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Neutrophil to Lymphocyte Ratio and Red Blood Cell Distribution Width Levels in Preterm vs. Term Births

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Abstract

Objective: Despite its broad etiology, preterm labor has been firmly linked to inflammatory/infectious processes. However, very few cases of preterm birth are preceded by acute signs and symptoms of clinical infection. Many studies have found neutrophil-to-lymphocyte ratio and red blood cell distribution width to be elevated in cases of subclinical infections. We performed a retrospective study to compare the levels of these two markers in preterm *vs.* term births.

Material and methods: Patient information was obtained retrospectively. Preterm and term birth patients were captured from our database during a three-year period. Neutrophil-to-lymphocyte ratio and red blood cell distribution width in the first trimester and on admission to labor and delivery was obtained. A sample size of 130 per group was required to find a 20% difference with 80% power (standard deviation=3.2). *p*-values less than 0.05 were considered significant.

Results: The preterm birth group contained 137 patients with an average gestational age of 32.4 ± 4.1 weeks and the term birth group included 145 patients with an average gestational age of 39.2 ± 1.1 weeks. The neutrophil-to-lymphocyte ratio at the time of delivery was found to be higher in the preterm birth group (5.9 ± 5.1 *vs.* 4.6 ± 3.2, *p*=0.007).

Red blood cell distribution width at delivery did not differ between groups (13.6 ± 0.9, 13.9 ± 1.8, *p*=0.09). Subgroup analysis of preterm patients with preterm premature rupture of membranes (n=52) or gestational age <35 weeks (n=72) did not result in significant difference when compared to term patients.

Conclusion: Neutrophil-to-lymphocyte ratio was significantly elevated in preterm birth patients when compared to term patients. No statistically significant difference in red blood cell distribution width was found between groups.

Introduction

Every year an estimated 15 million infants are born preterm, with nearly 1 million children dying annually due to complications of preterm birth [1]. Preterm birth (PTB) is the leading cause of neonatal mortality in the United States. The slow progress in reducing neonatal mortality secondary to PTB is related to the fact that PTB remains a complex condition where the underlying etiology and biological mechanisms remain unknown [2]. Despite its broad etiology, preterm labor has also been firmly linked to inflammatory/infectious processes. Proinflammatory cytokines are produced in gestational tissues in response to stressors and can prematurely induce uterine activation, which initiates the onset of preterm labor. Recent studies have demonstrated associations between preterm birth and elevated levels of interleukin (IL) 6, IL-1β and tumor necrosis factor alpha (TNF-α) [3]. However, very few cases of PTB are preceded by acute signs and symptoms of clinical infection. Unlike clinical infections, subclinical infections are identified by infiltration of tissue by neutrophils, macrophages, and lymphocytes without overt findings of clinical infection. This "sub-acute infection" is substantiated by the histological evidence of chorioamnionitis in the placentas of 20-70% of PTBs and positive membrane cultures in 30-60% of such patients [4-8].

Recently neutrophil-to-lymphocyte ratio (NLR) and red blood cell distribution width (RDW) have been found to signal the presence of subclinical infections. NLR might have prognostic significance of diseases related to chronic low-grade inflammation and can be easily obtained from the differential white blood cell count [9].

RDW is a measure of the variation of red blood cell volume (anisocytosis) and is routinely reported by automated laboratory equipment used to perform complete blood counts. RDW has classically been used to narrow the differential diagnosis of anemia; however, elevated levels have been associated with several disease processes [10]. Some have attributed this increase in RDW to occult inflammation. RDW has been extensively studied as a prognostic indicator in patients with various infectious processes [11].

We performed a retrospective study to examine the relationship of these two inexpensive, readily available infection markers in preterm and term births.

Materials and Methods

Patient information was obtained retrospectively from the delivery database of St Luke's University Hospital, an academic, tertiary-care institution. The study was approved by the Institutional Review Board prior to starting. Preterm birth was defined as delivery before

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37 completed weeks of gestation based on last menstrual period or ultrasound dating. Term births (TB) were defined as births occurring between 37 and 41 weeks. PTBs and TBs that fit selection criteria and satisfied the sample size requirement were captured from our database during the time period of July 1, 2013 and July 1, 2016. We collected demographic data from the individual charts including age, ethnicity, body mass index (BMI), gravity, parity, as well as additional comorbidities (e.g. diabetes, hypertension, cardiovascular disease, cancer, chronic kidney disease, chronic obstructive pulmonary disease, hypothyroidism, migraine and smoking history). We excluded individuals with known infection, inflammatory diseases, and hematological disorders. To control for the effect of steroids, patients who received steroid doses prior to two weeks before complete blood counts were drawn were also excluded. In addition, only term patients admitted in active labor prior to delivery were considered for the TB group.

Neutrophil and leukocyte counts as well as RDW were obtained using the XN-3000 Hematology Analyzer (Sysmex Corp, Lincolnshire, IL) in the first trimester and on admission to labor and delivery. NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count.

Statistical Analysis

Statistics were performed using SigmaStat software (Systat, San Jose, CA). A sample size of 130 was required to find a 20% difference with 80% power in NLR (standard deviation= 3.2). Patients with comorbidities were calculated as proportions and compared using chi squared test. Standard t-test was used to compare groups and, if not normally distributed, Mann-Whitney test was performed. Krustal-Wallis was employed to look for differences when more than two groups were compared. P-values less than 0.05 were considered significant. Correction for multiple measurements was not performed.

Results

The PTB study population contained 137 patients with an average gestational age of 32.4 ± 4.1 weeks, and the TB population included 145 patients with an average gestational age of 39.2 ± 1.1 weeks (Table 1). The PTB and TB groups did not differ in age or race. The PTB group had more pregnancies, while the TB group had more live births. Of the comorbidities reviewed, PTBs had significantly higher incidence of smoking (24.1% $vs.$ 11%, p=0.006), BMI >35 (13.8% $vs.$ 5.5%, p=0.029), and hypothyroidism (11% $vs.$ 2.5%, P=0.017). The other comorbidities (diabetes, hypertension, cardiovascular disease, cancer, chronic kidney disease, liver disease, pulmonary disease, neurologic disorders) did not differ between the groups.

The NLR at the time of delivery was found to be higher in the PTB than in the TB group (5.9 ± 5.1 $vs.$ 4.6 ± 3.2, p=0.007). The mean NLR in the first trimester did not differ significantly. Although both groups demonstrated an increase in mean NLR throughout pregnancy, the PTB group demonstrated a statistically significant increase in mean NLR change from the first trimester to delivery compared to the TB group (3.6 ± 2.2, 5.9 ± 5.1, p=0.005).

The PTB and TB patients mean RDW at delivery did not significantly differ (13.6 ± 0.9 $vs.$ 13.9 ± 1.8, p=0.09) (Table 2). Furthermore, the mean RDWs at 1st trimester were similar between groups (13.4 ± 1.2 $vs.$ 13.2 ± 1.4, p=0.11). Subgroup analysis of preterm patients with preterm premature rupture of membranes (n=52) or gestational age <35 weeks (n=72) did not result in significant difference when compared to TB patients. Both groups had an increase in RDW throughout pregnancy,

Variables	Preterm (n=137)	Term (n=145)	*p*-value
Age (years)	28.1 ± 5.6	27.3 ± 6.1	0.63
Gravity	2.8 ± 1.9	2.0 ± 1.4	0.001
Parity	0.8 ± 1.1	1.2 ± 0.83	0.001
Gestational Age	32.4 ± 4.1	39.2 ± 1.1	0.001
BMI > 35	19 (13.8%)	8 (5.5%)	0.029
Smoking	33 (24.1%)	16 (11%)	0.006
Race			
White	88 (64.2%)	98 (67.5%)	0.64
Hispanic	34 (24.8%)	31 (21.2%)	0.59
Black	12 (8.7%)	9 (6.2%)	0.56
other	3 (2.2%)	7 (4.8%)	0.39

Table 1: The PTB study population and the TB population.

Variables	Preterm (n=137)	Term (n=145)	*p*-value
N/L ratio	5.9 ± 5.1	4.7 ± 3.2	0.007
RDW	14.1 ± 1.9	13.5 ± 1.4	0.39

Table 2: The PTB and TB patients mean RDW at delivery.

but the TB patients demonstrated a statistically significant increase in mean RDW change from the first trimester to delivery compared to the PTB group (13.2 ± 1.4 $vs.$ 13.9 ± 1.9, p=0.02).

Discussion

The present study revealed neutrophil-to-lymphocyte ratio to be significantly higher in preterm patients. However, it found no significant elevation in red cell distribution width in the patients delivering preterm despite abundant evidence supporting maternal infection and inflammation as etiologies of PTB.

The rationale for using the NLR was to compare the inflammatory response, which would be represented by the neutrophil count, with the host immunity, as assessed by the lymphocyte count. Both direct and indirect evidence also support neutrophil activation in inflammatory states of pregnancy such as preeclampsia [12].

Several other investigators have employed NLR in the study of preterm birth. Akkar et al. conducted a prospective study in order to determine the relationship between NLR and women having early spontaneous preterm birth without clinical chorioamnionitis [13]. They reported a significant rise in NLR in women with spontaneous preterm birth as compared to those with term pregnancies. Similar to our study, they excluded patients with steroid exposure or infectious diseases; however, this study was limited by a small sample size of 35 in the preterm group and 44 in the term group.

Kim et al. noted that NLR may be used as a predictor of placental inflammatory response (PIR) which, in turn, is associated with PTB [14]. They noted a higher NLR in those patients with histologic evidence of chorioamnionitis. However, contrary to our study, they did not include a term birth group. The lack of an appropriate term control group limits the validity of their investigation.

Lastly, Daglar et al. reported a significantly elevated NLR in patients admitted in threatened preterm labor (PTL) or who had a PTB [15]. They did not find a difference in NLR between those that presented in threatened PTL who subsequently delivered at term and those that had a PTB. Contrary to the present study, they did not account for steroid-induced leukocytosis which may have led to erroneous results. They compared different groups, thus making a direct comparison to the current study difficult. In addition, lack of a difference between the patients with threatened preterm labor and those that delivered preterm may have been an effect of small sample size (n=55).

These studies support PTB as an inflammatory condition where an elevated NLR may act as its predictor, shifting the uterus from a state of quiescence to one of activity with the initiation of labor. Therefore, the significant difference found in the present study could result from inflammation present in preterm labor, which through a series of complex mechanisms stimulates neutrophil release from the bone marrow and from the attachment to the vascular endothelium.

Red blood cell distribution width is a simple and inexpensive measure that reflects the degree of heterogeneity of erythrocyte volume, traditionally used to characterize anemia [16]. Recent evidence has demonstrated a strong, graded association of RDW with high-sensitivity C-reactive protein (hsCRP) and erythrocyte sedimentation rate (ESR) indicating a possible role for RDW as a marker for inflammation [17]. Some studies have demonstrated an association of RDW and severity of preeclampsia, theorizing that the hypoperfused placenta produces reactive oxygen species and cytokines that induce an inflammatory response in the mother [18]. As preterm labor results from a similar local inflammatory response, we hypothesized in the present study that maternal RDW would be elevated in preterm patients as compared to term controls. However, no significant elevation was found in our PTB cohort. Furthermore, regardless of substantial evidence that supports reproductive tract infection-mediated inflammatory changes as a significant etiology for preterm premature rupture of membranes (PPROM), a subgroup analysis of patients with PPROM demonstrated no elevation in maternal RDW as compared to term controls, despite an increased microbial burden as compared to term patients with premature rupture of membranes (PROM) [19].

To explain this finding, one must acknowledge that maternal RDW has been shown to increase significantly between 34 weeks of gestation, as demonstrated by a study by Shehata et al. and the onset of labor in normal pregnancy, possibly related to a maternal need for reticulocytosis in preparation for the blood loss of parturition [20]. Moreover, the local inflammatory pathway that initiates PTL may not be sufficiently long enough in duration or great enough in amplitude to induce a compensatory erythropoiesis or sufficient oxidative stress that would manifest as anisocytosis. Additionally, RDW may not be as sensitive as NLR. We were unable to find other studies looking at RDW in PTL or PTB, thus we were unable to compare or contrast our results with other studies addressing this issue.

Conclusion

Limitations of the present study include retrospective design and single-center location, making it unable to establish causal or temporal relationships, as well as unable to avoid bias secondary to the demographic make-up of the center's clinic and private patients. Therefore, future randomized controlled trials at a large scale are required to establish casual or temporal relationships between NLR and PTB. Due to the heterogeneity of the cause of preterm labor, NLR might be more useful in predicting preterm birth in those patients with an infectious etiology.

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