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Original Article

Analyzing the Variation in Hematological Inflammatory Indicators Throughout Gestation

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ABSTRACT

Objective: Our study aimed to examine the inflammatory status, which is meticulously regulated during pregnancy, and the variations between trimesters and retrospectively assess the differences among trimesters by analyzing the maternal hematological-inflammatory-biomarker-data of healthy pregnant women.

Materials and Methods: The study population consisted of 42 uncomplicated pregnancies who were within 14 gestational weeks and applied for routine pregnancy follow-up at Karaman Training and Research Hospital between 01.08.2023 and 01.05.2024. Jamovi 2.6 was used for statistical analyses applying to the department.

Results: The study comprised 42 pregnant women with an average age of 26.5 years (range: 23.0-30.0), and their average body mass index was 27.9 (range: 25.9-33.1). In the second trimester, MCV, RDW, and PDW exhibit a statistically significant increase ($p<0.001$ for all). SIRI, AISI, NLR, and MLR were statistically substantially elevated in the second trimester compared to the other trimesters ($p=0.001$, $p=0.042$, $p=0.002$, $p=0.015$, respectively). No statistically significant difference was detected between SII and the trimesters. Upon examining the correlation of hematological inflammatory indices with advancing gestational age and among themselves, it was found that SIRI, NLR, and MLR exhibited a weak positive correlation with gestational weeks (Spearman's; rho=0.228, $p=0.01$; 0.191, $p=0.032$; 0.205, $p=0.022$, respectively).

Conclusion: We have determined the standard range of inflammatory indicators in healthy pregnant individuals across each trimester.

Keywords: AISI, MLR, NLR, pregnancy, SIRI

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INTRODUCTION

Pregnancy is a unique, highly coordinated physiological process characterized by dynamic immunological interactions between the mother and fetus, as well as a semi-allogeneic placenta. During implantation and trophoblast invasion, as well as interactions with nearby immune cells, different hormones, cytokines, and protein factors are released from both the fetal tissues and the immune cells of the mother. These factors may synergistically contribute to sterile inflammation within the placental microenvironment. The mother's immune system regulates this sterile inflammatory state.

Blood tests can identify significant alterations in a woman's immune system adaptability and inflammatory response during gestation [1, 2]. To keep the fetus safe from germs and to control the mother's immune system so the fetus doesn't reject the mother, the body makes a lot of changes that cause higher levels of different inflammatory markers during pregnancy [1, 2]. An exacerbated inflammatory response during gestation has been associated with several complications of pregnancy, including hypertension, preeclampsia, premature birth, intrauterine growth restriction, depressed symptoms, and repeated abortion. Systemic inflammation normally occurs during pregnancy [1]. After an irritating stimulus, the body initiates the immune response to restore homeostasis [3]. Inflammation occurs also during the menstrual cycle, during early pregnancy, and at the time of birth [4].

Recent investigations have utilized inflammatory markers derived from common hemogram tests [5-10]. The markers include red cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR) and platelet-to-lymphocyte ratio (PLR). Given the complexity of inflammation, combined inflammatory indices SII (Systemic immune-inflammation index), SIRI (Systemic inflammatory response index), AISI (Aggregate index of systemic inflammation) have been established alongside the assessment of one or two inflammatory cells [11].

While research discussing alterations in inflammatory indicators during pregnancy exists [12], investigations examining the variations in hematological inflammatory biomarkers across trimesters throughout the entirety of pregnancy are scarce in the literature [13-15]. Our study aimed to examine the inflammatory status, which is meticulously regulated during pregnancy, and the variations between trimesters.

Consequently, our study aimed to retrospectively assess the differences among trimesters by analyzing the maternal hematological-inflammatory-biomarker data of healthy pregnant women.

MATERIALS AND METHODS

The study population consisted of 42 uncomplicated pregnancies who were within 14 gestational weeks and applied for routine pregnancy follow-up at Karaman Training and Research Hospital between 01.08.2023 and 01.05.2024. All participants provided written informed consent upon enrollment. The study accords with the Declaration of Helsinki and was conducted with the approval of the Karamanoglu Mehmetbey University Faculty of Medicine Clinical Research Ethics Committee on 29/05/2024, under decision number E-11095095-050.04-194274.

The study was planned retrospectively. The inclusion criteria were being pregnant over the age of 18, living in the Karaman region, who were citizens of the Republic of Türkiye, and regular follow-ups in every trimester. Gestational age was calculated based on the last menstrual period and confirmed via ultrasound examination. Pregnant women with age <18, accompanying diseases that could affect inflammation (history of hyperemesis gravidarum or bleeding during pregnancy), multiple pregnancies, type 1 or 2 Diabetes Mellitus, kidney or liver diseases, chronic inflammatory diseases, congenital fetal anomalies, corticosteroid use, and any inflammatory or malignant hematological disorders were not included in the study. The participants who did not attend polyclinic check-ups regularly and had lacked complete blood count (CBC) at enrollment or whose pregnancies ended for unexpected reasons (abortion, preterm delivery, etc.) were also excluded from the study. Finally, data from 42 participants with complete CBC results for all trimesters were analyzed.

Patient Population

Participant characteristics and maternal demographics, such as age, height, weight, body mass indices, disease and obstetric history and venous blood sample results for each trimester were mostly obtained via the hospital's computerized medical records system.

To evaluate the combined inflammation indices during pregnancy, we obtained the CBC parameters of every trimester and recorded the values of white blood cell (WBC), neutrophils (N), monocyteS (M), lymphocytes (L), platelet (P), hemoglobin (Hb), hematocrit count (Hct), red cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW) by scanning the hospital database. Specifically, the SII is calculated as PxN/L, the SIRI is calculated as NxM/L, and the AISI is calculated using the formula as NxPxM/L. In addition to these combined parameters, we explored three traditional inflammatory indicators: NLR (N/L), PLR (P/L) and MLR (M/L).

Statistical Analysis

The data set was created in the Excel program. Statistical analysis was performed using Jamovi (version 2.3). First, the fit of the parameters in the data set to the normal distribution was evaluated. Kolmogorov-Smirnov test was used for this purpose. Categorical data were presented as numbers and percentages. Since our study parameters did not fit the normal distribution, continuous data were given using medians and 25th and 75th percentiles. Neutrophil-lymphocyte ratio was included in the analyses as a reference index. Hematological data and indices of the control group

and case group were compared using the Mann-Whitney U test, one of the univariate tests. ROC analysis was performed to evaluate the indices' ability to predict acute cholecystitis. The area under the curve and cut-off values were calculated by ROC analysis. The results are presented as AUC, cut-off value, sensitivity, specificity, positive predictive value and negative predictive value. Two methods were used to compare the indices' ability to predict acute cholecystitis. One was to compare AUCs, for which the DeLong test was used. The other was odds ratios. According to the cut-off value obtained as a result of the ROC analysis, the indexes of the case group and control group were dichotomized. Odds ratios were calculated with dichotomized indexes. Chi-square test was used to compare groups in terms of categorical data.

RESULTS

The study comprised 42 pregnant women with an average age of 26.5 years (range: 23.0-30.0), and their average body mass index was 27.9 (range: 25.9-33.1). The average gestational age at delivery was 36.2 weeks for the vaginal birth group and 35.4 weeks for the cesarean section group. The mean weight of newborns was 3215.0 grams (range: 2864.0-3419.0 grams). Thirty-one (73.8%) of the pregnant women experienced normal vaginal deliveries, while eleven (26.2%) underwent cesarean sections.

Table 1 presents the demographic features and mean combined inflammatory index values of pregnant women according to their way of delivery. The research showed no statistically significant difference in the combined inflammatory markers of the third trimester between the delivery groups (p>0.01).

Upon separate comparison of the values in the first, second, and third trimesters, it was noted that neutrophil levels rose in the second trimester, although basophil values remained unchanged (p=0.017, p=0.021, respectively).

In the second trimester, there is a statistically significant decrease in RBC, hemoglobin, hematocrit, and platelet values (p<0.001, p=0.004, p=0.006, p=0.026, respectively), while MCV, RDW, and PDW exhibit a statistically significant increase (p<0.001 for all).

SIRI, AISI, NLR, and MLR were statistically substantially elevated in the second trimester compared to the other trimesters (p=0.001, p=0.042, p=0.002, p=0.015, respectively). No statistically significant difference was detected between SII and the trimesters. The hematological index values for the first, second, and third trimesters are presented in Table 2.

Upon examining the correlation of hematological inflammatory indices with advancing gestational age and among themselves, it was found that SIRI, NLR, and MLR exhibited a weak positive correlation with gestational weeks (Spearman's; rho=0.228, p=0.01; 0.191, p=0.032; 0.205, p=0.022, respectively) (Table 3).

Table 1. Demographic data and relationship between delivery mode and 3rd trimester inflammation indices

	Vaginal birth	Cesarean section	Total	p
Total N (%)	31 (73.8)	11 (26.2)	42	
Median (IQR)				
Weight of the newborn-gr	3235.0 (2867.5-3497.5)	3190.0 (2737.5-3362.5)	3215.0 (2863.8-3418.8)	0.511
Height of the newborn-cm	50.0 (49.0-50.0)	50.0 (49.5-50.0)	50.0 (49.0-50.0)	0.805
Gestational age	36.2 (35.2-36.5)	35.4 (35.0-36.2)	36.1 (35.1-36.4)	0.156
SIRI	2.0 (1.7-2.6)	1.6 (1.2-3.2)	2.0 (1.5-2.7)	0.399
SII	778.4 (602.6-982.1)	826.7 (655.2-906.0)	799.8 (623.6-943.4)	0.637
AISI	435.1 (385.4-545.7)	413.3 (378.6-751.6)	427.1 (382.3-627.6)	0.852
NLR	3.6 (2.8-4.1)	3.2 (2.8-3.7)	3.4 (2.8-4.0)	0.415
MLR	0.3 (0.2-0.3)	0.2 (0.2-0.3)	0.3 (0.2-0.3)	0.112
PLR	105.0 (80.4-127.9)	98.0 (86.7-126.6)	104.3 (83.2-128.0)	0.742

SIRI: Neutrophil*monocyte/ lymphocyte; SII: Systemic immune-inflammation index; AISI: Neutrophil*platelet*monocyte/ lymphocyte; NLR: Neutrophil/ lymphocyte ratio; MLR: Monocyte/ lymphocyte ratio; PLR: Platelet/lymphocyte ratio.

Table 2. Average hemogram and hematological index values of the first, second and third trimesters

Parameters	1.Trimester, n (%)	2. Trimester, n (%)	3. Trimester, n (%)	Total, n (%)	p
Median (IQR)	42 (33.3)	42 (33.3)	42 (33.3)	126	
WBC (x10^3/µL)	9.7 (8.1-11.7)	10.5 (9.3-12.7)	10.5 (9.1-11.8)	10.3 (8.8-11.9)	0.078
NEU (x10^3/µL)	6.7 (5.2-8.3)	7.4 (6.5-9.8)	7.4 (6.1-8.6)	7.2 (6.1-8.6)	**0.017**
LYMPH (x10^3/µL)	2.2 (2.0-2.6)	2.1 (1.7-2.4)	2.2 (1.9-2.5)	2.2 (1.9-2.5)	0.306
MONO (x10^3/µL)	0.5 (0.4-0.6)	0.6 (0.5-0.6)	0.6 (0.5-0.7)	0.6 (0.5-0.7)	**0.035**
EOS (x10^3/µL)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.1 (0.1-0.1)	0.1 (0.1-0.2)	0.330
BASO (x10^3/µL)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	**0.021**
NEU %	69.8 (65.0-71.4)	73.0 (69.2-77.0)	72.0 (67.7-74.7)	71.0 (67.9-74.5)	**0.002**
LYMPH %	23.5 (21.3-27.0)	19.8 (15.8-23.6)	21.0 (18.4-24.4)	21.7 (18.4-24.4)	**0.002**
MONO %	5.2 (4.8-6.3)	5.2 (4.5-5.9)	5.8 (4.8-6.7)	5.4 (4.5-6.4)	0.143
EOS %	1.3 (0.6-2.0)	1.3 (0.9-1.7)	1.1 (0.7-1.6)	1.2 (0.7-1.8)	0.452
BASO %	0.3 (0.2-0.4)	0.2 (0.2-0.3)	0.2 (0.2-0.3)	0.3 (0.2-0.3)	**<0.001**
RBC (x10^12/L)	4.6 (4.4-4.8)	4.1 (3.8-4.4)	4.3 (4.1-4.4)	4.3 (4.0-4.6)	**<0.001**
HGB (g/dL)	12.8 (12.2-13.5)	12.1 (11.2-12.6)	12.4 (11.5-13.4)	12.5 (11.6-13.1)	**0.004**
HCT %	38.5 (36.7-40.2)	36.3 (34.2-38.6)	37.5 (35.3-39.8)	37.5 (35.1-39.6)	**0.006**
MCV (fL)	86.9 (81.2-88.3)	89.2 (85.7-93.0)	90.1 (84.8-92.7)	88.3 (84.3-91.7)	**<0.001**
MCH (pg)	28.9 (26.7-29.8)	29.6 (28.5-30.9)	29.6 (28.2-31.4)	29.4 (27.8-30.6)	**0.037**
MCHC	33.5 (32.8-33.8)	33.1 (32.7-33.5)	32.9 (32.5-33.6)	33.1 (32.6-33.7)	0.143
RDW-CV (fl)	13.6 (13.0-14.6)	13.8 (13.2-14.9)	13.8 (13.4-15.1)	13.7 (13.2-14.9)	0.321
RDW-SD (fL)	41.8 (40.4-44.0)	45.0 (43.1-48.4)	44.5 (43.4-46.9)	44.0 (41.5-46.6)	**<0.001**
PLT (x10^3/µL)	259.0 (227.5-302.2)	242.5 (196.5-285.2)	228.5 (182.5-264.0)	245.0 (202.0-285.8)	**0.026**
MPV (fL)	9.9 (9.4-10.9)	10.1 (9.1-10.5)	10.2 (9.3-10.9)	10.1 (9.3-10.8)	0.355
PDW %	16.1 (15.7-16.2)	16.2 (16.0-16.5)	16.4 (16.2-16.7)	16.2 (15.9-16.5)	**<0.001**
SIRI	1.4 (1.2-1.9)	1.9 (1.6-2.9)	2.0 (1.5-2.7)	1.8 (1.3-2.6)	**0.001**
SII	754.3 (621.8-934.4)	870.3 (684.3-1268.1)	799.8 (623.6-943.4)	796.7 (638.0-1005.0)	0.111
AISI	389.5 (292.8-500.0)	510.0 (335.1-712.0)	427.1 (382.3-627.6)	445.3 (317.0-619.0)	**0.042**
NLR	3.0 (2.5-3.3)	3.7 (3.0-4.9)	3.4 (2.8-4.0)	3.3 (2.8-4.0)	**0.002**
MLR	0.2 (0.2-0.3)	0.3 (0.2-0.3)	0.3 (0.2-0.3)	0.3 (0.2-0.3)	**0.015**
PLR	121.9 (103.7-141.9)	116.8 (94.4-134.6)	104.3 (83.2-128.0)	115.7 (93.0-139.8)	0.144

WBC: White blood cell; NEU: Neutrophil; LYMP: Lymphocyte; MONO: Monocyte; EOS: Eosinofil; BASO: Basophile; RBC: Red blood cell; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; RDW: Red cell distribution width; PLT: Platelet; MPV: Mean platelet volume; PDW: Platelet distribution width, SIRI: Neutrophil*monocyte/ lymphocyte; SII: Systemic immune-inflammation index; AISI: Neutrophil*platelet*monocyte/ lymphocyte; NLR: Neutrophil/lymphocyte ratio; MLR: Monocyte/lymphocyte ratio; PLR: Platelet/lymphocyte ratio.

DISCUSSION

The maternal immune response is an essential factor in the development and continued existence of a healthy pregnancy. Considering that the fetus functions as a semi-graft, a successful pregnancy necessitates systems that avoid rejection of the allograft. The adaptation of the immune system is essential for protecting against the rejection of the semi-allograft conceptus during gestation [16].

Elevated inflammation in pregnancy has been associated with multiple adverse pregnancy outcomes and potentially an increased susceptibility to future chronic disease. But studies on inflammation markers in healthy pregnant women are

Table 3. Correlation of hematologic inflammatory indices with trimester increase

	Trimester	SIRI	SII	AISI	NLR	MLR	PLR
Trimester							
Pearson's r	—						
p	—						
SIRI							
Pearson's r	0.228	—					
p	0.010	—					
SII							
Pearson's r	0.070	0.678	—				
p	0.433	<0.001	—				
AISI							
Pearson's r	0.122	0.869	0.836	—			
p	0.173	<0.001	<0.001	—			
NLR							
Pearson's r	0.191	0.795	0.813	0.653	—		
p	0.032	<0.001	<0.001	<0.001	—		
MLR							
Pearson's r	0.205	0.801	0.411	0.619	0.584	—	
p	0.022	<0.001	<0.001	<0.001	<0.001	—	
PLR							
Pearson's r	-0.121	0.188	0.696	0.428	0.413	0.296	—
p	0.178	0.035	<0.001	<0.001	<0.001	<0.001	—

SIRI: Neutrophil*monocyte/lymphocyte; SII: Systemic immune-inflammation index; AISI: Neutrophil*platelet*monocyte/ lymphocyte; NLR: Neutrophil/ lymphocyte ratio; MLR: Monocyte/lymphocyte ratio; PLR: Platelet/lymphocyte ratio.

quite limited. Our study investigated the alterations of these hematological indices of maternal immune-inflammatory status throughout trimesters, revealing a significant rise in SIRI, AISI, NLR, and MLR during the second trimester. However, we observed a weak positive correlation among SIRI, AISI, MLR, and gestational age.

Inflammatory processes are key determinants of pregnancy outcomes, independent of infection. Cintesun et al. [5] investigated subclinical inflammation markers in hyperemesis gravidarum (HEG) (n=94), one of the pregnancy-related disorders in the first trimester, and the control group (n=100), finding a significant elevation of NLR and PLR in the HEG group [5]. In another study, the results of the hospitalized HEG cases, including NLR and PLR, were also high, consistent with the results of the previous study ($p<0.05$). They also found the SII and SIRI levels increased in the HEG group ($p<0.05$) [17]. The first trimester results of our research, including NLR, PLR, MLR, SIRI, and SII, were very similar to the results of the first trimester control groups in these two investigations.

Zhang et al. [13] conducted a study with over seventeen thousand pregnant women, which consisted of a control group and a GDM-diagnosed group. They researched SII and SIRI levels in all three trimesters of these groups. When they evaluated the SII, they found that the results increased in the second trimester but declined in the third ($p>0.05$). Additionally, both groups experienced an increase in SIRI results throughout pregnancy ($p>0.05$) [13]. When we consider the control group of this study, the results obtained from our research correlate with them. Furthermore, our investigation revealed that the increase in SIRI was statistically significant ($p<0.05$). However, the change in SII levels didn't have statistical significance.

In the first trimester, the effectiveness of NLR and PLR values in predicting pregnancy outcomes was investigated in pregnant women diagnosed with threatened abortion who were

hospitalized and received medical treatment. According to the results, the neutrophil counts in cases that resulted in abortion were found to be significantly higher when compared to cases that continued beyond the 24th week of pregnancy. Additionally, lymphocyte counts were significantly lower in the abortion group ($p<0.05$). Based on these findings, NLR was found to be higher in the group that resulted in abortion, but no significant difference was observed between the groups in terms of PLR ($p>0.05$) [8].

In another study conducted for the same purpose, the SII values in the group that resulted in abortion were found to be significantly higher, but no differences were observed between the groups in terms of SIRI, NLR, or PLR values [18]. In another research evaluating the potential role of complete blood count parameters in predicting preterm birth, pregnant women who delivered before and after 37 weeks were categorized. When neutrophil, leukocyte, lymphocyte, NLR, and PLR values were considered, no significant differences were found between the first and third trimester values among the groups [19].

Zheng et al. [14] evaluated neutrophil, monocyte, LDL, SII, and SIRI parameters in 223 pregnant women diagnosed with thrombophilia and compared these parameters with adverse pregnancy outcomes in this group. The presence of fetal death after the 12th week of gestation, neonatal asphyxia, preterm birth, pregnancy termination before the 36th week due to hypertension, preeclampsia, or placental insufficiency, and the presence of SGA (small for gestational age) were highlighted as predictive parameters for SII and LDH values [14].

The studies mentioned above have linked inflammatory markers with various pathological conditions seen during pregnancy. However, in our study, we prioritized trimester distributions of test results for completely normal pregnancies. In another study comparing the cesarean scar pregnancy group (n=23) with normal pregnancies (n=126), only the SII parameter was found to be significantly predictive for CSP, with a sensitivity of 73.9% and a specificity of 66.2%. This study highlighted that cesarean deliveries were associated with outcomes distinct from those of normal vaginal deliveries [15]. In our study, we compared the outcomes of cesarean deliveries and normal vaginal deliveries and determined that there were no significant differences in SII, SIRI, ASI, or other parameters.

As noted in studies related to pregnant women, normal pregnancies and complicated pregnancy groups were compared. For example, Forget et al. [10] in 2017 established reference values for NLR, a simple method for determining inflammation levels, in a non-geriatric population without acute or chronic diseases. In their study conducted with 413 participants aged 21–66 years, the mean NLR was determined to be 1.65, with a range of 0.78–3.58 [10]. In our study, the NLR values ranged from 2.5 to 4.9 across all trimesters, with a mean of 3.3. This difference may be attributed to the inflammatory adaptation process that we physiologically observed during pregnancy.

Limitations

The study possesses certain limitations. The parturients were from a single institution; thus, the sample size is restricted. Therefore, our findings may not accurately reflect the broader population. Future multicenter research with bigger patient cohorts may yield the cut-off values for inflammatory indices in pregnant women without comorbidities.

CONCLUSION

We have determined the standard range of inflammatory indicators in healthy pregnant individuals across each trimester. These data may assist the researcher and physicians in identifying cut-off values for inflammatory markers.

DECLARATIONS

Ethics Committee Approval: The Karamanoglu Mehmetbey University Faculty of Medicine Ethics Committee granted approval for this study (date: 29.05.2024, number: E-11095095-050.04-194274).

Author Contributions: Concept – OD, BI, HSA, AO; Design – OD, BI, HSA, AO; Supervision – OD, BI, HSA, AO; Resource – HSA, OD; Materials – OD; Data collection and/or processing – OD; Analysis and/or interpretation – HSA, AO; Literature review – BI, OD, HSA; Writing – HSA, OD; Critical Review – BI, HSA.

Conflict of Interest: The authors declared no conflict of interest.

Use of AI for Writing Assistance: The authors declared that they did not use AI-powered technologies such as big language models (LLMs), chat robots, or image creators, Chat GPT in this current study.

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