ORIGINAL ARTICLE

Positive Correlation between Monocyte-to-Lymphocyte Ratio and C-Reactive Protein in Vitamin D Deficient Preterm Infants with Respiratory Distress Syndrome

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ABSTRACT

INTRODUCTION: Respiratory distress syndrome (RDS) is one of the leading causes of preterm infant mortality. Vitamin D deficiency is known as a risk factor for RDS related to infants with immature lungs leading to inflammatory exacerbations. Therefore, early diagnosis is needed to prevent RDS. This study evaluated white blood cells (WBC) ratios and C-reactive protein (CRP) among vitamin D deficient preterm infants with RDS to identify simple and prompted inflammatory markers. **MATERIALS AND METHODS**: A cross-sectional study involving forty preterm infants (28 to 34-weeks gestational age) with low vitamin D levels (25(OH)D <20 ng/dL) was conducted in a neonatal ward of a hospital in Bandung, Indonesia, from February to October 2018. Two consecutive blood collections (cord blood at birth and venous blood at first week) were performed to measure cord blood vitamin D (25(OH)D) levels, complete blood count (CBC), and CRP levels. White blood cell ratios (Monocyte-to-Lymphocyte ratio: MLR, Neutrophil-to-Lymphocyte ratio: NLR, and Platelet-to-Lymphocyte ratio: PLR) were calculated and analyzed. Association analysis was performed using Student's t-test, Mann-Whitney U and Spearman correlation tests. **RESULTS:** The median of 25(OH)D levels were 9.96 (7.58-15.81) ng/dL. A significant positive correlation between MLR and CRP was found in the RDS group (r=0.576, p=0.004) and all the subjects (r=0.491, p=0.002); the WBC ratio and CRP levels showed insignificant higher trends when compared to the non-RDS group. **CONCLUSION**: There was a positive correlation between MLR and CRP among vitamin D deficient preterm infants with RDS.

KEYWORDS: Monocyte-to-Lymphocyte ratio (MLR), preterm infants, Respiratory Distress Syndrome, Vitamin D

INTRODUCTION

Respiratory distress syndrome (RDS) is the most common complication that leads to mortality in preterm infants and is due to a lack of the surfactant in the alveoli.[1,2] Previous studies reported that preterm infants are born with lower vitamin D (25(OH)D) levels compared to term infants.[3-7] Vitamin D has a role in the surfactant secretion from the type II pneumocyte of foetal lungs and also the development of the immune response. [8] According to previous studies, low vitamin D levels is believed to be a significant risk factor for preterm infants to develop immune deregulation.[9, 10]

As RDS is commonly discovered among lower gestational age infants, it is frequently seen in preterm infants with severe vitamin D deficiency.[11, 12] Vitamin D deficiency is, in fact, a global problem, even in Indonesia as a country with routine and sufficient sunlight throughout the year. Additionally, the

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deficiency is not always diagnosed early to ensure specific and timely treatments in many countries.

Vitamin D also inhibits the production of inflammatory cytokines in immune cells, therefore, low vitamin D levels can increase inflammatory reactions.[10, 13] RDS is accompanied by an imbalanced inflammatory response, therefore by following the response of the immune cells, their levels can be a predictive indicator to RDS. Monocyte-to-lymphocyte ratio (MLR), neutrophil-to-lymphocyte ratio (NLR), and Platelet-to-lymphocyte ratio (PLR) have been proposed as easy and inexpensive inflammatory markers; while C-reactive protein (CRP) is known as an acute phase reactant in inflammatory reactions. [14-17]

Therefore,, our study was aimed to evaluate the correlation between MLR, NLR, PLR, and CRP among preterm infants with low vitamin D levels.

MATERIALS AND METHODS

A cross-sectional study which involved neonatal with 28 -34-week gestational age, 25(OH)D level of ≤20 ng/ dL[18] was conducted at the Neonatology Division, Department of Child Health, Faculty of Medicine Universitas Padjadjaran and Dr Hasan Sadikin General Hospital, Bandung, Indonesia, from February to October 2018. This tertiary referral hospital facilitated 563 preterm births (28-24 weeks of gestational age) during the study period. Infants with major congenital anomaly, twins, and born from infected or diabetic mothers were excluded. Subjects who had complete vitamin D level and complete blood count (CBC) data were only 53 subjects. Among the subjects, there were 40 infants with low vitamin D levels. Hence, the 40 subjects of this study consisted of 27 RDS and 13 non-RDS infants.

Verbal consents were obtained from the subjects' parents or caregivers before enrolment into the study. We conducted complete history taking and detailed clinical examination for assessment of signs of respiratory distress, recorded the mode of delivery, and birth weight. Gestational age assessment was based on the date of the last menstrual period. Clinical diagnosis of the RDS was marked when the following were observed: respiratory rate >60 per-minute, chest wall retraction, grunting, cyanosis in room air, radiological (+) towards RDS, PaO2 (Partial Pressure of Oxygen) levels of 50 mmHg based on blood gas analysis, and when the infant did not recover from more than four criteria and clinical states within 24 hours.[19, 20] A chest X-ray of the postero-anterior view was performed on infants with RDS. The X-ray images were interpreted consecutively to identify the RDS by the radiologist on duty at the Radiology Department.

Cord blood and peripheral blood were collected from each eligible subject. A total of 3 mL cord blood was collected immediately after birth. The cord blood was directly processed in the laboratory and prepared for analysis. Sera from cord blood were stored at -80^{o}C for further analysis of vitamin D levels when blood collection was completed. A total of 1 mL venous blood was collected using a routine venipuncture procedure in the first week of life (day 2-7) and was examined for CBC and CRP.

CBC was performed using an automated flow-cytometry haematology analyzer (Sysmex XN series, Sysmex Corp., Japan). The ratios of circulating monocyte, neutrophil, lymphocyte, and platelet count which were marked as MLR, NLR, and PLR respectively were computed using the absolute count of each parameter from CBC.[19,20] The level of 25(OH)D was measured using a competitive enzyme immunoassay method (Cloud-Clone Corp, USA). The serum CRP level was measured using the particle-enhanced turbidimetric immunoassay (PEITA, Siemens Dimensions Series).

Collected data were analysed using the Statistical Package for Social Science (SPSS version 23 for Windows; SPSS Inc, Chicago) and then stratified into two groups, the RDS and non-RDS groups. The primary analysis of the data was aimed to access the differences in laboratory parameters between the two groups. This analysis was carried out using the Student's t-test or Mann-Whitney U statistical test.

The secondary analysis was performed to observe the correlation between MLR, NLR, PLR, and CRP level, stratified by two groups using the Spearman correlation test. A significant statistical difference was defined when the p-value was less than 0.05.

All procedures were conducted in compliance with policies of the Faculty of Medicine, Universitas Padjadjaran and Dr Hasan Sadikin General Hospital, Bandung, West Java, Indonesia. This study was approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Padjadjaran Bandung (approval number: 303/UN6.KEP/EC/2018).

RESULTS

Preterm infants with RDS had significantly younger gestational age and lower birth weight than those in the non-RDS group (Table I). Vitamin D levels in both groups were lower than the normal limit (<20 ng/mL), but there was no significant difference between both groups.

Table I. Clinical and Laboratory Characteristics of Preterm infants

Variables	RDS N = 27	Non-RDS N =13	*P*-Value
Gestational Age (weeks)	32 (30-33)	34 (32.5-34)	0.025[a]
Birth Weight (grams)	1531± 294	1822± 292	0.006[b]
25(OH)D (ng/mL)*	11.7± 5.7	11.0±4.3	0.708[b]
Hemoglobin (g/dL)	15.3±3.2	16.3±2.7	0.307[b]
Red Blood Cell (10^6/µL)	4.5± 0.7	4.9± 8.8	0.312[b]
Hematocrit (%)	45.7± 10.6	48.7± 8.5	0.434[b]
RDW (CV%)	16.0 (15.6-17.7)	17.7 (16.0-18.9)	0.118[a]
Leukocyte (10^3/µL)	9.3± 4.2	11.5± 4.0	0.132[b]
Basophil (10^3/µL)	0.01 (0.00-0.03)	0.01 (0.00-0.02)	0.711[a]
Eosinophil (10^3/µL)	0.12 (0.06-0.26)	0.26 (0.05-0.50)	0.530[a]
Neutrophil (10^3/µL)	4.7± 3.0	6.1± 3.7	0.190[b]
Lymphocyte (10^3/µL)	2.9± 1.6	3.5± 1.5	0.231[b]
Monocyte (10^3/µL)	1.5± 0.7	1.5± 4.8	0.974[b]
Platelet (10^3/µL)	233± 107	236± 58	0.874[b]

Abbrev: (a) Mann-Whitney U test, data as mean±SD (b) Student's t-test, data as median-IQR; IQR, Inter Quartile Range; SD, Standard Deviation; RDW, Red Cell Distribution Width;
*Cord blood sample

Table I also shows the comparison of hematologic parameters in the first week of life between preterm infants with and without RDS. All haematologic parameters in the RDS group in the first week of life were lower but were not significantly different from the non-RDS group.

The preterm infants with RDS showed a higher trend of MLR, NLR, PLR, and CRP level but were not significantly different from the non-RDS group as depicted in Table II.

Table II. White Blood Cell Ratio and C-reactive protein Levels of Preterm Infants

Variables	RDS (N = 27)	Non-RDS (N = 13)	*P*-Value
MLR	0.62± 0.28	0.50 ±0.29	0.142[a]
NLR	1.89 (0.89-3.54)	1.18 (1.06-2.68)	0.798[b]
PLR	94.40 (58.82-111.66)	71.72 (42.43-92.71)	0.197[b]
CRP	0.20 (0.09-0.39)	0.16 (0.12-0.42)	0.755[b]

Abbrev: (a) Student's t-test, data as mean±SD (b) Mann-Whitney U test, data as median-IQR; RDS, Respiratory Distress Syndrome; IQR, Inter Quartile Range; SD, Standard Deviation; MLR, Monocyte-to-lymphocyte ratio; NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; CRP, C-reactive protein.

Therefore, the RDS group and all subjects (RDS and non-RDS) had a significant positive correlation between MLR and CRP as displayed in Table III. The RDS group, non-RDS group, and all subjects showed trends of a positive correlation between NLR, PLR, and CRP level. However, the NLR had a weak negative correlation to CRP level among the non-RDS group.

DISCUSSION

Preterm birth is a significant global health issue, as well as is vitamin D deficiency. This double burden contributes to RDS associated to lung immaturity. Preterm infants are born before their lungs are fully developed, while maturation is completed.[1,2,21] Immature lungs inadequately produce the surfactant, which needed for reduces surface tension in the alveoli.

Table III. Correlation between CRP level to MLR, NLR, and PLR in preterm infants

	MLR	p Value*	NLR	p Value*	PLR	p Value*
RDS group	0.557	0.009	0.286	0.208	0.283	0.210
Non-RDS group	0.374	0.287	-0.031	0.933	0.350	0.322
Over all	0.485	0.006	0.245	0.183	0.303	0.098

*Spearman Correlation test

RDS is the most common complication of preterm infants and occurs due to the lack of surfactant in the alveoli.[1,2] There is evidence that preterm infants are often born with lower vitamin D levels compared to term infants.[3-6] Vitamin D is suggested to have a role in surfactant secretion from the type II pneumocyte (AT II cells, Alveolar Type II cells) of foetal lungs.[22] On the other hand, immature fibroblast in immature lungs cannot express vitamin D adequately leading to inadequate surfactant secretion from the AT II cells.[8] In preterm infants, RDS disease was suggested to be induced by low vitamin D levels.[23] Based on these facts, the vitamin D status of preterm infants is considered to be associated with immune regulation in neonates.[11] Therefore, in our study, subjects with low vitamin D levels were included to assess the inflammation process due to RDS in preterm infants. In RDS, there is the involvement of the inflammatory process and the inflammation is caused by lung immaturity and lack of surfactant secretion. The process cascade of RDS is as follows: lack of pulmonary compliance, atelectasis, reduced gas exchange, severe hypoxemia and hypercarbia. The integrity of the endothelial and epithelial are subsequently damaged, and this results in the stimulation of local and systemic inflammation.[24-26]

Our study showed that preterm infants with RDS were of younger gestational age and had lower birth weight than those in the non-RDS group. This finding is in accordance with previous studies which revealed that the risk of RDS decreases with increasing gestational age.[27-29] The proportion of RDS infants in our study (27/40 subjects) was higher than in a previous study which reported the RDS affects in one-third of infants born between 28 to 34 weeks' of gestation.[20] The proportion of RDS infants was higher because only infants with low vitamin D levels were included in this study.

his study also showed that all haematologic parameters in preterm infants with RDS tended to be lower compared with the non-RDS group. This finding is similar to a previous study which revealed that because RDS was commonly found in younger gestational age, lower haemoglobins and fewer immune cells, such as neutrophils, lymphocytes, and monocytes were reported.[30,31] Our study also demonstrated lower platelet counts in the RDS group compared to the non-RDS group. Additionally, similar to the findings of a prospective study by Kohelet *et al* (1990), RDS severity was strongly associated with platelet count reduction in the first few days after birth. Platelet count was often decreased in high-risk neonates, such as those with perinatal asphyxia, umbilical catheters, hyperbilirubinemia, polycythaemia, sepsis, intracranial bleeding and undergoing phototherapy.[32] On the contrary, Chen *et al* (2019), in a retrospective study reported different results from Kohelet *et al* (1990), . Here it was demonstrated that platelet, neutrophil and monocyte counts at birth are higher in infants with RDS but basophil and lymphocyte counts were lower.[33]

Essential components of the innate immune response are circulating white blood cells (WBC), e.g. neutrophil, lymphocyte, and monocyte. Ratios of the WBC (NLR, MLR, and PLR) have been proposed as potential markers of general inflammatory responses for predicting survival of patients with various diseases, such as malignancies, autoimmune and infectious diseases.[34,14-16,35] Interestingly, our study demonstrated that MLR, NLR, PLR and CRP level of the RDS group were higher compared to the non-RDS group (Table II). Our study also showed a significant correlation between MLR and CRP level, which are inflammation-related evidence that bridges RDS and lung immaturity (Table III). These findings also suggested that the innate immunity is crucial during the neonate period, since the adaptive immunity is not fully developed.[13] Until today, the notions regarding MLR, NLR, and PLR in preterm infants are still scarce, while the CRP is considered as a primary inflammatory marker. Monocytes are an important component of peripheral blood as well, hence the increase of monocyte count describes increased monocyte migration from the bone marrow to the peripheral blood due to the inflammatory response.[30, 36] The ratio of monocyte to lymphocytes will be altered and will reflect an unfavourable balance involving

monocytes and a favourable prognostic outcome involving lymphocytes, hence MLR is considered as an indicator of systemic inflammation.[37]

The correlation between MLR and CRP level suggests the strengthening of MLR utilization as an alternative inflammatory marker. This finding is expected to improve the health service because many hospitals in developing countries do not have the facilities to examine CRP as a common inflammatory marker. The NLR associated as a marker of a pro-inflammatory state is also widely known as a haematological marker for systemic inflammation. There are associations between a higher neutrophil percentage or lower lymphocyte percentage with the increased inflammatory response in several diseases. [38, 39] The use of NLR which quantitates neutrophilia (an indicator of inflammation) and lymphopenia (an indicator of physiologic stress) is a valuable prognostic gage for evaluating patients with systemic inflammation.[40, 41] Porata and Tse *et al.* (2012) reported that PLR is another indicator of systemic inflammation that has been validated as a prognostic predictor in some tumours .[42] Meanwhile, previous studies also reported platelet in the involvement of the inflammatory process.[43-46] Platelets play an important role in premature birth-related diseases, including RDS.[47,48] The higher trend in CRP level as the most frequently used inflammatory marker defined a higher inflammatory process in the RDS group compared to the non-RDS group.

One limitation of this study is that it was a cross-sectional study which only involved 40 preterm infants with complete CBC data and low vitamin D levels. Further research is mandatory to explore the leucocyte activity related to inflammation and low vitamin D levels.

In conclusion, WBC ratios involved in inflammation process show correlation with the CRP among vitamin D deficient preterm infants with RDS. Therefore, we propose that performing WBC ratio analysis are important in recognising RDS in preterm infants.

RECOMMENDATIONS

Monocyte-to-Lymphocyte Ratio is a potential marker to assess the inflammatory process leading to RDS, particularly among vitamin D deficient preterm infants. Further research is imperative to involve other perinatal related variables to have an integrated prevention and treatment approach.

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Author Contributions

Reni Ghrahani conceptualized, conducted the study, provided instruments of the data collection, coordinated and supervised data collection, performed the analyses, composed and edited the manuscript.

Mohammad Ghozali, Adhi Kristianto Sugianli, Raden Tina Dewi Judistiani, and Tetty Yuniati designed the study, checked, and revised the manuscript;

Budi Setiabudiawan conceived the study and reviewed the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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