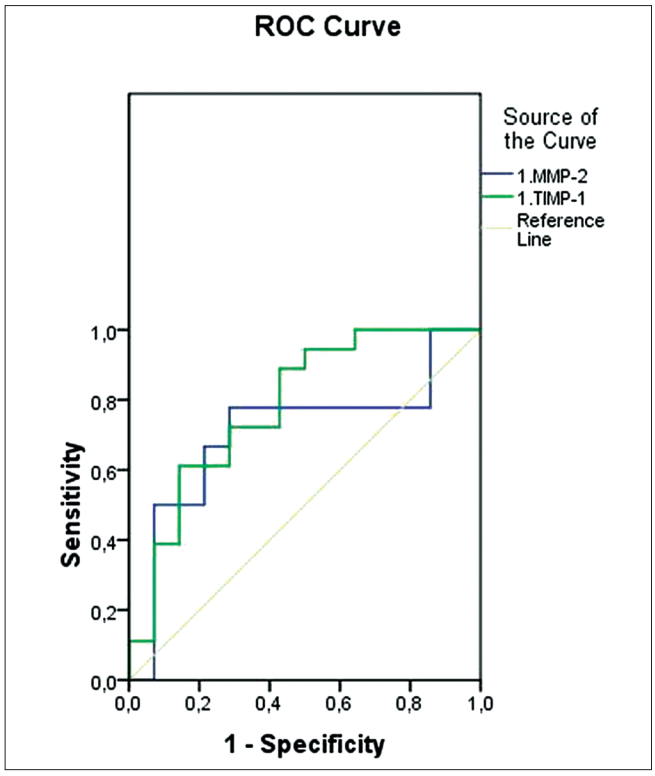

Figure 2: Receiver operating curve for the specificity and sensitivity of matrix metalloproteinases-2 and tissue inhibitors of matrix metalloproteinases-1 measurements

between the proteinases and age and weight at the time of study, heart rate, oxygen saturation at admission, duration of hospitalization, white blood cell count, hemoglobin, pH, PCO_2 or HCO_3 levels.

Potential confounders that may affect proteinase levels include age, gender, birth weight, CRP level, complete blood count and blood gases analysis. These clinical parameters were not significantly different between the preterm and term groups.

Discussion

The principle finding of this study was that MMP-2 and TIMP-1 serum concentrations were elevated in preterm infants who presented for a first episode of wheezing. However, MMP-9 and the MMP-9/TIMP-1 ratio were not elevated in these infants.

MMPs includes collagenases (MMP-1, MMP-8 and MMP-13), stromelysins (MMP-3, MMP-7 and MMP-10) and gelatinases (MMP-2 and MMP-9).[5,6] Gelatinases degrade the extracellular matrix, regulate proinflammatory cytokines and contribute to tissue remodeling in the lungs. MMP-2 is secreted by many cells of mesenchymal origin and is unique among MMPs in that it is not activated with elastase, cathepsin G, plasmins or urokinase. It is activated only on the cell surface.[6] MMP-9, also known as gelatinase-B, is a type 4 collagenase secreted by leukocytes,[6,13] and excess MMP-9 activity and hyperoxic lung damage are closely linked.[5,8,13-15] TIMP-1 is produced by connective tissue cells and macrophages and irreversibly binds and inactivates MMP-9.[6,16] It has been suggested that TIMP-1 may have a role in postnatal lung adaptation. Furthermore, a high ratio of MMP-9 to TIMP-1 is thought to be a risk factor for

Table 1: Comparison of the laboratory results and MMP-2, MMP-9, TIMP-1 levels in preterm and term infants

Parameters	Preterm group (*n*=18)	Term group (*n*=14)	*P* value
Age (months) mean±SD	3.2±1.4	3.5±1.4	0.4
Leucocyte counts			
Mean±SD/mm^3	10,550±5,600	9,400±4,400	0.6
Percentages of neutrophils	37.8±15.9	38.3±17.9	0.9
Hemoglobin levels			
Mean±SD (g/dl)	9.7±1.7	10.1±0.8	0.4
Platelet counts			
Mean±SD/mm^3	346,000±149,000	363,000±157,000	0.8
CRP levels			
Mean±SD (mg/dl)	1±1.2	0.6±0.3	0.5
MMP-2 concentrations			
Median (ng/ml)			
1st levels	1846	5	0.04
2nd levels	542		0.1
TIMP-1 concentrations			
Median (ng/ml)			
1st levels	2493	141	0.007
2nd levels	3120		0.002
MMP-9 concentrations			
Median (ng/ml)			
1st levels	1956	2426	0.6
2nd levels	80		0.02

MMP = Matrix metalloproteinases, TIMP = Tissue inhibitors of matrix metalloproteinases, CRP = C-reactive protein, SD = Standard deviation

lung damage.[5,16] One study that examined the longitudinal profile of MMP-9 and the MMP-9/TIMP-1 ratio showed episodic peaks in the level of this proteinase and its inhibitor.[11] Infections triggering the proteinase cascade lead to lung injury and CLD development.[11] The negative proportion of MMP-9 to TIMP-1 that we detected in term infants was not present in preterm infants. The balance of MMP-9 to TIMP-1 may have been disrupted by lung damage in the premature infants.

Previous studies examined MMP-9 and the MMP-9/TIMP-1 complex in bronchoalveolar lavage specimens of ventilated preterm infants and identified relationships between CLD development and proteinase release.[11] Studies of MMP levels in RDS and CLD patients have produced conflicting results.[8,9,14,15] Dik *et al*.[14] reported increased MMP-9 levels in patients with RDS, but Sweet *et al*.[8] and Ekekezie *et al*.[15] did not find a difference. In another study, median MMP-9 levels were significantly higher in infants with RDS up to 4 days after birth, but in infants with CLD, levels increased after a week.[14] Increased MMP-9 activity is associated with better pulmonary outcomes in infants with RDS, suggesting that MMP-9 plays a protective role.[14] A higher ratio of MMP-9 to TIMP-1 in bronchoalveolar lavage specimens from infants with CLD was reported.[8,15] An imbalance in MMP-9 and TIMP-1 can lead to neonatal lung disease and the development of CLD.[8,15] These controversial results on the harms and benefits associated with MMP-9 should be highlighted. In this study, we

compared two groups of infants that were matched for age and gender and differed only with respect to their gestational age at birth and postnatal history. We studied proteinase levels in the peripheral blood so that the results could be related to the timing of the study and to the type of specimen tested. Moreover, most of the previous studies were performed in the 1st weeks of life of the preterm newborns. Our study included the infancy period, which has not been studied before.

A study of 1059 preterm infants with birth weights <1000 g detected a significant negative correlation between MMP-9 levels on day one of life and administered FiO_2 on day three.[17] This was contrary to studies that had shown a relationship between high MMP-9 levels and hyperoxia.[18] It is not appropriate to compare studies that have used different technologies such as zymography, ELISA and luminex. In addition, there are limited normative data on the plasma activity of the MMPs.[19]

Endotracheal aspirates of children with respiratory failure have revealed increased MMP-9 concentrations compared with the controls, yet MMP-2 levels did not differ.[20] MMP-2 levels were significantly increased from day zero to day one in the tracheal secretions of infants who subsequently developed CLD, suggesting that MMP-2 plays a role in the pathogenesis of CLD.[21] Compared with term patients, we detected increased MMP-2 levels, but no difference in MMP-9 levels, in preterm infants in the acute phase of wheezing. The inverse correlation may be due to the active secretion of MMPs from cells of the lungs that was not reflected by serum levels.

One limitation of our study is that we studied systemic rather than tissue MMP and TIMP levels. Nonetheless, we demonstrated significant differences in serum MMP-2 and TIMP-1 levels. Our patient group was not on mechanical ventilation, so obtaining tracheal samples would not have been ethical. In addition, the viral etiology for the episode of wheezing was not detected, which may have altered serum proteinase levels. Furthermore, we do not know the baseline MMP and TIMP levels before the wheezing attack. Despite our limited sample size and lack of a control group of premature born infants without a wheezing episode, we observed significantly higher levels of MMP-2 and TIMP-1 in preterm infants than in term infants, which may be useful for predicting lung morbidity during early infancy.

Disruption of the balance between MMP-9 and TIMP-1 and overproduction of MMP-2 and TIMP-1 in the serum may be associated with the pathogenesis of wheezing in preterm infants recovering from RDS and/or CLD.

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