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Systemic inflammatory status – a bridge between gestational weight gain and neonatal outcomes (STROBE-compliant article)

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Abstract

Pregnant women with excessive gestational weight gain express an inflammatory status with multiple negative effects on birth outcomes.

The aim of this study was to identify the relationship between gestational weight gain at different gestational ages and inflammatory status in pregnant women and their newborns assessing both interleukin 6 and 8, as well as hepcidin in these couples.

Our study included 170 pregnant women and their newborns. Pregnant women were clinically assessed at the end of the 1st trimester and at term, whereas the newborns were assessed over the first 3 days of life. The levels of interleukin 6, 8 and hepcidin were measured in both pregnant women and their newborns.

We noticed higher levels of interleukin 6, interleukin 8 and hepcidin in pregnant women at the time of delivery as compared to the end of the 1st trimester. We observed a direct significant correlation between gestational weight gain at the time of delivery and interleukin 8 in both mothers [$r = 0.1834$, 95% CI: 0.0293–0.3290, ($P = .0167$)] and newborns [$r = 0.1790$, 95% CI: 0.0248–0.3249, ($P = .0195$)]. Our study underlined that a higher gestational weight gain resulted in a significantly higher birth weight [$r = 0.2190$, 95% CI: 0.0663–0.3617, ($P = .0041$)].

Our findings suggest that interleukin 8 might be an important indicator of inflammatory status in both mothers and newborns. Moreover, excessive gestational weight gain was associated with an increase in birth weight.

Abbreviations: BMI = body mass index, BW = birth weight, CBC = complete cellular blood count, CDC = Center for Disease Control, CI = confidence interval, CRP = C reactive protein, ELISA - enzyme Linked Immunosorbent assay, ESR = erythrocyte sedimentation rate, GWG = gestational weight gain, Hgb = hemoglobin, HDL = high density lipoprotein, IL-6 = interleukin 6, interleukin-8 = interleukin 8, IOM = Institute of Medicine, LDL = low density lipoprotein, n = number, r = correlation coefficient, statistical significance ($P < .05$), TNF = alfa – tumor necrosis alfa.

Keywords: birth weight, gestational weight gain, hepcidin, interleukins, pregnant woman

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1. Introduction

Weight gain during pregnancy is influenced by both genetic and environmental factors.[1] Despite the fact that genetic predisposition carries a great contribution in the determinism of gestational weight gain (GWG),[2–7] genetic factors are not modifiable, at least until now, by any external approaches. Therefore, in terms of prevention and therapeutic strategies, environmental factors become useful tools for decreasing the incidence of excessive GWG since they can be externally modified. Among environmental factors resulting in excessive GWG, dietary habits are probably the most important since they are easily changeable. Pregnant women must be aware that their inappropriate diet could result in either malnutrition or excessive GWG. It is also worth mentioning that inappropriate GWG results in negative impact on both mother and offspring.

It is well-documented that malnutrition during pregnancy is associated with multiple nutritional deficiencies. Nevertheless, excessive GWG due to a poor-quality diet might also lead to a wide-variety of nutritional deficiencies. Hepcidin is a hepatocyte-derived peptide hormone involved in iron homeostasis.[8] At the same time, hepcidin acts and an acute phase protein and inflammation positive regulator, its bioavailability being regulated by multiple systemic stimuli like anemia and iron concentration.[9,10] Its acting mechanism is based on its binding to ferroprotein, the essential transmembrane cellular iron efflux

protein, resulting in its degradation, preventing iron absorption in the bowel and its release from macrophages.[11] A study performed on pregnant women aiming to assess the correlation between GWG and iron serum levels proved that obese pregnant women express a higher risk for developing iron deficiency and that hepcidin might be a negative regulating factor of intestinal iron absorption and macrophage iron efflux.[12] Contrariwise, other findings revealed that serum iron is not associated with either pre pregnancy body mass index (BMI) nor with GWG, while interleukin (IL)-6 is positively associated with hepcidin at delivery time, but not at midgestation.[13] Nevertheless, the findings in the literature remain contradictory regarding this aspect since Dosch et al pointed a reverse correlation between ferritin and IL-6 in pregnant women with a BMI $> 35\,kg/m^2$. Moreover, the authors found a positive correlation between ferritin and hepcidin, but not between the latter one and maternal BMI or other inflammatory markers.[14]

Recent studies performed on different populations proved that obesity is associated with a low-grade systemic inflammatory status.[15,16] Similarly, it was hypothesized that pregnant women with excessive GWG might also express an inflammatory status with multiple negative effects on birth outcomes. Regarding this aspect, a recent study performed on 671 pregnant women assessed the correlations between the pregnant woman's diet, quantified through a questionnaire during the 30th week of pregnancy, GWG and certain inflammatory markers like C reactive protein (CRP), serum amyloid A, IL-6, IL-8, IL-1β, and tumor necrosis factor (TNF)-alpha. The authors underlined that a high intake of animal proteins and excessive GWG result in higher serum concentrations of inflammatory markers defining the so-called gestational inflammatory status proving that GWG was positively correlated with CRP and serum amyloid levels and negatively correlated with IL-8.[17] This inflammatory status was also proved to be associated with negative delivery outcomes since it might induce preterm labor. Thus, the same study mentioned above, concluded also that premature membrane rupture might be related to this systemic inflammatory status showing an association between this pathology and high levels of serum TNF-alpha and vascular endothelial growth factor in both maternal blood flow and newborns umbilical cord.[17] These findings suggest that newborns whose mothers express a systemic inflammatory status during pregnancy, might also be found with this systemic inflammation at the time of birth. Contrariwise, another study reported in the literature with a similar objective found low levels of IL-1 beta in both mothers and newborns.[18] Moreover, Zak et al proved a positive association between TNF alpha levels and the risk of preeclampsia and a negative one between TNF-alpha, IL-6, IL-10 and birth weight (BW).[19] Cao et al showed that serum leptin along with CRP and IL-6 have higher levels in obese pregnant women versus normal weight ones at midgestation.[13] The same study noticed that the levels of these cytokines tend to change during different stages of pregnancy showing that at time of delivery, leptin levels remain high, but IL-6 levels become similar to those of normal GWG women. Contrariwise, the study of Perng et al found no correlation between IL-6, TNF-alpha and GWG suggesting that post-partum inflammation is not related to maternal weight or GWG.[20]

The aim of this study was to identify the relationship between GWG at different gestational ages (after the 1st trimester and at delivery time) and inflammatory status of both pregnant women and their newborns through the assessment of IL 6, IL 8 and hepcidin in these couples.

2. Material and methods

2.1. Study sample selection

We performed a prospective observational study on 170 pregnant women aged between 19 and 40 years and their newborns, referred to an Emergency County Hospital from Romania, between July 2017 and December 2019. The pregnant women with the age range mentioned above were included in the study based on the inclusion and exclusion criteria at the end of the 1st trimester of pregnancy. Pregnant women were assessed at the end of the 1st trimester of pregnancy and at the time of delivery, while newborns were assessed on the 2nd or 3rd day of life. We applied the Institute of Medicine (IOM) classification,[21] for GWG: for BMI $< 18{,}5\,kg/m^2$ - underweight women at the beginning of the pregnancy, the recommended GWG is 12.5–18 kg; for BMI = 18, 5–24.9 kg/m^2 - normal weight women, the recommended GWG is 11.5–16 kg; for BMI = 25–29.9 kg/m^2 - overweight women, the recommended GWG is 7.00–11.5 kg; while for BMI $> 30\,kg/m^2$ - obese women, the recommended GWG is 5–9 kg.[21]

The inclusion criteria consisted of full-term pregnancies and women with a single pregnancy. The exclusion criteria were preterm delivery, maternal underweight at the beginning of the pregnancy, suspect or documented intrauterine infection, chromosomal disorders, fetal congenital syndromes, maternal history of chronic disorders such as high value of glycemia, arterial hypertension, premature rupture of membranes (excluded by vaginal exam and ultrasound assessment), incomplete data, and pregnant women who refused to sign the informed consent form. All pregnant women underwent a thorough anamnesis in order to rule out potential risk factors (drugs use, alcohol consumption, congenital or acquired maternal hypoxia, exposure to environmental toxins), and clinical exam during each routine consult including weight, height and blood pressure monitoring, as well as ultrasound exam. According to the recommendation of our health care system, all pregnant women underwent a serological screening for the most common infections with a major negative impact on fetal development (e.g., toxoplasmosis, cytomegalovirus, Rubella, Herpes virus, etc.), as well as a genetic screening for the most frequent chromosomal disorders (e.g., trisomy 13, trisomy 21, etc.). Moreover, all newborns were clinically assessed and monitored at the time of birth and during their hospitalization (ranging between 3–5 days) ruling out those with sings of perinatal or postpartum infection, with clinical signs of necrotizing enterocolitis, and those with a positive gastric aspirate (harvested if rupture of membranes occurred more than 24 hours prior to birth) or elevated inflammatory biomarkers (CRP, ESR). According to the Centers for Disease Control and Prevention (CDC), newborns with the birth weight between 2500 to 4000 grams are considered appropriate for gestational age if born at term,[22,23] as in our study.

We assessed different laboratory parameters (Hemoglobin – Hgb, CRP, ESR, Iron, protein, cholesterol, triglyceride, HDL – high density lipoprotein, LDL – low density lipoprotein), and the serum levels of IL-6, IL-8 and hepcidin in pregnant women after the 1st trimester of pregnancy and at the time of delivery, but also in their newborns. Cholesterol, triglycerides, and protein levels

were measured by spectrophotometry on a Cobas Integra 400 plus automated analyzer for all surveyed mothers and newborns. The IL-6 analysis was performed by chemiluminescence with the analyzer Immulite 2000 Siemens Healthcare system (detection limit 2 pg/ml, coefficient of variation: intra-run <5% and inter-run <7.5%). The IL-8 and Hepcidin determination were realized by sandwich ELISA technique (enzyme Linked Immunosorbent assay) using an automate ELISA DSX Dynex analyzer Technologies, USA (DRG Instruments GmbH Germany). The detection limit for IL-8 was 1.1 pg/ml, coefficient of variation: intra-run <4% and inter-run <13.5% with an indicative reference value for healthy persons about 132 pg/ml, while for hepcidin the measurement interval was 0.153 to 81 ng/ml, with a coefficient of variation: intra-run and inter-run <15% with an indicative reference value for healthy persons about 0.25 to 47.66 ng/ml.

All pregnant women signed the informed consent prior to the inclusions in the study on their behalf and their children's. The study was approved by the Ethics Committee of the University of Medicine, Pharmacy, Sciences and Technology from Târgu Mureş (No 138/05.07.2018), and it was performed according to the principles of the Helsinki Declaration.

2.2. Statistical analysis

The statistical analysis comprised elements of descriptive statistics (frequency, percent, mean, median, standard deviation), as well as inferential statistics ones. The Shapiro–Wilk test was applied in order to assess the distribution of the analyzed series. We applied the Pearson correlation (most appropriate for measurements taken from an interval scale) in order to measure the strength of association between GWG variables and cytokine variables and the direction of the relationship and linear regression to evaluate the impact of a unit change in the independent variables on the dependent variables. The chosen significance threshold for P value was .05. The statistical analysis was performed using the program Graph Pad Prism, trial variant.

3. Results

3.1. Demographic aspects and descriptive analysis of laboratory parameters and cytokines levels

The mean age of the pregnant women included in our study was 29.35 ± 4.28 years, with a median age of 29 years. Concerning the environment, 71.76% of the pregnant women were from urban area and 28.24% from rural area. Regarding the educational level, we noticed that 54.71% (93) of the pregnant women had superior level of education as compared to 45.29% (77), who only graduated high school. Assessing the parity, our study underlined that more than half of the pregnant women (52.94%) were primipara, 37.65% were secundipara and only 9.41% were tertipara. The mean GWG at the end of 1st trimester was 2.04 ± 2.50 kg, with a median value of 2 kg, while at the time of delivery; we noticed a mean GWG of 15.56 ± 4.85 kg, with a median value of 15 kg (Table 1).

The descriptive analysis of the laboratory parameters was detailed in Table 1. Thus, regarding the lipid profile parameters, we noticed that both cholesterol and triglycerides increased during gestation: the level of cholesterol increased from 190.0 ± 36.33 mg/dl at the end of the 1st trimester to 245.9 ± 52.14 mg/dl at the time of delivery, while the triglycerides doubled their value during gestation (113.1 ± 44.07 mg/dl at the end of the 1st trimester versus 223.3 ± 92.05 mg/dl higher at the time of delivery). Concerning the inflammatory biomarkers, both CRP and ESR showed an increase in value at the time of delivery when compared to the end of the 1st trimester: CRP was 7 folds higher at the time of delivery, 37.06 ± 50.36 mg/l vs 5.013 ± 4.215 mg/l at the end of the 1st trimester; while ESR values were more than 2 folds higher at the end of gestation, 38.76 ± 15.25 mm/hour as compared to 16.82 ± 9.595 mm/hour at the end of the 1st trimester (Table 1).

We noticed a doubling of IL-8 value at the time of delivery as compared to the end of the 1st trimester: 36.34 ± 33.70 pg/ml at the end of the 1st trimester versus 82.05 ± 71.85 pg/ml at the end of pregnancy. In terms of IL-6, our study pointed out a triple value at the time of delivery as compared to the end of the 1st trimester: 2.77 ± 1.74 pg/ml at the end of the 1st trimester and 10.59 ± 11.80 pg/ml at term. Similarly, hepcidin levels were also higher at the time of delivery when compared to the initial assessment: 8.11 ± 10.04 ng/ml at the end of 1st trimester vs 12.03 ± 20.76 ng/ml at delivery time (Table 1).

Table 1

The descriptive analysis of laboratory parameters and cytokines levels.

Variables (n = 170)	Mean ± SD	Median value
Pregnant women		
Age of pregnant women (years)	29.35 ± 4.28	29.00
At the end of first trimester		
GWG (kg)	2.04 ± 2.50	2.00
IL 8 (pg/ml)	36.34 ± 33.70	28.41
Hepcidin (pg/ml)	8.11 ± 10.04	5.15
IL 6 (pg/ml)	2.77 ± 1.74	2.14
Hemoglobin (g/dl)	12.30 ± 0.9048	12.30
Total proteins (g/dl)	6.829 ± 0.3968	6.805
Cholesterol (mg/dl)	190.0 ± 36.33	187.5
LDL cholesterol (mg/dl)	115.9 ± 32.90	113.3
HDL cholesterol (mg/dl)	74.29 ± 15.18	73.35
Triglycerides (mg/dl)	113.1 ± 44.07	105.7
Iron (mg/dl)	105.9 ± 32.88	107.9
CRP (mg/l)	5.013 ± 4.215	3.56
ESR (mm/h)	16.82 ± 9.595	14.00
At delivery time		
GWG (kg)	15.56 ± 4.85	15.00
IL 8 (pg/ml)	82.05 ± 71.85	64.36
Hepcidin (pg/ml)	12.03 ± 20.76	4.65
IL 6 (pg/ml)	10.59 ± 11.80	7.08
Hemoglobin (g/dl)	11.80 ± 1.149	11.90
Total proteins (g/dl)	6.187 ± 0.5663	6.285
Cholesterol (mg/dl)	245.9 ± 52.14	240.5
LDL cholesterol (mg/dl)	157.7 ± 50.59	151.0
HDL cholesterol (mg/dl)	70.97 ± 25.02	68.80
Triglycerides (mg/dl)	223.3 ± 92.05	213.8
Iron (mg/dl)	83.87 ± 68.31	68.77
CRP (mg/l)	37.06 ± 50.36	16.46
ESR (mm/h)	38.76 ± 15.25	36.50
Newborns		
Birth weight (gr)	3389 ± 331.80	3375
IL 8 at birth (pg/ml)	385.4 ± 429.2	233.40
Hepcidin at birth (ng/ml)	41.08 ± 20.42	37.30
IL 6 at birth (pg/ml)	19.77 ± 20.29	13.40

CRP = C reactive protein, ESR = erythrocyte sedimentation rate, GWG = gestational weight gain, HDL = high density lipoprotein, IL = interleukin, n = number, LDL = low density lipoprotein, SD = standard deviation, statistical significance ($P < .05$).

Table 2

The correlations between GWG and laboratory parameters in pregnant women.

	GWG		
Variables (n = 170)	**r coefficient**	**95% Confidence Interval**	***P* value**
At the end of first trimester			
Hemoglobin (g/dl)	0.03759	−0.1136 to 0.1871	.6265
CRP (mg/l)	−0.003476	−0.1539 to 0.1472	.9641
ESR (mm/h)	−0.1285	−0.2737 to 0.02253	.0950
Iron (mg/dl)	0.001278	−0.1493 to 0.1518	.9868
Total proteins (g/dl)	−0.09352	−0.2407 to 0.05785	.2251
Cholesterol (mg/dl)	−0.01550	−0.1657 to 0.1354	.8410
LDL-Cholesterol (mg/dl)	−0.05942	−0.2081 to 0.09196	.4415
HDL-Cholesterol (mg/dl)	0.1612	0.01089 to 0.3043	.0357
Triglycerides (mg/dl)	−0.08072	−0.2285 to 0.07069	.2954
At delivery time			
Hemoglobin (g/dl)	0.005365	−0.1453 to 0.1558	.9446
CRP (mg/l)	−0.08524	−0.2328 to 0.06616	.2691
ESR (mm/h)	0.02515	−0.1259 to 0.1750	.7448
Iron (mg/dl)	0.01473	−0.1361 to 0.1649	.8488
Total proteins (g/dl)	0.003350	−0.1473 to 0.1538	.9654
Cholesterol (mg/dl)	0.1071	−0.04412 to 0.2536	.1643
LDL-Cholesterol (mg/dl)	0.07685	−0.07457 to 0.2248	.3192
HDL-Cholesterol (mg/dl)	0.04678	−0.1045 to 0.1959	.5447
Triglycerides (mg/dl)	0.1050	−0.04626 to 0.2516	.1729

CRP = C reactive protein, ESR = erythrocyte sedimentation rate, GWG = gestational weight gain, Hgb = hemoglobin, HDL = high density lipoprotein, LDL = low density lipoprotein, n = number, r = correlation coefficient, statistical significance ($P < .05$).

Regarding the assessment of newborns, we noticed a mean BW of 3389 ± 331.80 g, a mean value of IL-8 of 385.40 ± 429.20 pg/ml, a hepcidin mean value of 41.08 ± 20.42 ng/ml, and an IL-6 mean value of 19.77 ± 20.29 pg/ml (Table 1).

Correlating laboratory parameters with GWG at the end of the 1st trimester of pregnancy and at the time of delivery, we noticed a direct significant correlation only for HDL-cholesterol at the end of the 1st trimester [$r = 0.1612$, 95% CI: 0.01089–0.3043, ($P = .0357$)], proving that a higher value of GWG results in a higher value of HDL-cholesterol in mothers, i.e., for every 1 kg in GWG, HDL-cholesterol increases with 0.978 mg/dl (Table 2). The findings are described in Table 2.

3.2. The correlations between GWG at the end of 1st trimester, BW and cytokines values

We observed a reverse dependency (negative relationship) between the GWG at the end of the 1st trimester of pregnancy and both IL-8 and hepcidin ($r = -0.06807/-0.06838$), and a direct dependency (positive relationship) between the GWG at the end of the 1st trimester of pregnancy and IL-6 in pregnant women ($r = 0.08695$), but without statistical significance ($P = .3777/.3756/.2595$) (Table 3). We observed a direct dependency between the GWG at the end of the 1st trimester of pregnancy and both BW and IL-6 levels in newborns ($r = 0.07502/0.003291$), and a reverse dependency in terms of GWG and both IL-8 and hepcidin levels ($r = -0.07650/-0.01629$), all without statistical significance ($P = .3309/.3214/.8330/.9660$) (Table 3).

3.3. The correlations between GWG at the time of delivery, BW and cytokines values

We observed a direct significant correlation between the GWG at the time of delivery and IL 8 [$r = 0.1835$, 95% CI: 0.03388–0.3251, ($P = .0166$)], proving that a higher value of GWG results in a higher value of IL-8 in mothers (Table 4), and for every 1 kg in GWG values we noticed an increase in IL-8 value of 2.721 pg/ml. Contrariwise, we found a reverse dependency between GWG and both hepcidin and IL-6 at the time of delivery in pregnant women ($r = -0.07520/-0.02920$), but without statistical significance ($P = .3298/.7055$) (Table 4).

Our study underlined that excessive GWG significantly increases BW, pointing out a direct significant correlation between GWG at the time of delivery and BW [$r = 0.2171$, 95% CI: 0.06883–0.3560, ($P = .0045$)] (Table 3), and for every 1 kg in GWG values we found an increase in BW value of 14.866 grams.

Regarding the relationship between GWG at the time of delivery and the newborns' inflammatory status assessed in our study, we obtained a direct correlation for IL-8 in newborns [$r = 0.04028$], and a reverse dependency between GWG and both hepcidin and IL-6 at the time of delivery in newborns but without statistical significance ($P = .6020/.3304/.9678$) (Table 4).

Table 3

The correlations between GWG at the end of 1st trimester, BW and cytokines values.

	GWG at delivery time		
Variables (n = 170)	**r coefficient**	**95% Confidence Interval**	***P* value**
Pregnant women at the end of first trimester			
IL 8 (pg/ml)	−0.06807	−0.2164 to 0.08333	.3777
Hepcidin (ng/ml)	−0.06838	−0.2167 to 0.08302	.3756
IL 6 (pg/ml)	0.08695	−0.06444 to 0.2344	.2595
Newborns			
Birth weight (gr)	0.07502	−0.07639 to 0.2231	.3309
IL 8 (pg/ml)	−0.07650	−0.2245 to 0.07491	.3214
Hepcidin (ng/ml)	−0.01629	−0.1664 to 0.1346	.8330
IL 6 (pg/ml)	0.003291	−0.1473 to 0.1538	.9660

GWG = gestational weight gain, IL = interleukin, LDL = low density lipoprotein, n = number, r = correlation coefficient, statistical significance ($P < .05$).

3.4. Correlations between inflammatory biomarkers, lipid profile parameters and cytokine levels

We observed a direct significant correlation between CRP values at the time of delivery and both hepcidin and IL-6 at the time of delivery in pregnant women ($P < .0001$), showing that a higher value of CRP results in a higher values of hepcidin, respectively

Table 4

The correlations between GWG at delivery time, BW and cytokines values.

	GWG at delivery time		
Variables (n = 170)	**r coefficient**	**95% Confidence Interval**	***P* value**
Pregnant women at delivery time			
IL8 (pg/ml)	0.1835	0.03388 to 0.3251	.0166
Hepcidin (ng/ml)	−0.07520	−0.2232 to 0.07622	.3298
IL6 (pg/ ml)	−0.02920	−0.1790 to 0.1219	.7055
Newborns			
Birth weight (gr)	0.2171	0.06883 to 0.3560	.0045
IL 8 (pg/ ml)	0.04028	−0.1109 to 0.1897	.6020
Hepcidin (ng/ml)	−0.07510	−0.2231 to 0.07632	.3304
IL 6 (pg/ ml)	−0.003123	−0.1536 to 0.1475	.9678

GWG = gestational weight gain, IL = interleukin, n = number, r = correlation coefficient, statistical significance ($P < .05$).

IL-6 in mothers [$r = 0.5489$, 95% CI: 0.4342–0.6461; $r = 0.3018$, 95% CI: 0.1585–0.4327]. Assessing the influence of CRP at the time of delivery on cytokine variables, we noticed that in case of hepcidin every 1-unit increase in CRP values led to an increase in hepcidin values of 0.226 ng/ml. Similar results were obtained for IL-6, i.e., for every 1-unit increase in CRP values, we noticed an increase in IL-6 values of 0.071 pg/ml. Nevertheless, in terms of IL-8, we found that CRP values do not significantly predict the IL-8 values in mother. Evaluating ESR values at the time of delivery we observed a direct significant correlation only with hepcidin [$r = 0.2605$, 95% CI: 0.1144–0.3955; $P = .0006$], showing that for every 1-unit increase in ESR values, hepcidin values consecutively increase with 0.355 ng/ml.

4. Discussions

Low-grade systemic inflammatory status represents the main focus of multiple recently reported studies and it has been associated to multiple conditions.[15,24] Nevertheless, obesity is one of the most important conditions that induces the production of proinflammatory cytokines resulting in a low-grade systemic inflammation.[16] Moreover, the incidence of this condition is increasing worldwide regardless of the age, being the most common issue in obstetrics with negative outcomes for both mother and her newborn.[25] The transfer of inflammation from mother to fetus in the setting of obesity might be produced directly through placenta, and indirect due to excessive lipid transfer from mother to fetus inducing the fetal secretion of proinflammatory cytokines.[26] The newborns whose mothers are obese have a long-term risk for developing obesity and metabolic dysfunction.[25]

Weight gain during pregnancy not only differs during the 3 trimesters, but these differences were proven to be related with certain complications. Thus, weight gain during the 1st and 2nd trimesters was associated with the development of excessive GWG,[27] gestational diabetes mellitus,[28] and postpartum weight retention.[29] It is well-known that inflammation associated with weight gain is a result of the synthesis of proinflammatory biomarkers from adipocytes.[16] Our study also assessed the relationship between GWG at the end of the 1st trimester and inflammatory status in both mothers and newborns. Despite the fact that we found no significant association, we noticed that an increased GWG is associated with higher levels of IL-6, IL-8 and hepcidin in mothers. Our findings suggest that an early gestational inflammatory status might be detected even at the end of the 1st trimester of pregnancy providing the ideal opportunity to intervene before further excessive GWG occurs. Thus, we might hypothesize that proper nutritional interventions implemented early during pregnancy might reduce the risk for systemic inflammation in both mother and offspring.

It is a well-documented fact that IL-6 is commonly elevated in individuals with excessive weight gain.[30] Moreover, this cytokine was proved to be involved in the synthesis and up-regulation of CRP.[16] IL-6 is a particular cytokine since it owns a dichotomous role expressing both pro-inflammatory response present in different pathologies such as obesity, triggered by a trans-signaling mode; and an anti-inflammatory response mediated by the classic signaling mode.[31] Our study underlined a direct dependency between CRP levels and IL-6, proving that an increase in CRP led to a consecutive increase in IL-6 levels in pregnant women. These findings suggest that inflammatory biomarkers and cytokines are strongly related in the setting of low-grade inflammation associated to excessive GWG. Contrariwise, other studies that assessed IL-6 levels at different gestational ages concluded that even though they might be initially high in obese pregnant women, they were similar to those of normal weight women at the end of pregnancy.[13] Moreover, other authors found no relationship between IL-6 and GWG.[20] Despite the lack of significance, our study also suggested that GWG at term might be reversely correlated with IL-6 levels in both mothers and newborns.

As we already mentioned above, hepcidin is a negative regulating factor that might result in iron deficiency. In the setting of a healthy pregnancy, this marker is low, enabling iron transfer to the fetus.[32,33] Additionally, hepcidin might be considered a positive regulator of inflammation,[10] fact sustained also by our findings since we noticed that increased levels of CRP and ESR result in elevated levels of hepcidin. Nevertheless, iron deficiency is commonly noticed in obese pregnant women.[34] Contrariwise, other studies found no relationship between hepcidin levels and obesity in pregnant women.[13,35] Thus, Anelli et al noticed that lower levels of hemoglobin were correlated with obesity in pregnant women, but not related to the increase in hepcidin.[10] The authors suggested a potential compensatory mechanism to obesity-associated inflammation defined by the elevated hepcidin levels encountered in obese mothers. Our study supports the findings of Anelli et al[10] since we noticed a reverse dependency between GWG at the time of delivery and hepcidin levels in both mothers and newborns, but without statistical significance.

IL-8 is a chemokine synthesized by different tissues and blood cells, which induces both chemotaxis and phagocytosis at the site of inflammation.[36] This proinflammatory cytokine was related to negative birth outcomes as it was emphasized by a recent study performed on 160 pregnant women, which proved that IL-8 expression was positively associated with the severity of preeclampsia.[37] Similarly, our study also revealed a significant direct correlation between GWG and IL-8 in both mothers and offspring suggesting that a higher weight gain during pregnancy results in increased levels of IL-8 in these couples. Nevertheless, the reports from the literature remain contradictory since other studies noticed a negative correlation between GWG and IL-8 levels.[17]

The limitations of our study consist mainly in the relatively small number of cases and the fact that we did not assess the levels of proinflammatory cytokines before pregnancy in order to have a more accurate comparison. Moreover, it would have been useful to assess also different gene polymorphisms of these cytokines in both mothers and newborns. Another potential limitation might be represented by the fact that our sample of pregnant women originated from a single area of Romania.

Nevertheless, our study is among the few that aimed to assess the inflammatory status in pregnant women and their newborns. One of the most important strengths is that we assessed this inflammatory status in both pregnant women and newborns taking into account GWG as the main connection between them. Moreover, our findings might represent a strong basis for further studies on the same topic.

5. Conclusions

Gestational inflammatory status is closely related to the neonatal one. Our findings revealed that excessive GWG at the end of the pregnancy might result in higher levels of IL-8 in mothers. Moreover, we might state that excessive GWG could lead to a

consecutively higher BW and a potential neonatal inflammatory status revealed by elevated levels of IL-8 in newborns. Thus, our results suggest that IL-8 might be an important indicator of systemic inflammatory status in both mothers and newborns. We also noticed a significant positive relationship between inflammatory biomarkers and IL-6 and hepcidin. Nevertheless, further studies are needed on bigger cohorts taking into account also other genetic and environmental parameters in order to define precisely the determinism of both gestational and neonatal inflammatory status.

Author contributions

Dr Rugină Cosmin, Prof Cristina Oana Mărginean, Dr Lorena Elena Meliţ, Dr Viviana Modi and Dr Mărginean Claudiu conceptualized and designed the study, drafted the initial manuscript, and revised the manuscript.
Dr. Adina Huţanu performed the laboratory analysis
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References

[1] Mărginean CO, Mărginean C, Meliţ LE. New insights regarding genetic aspects of childhood obesity: a mini review. Front Pediatr 2018;6:271.
[2] Mărginean C, Mărginean CO, Bănescu C, et al. Impact of demographic, genetic, and bioimpedance factors on gestational weight gain and birth weight in a Romanian population: a cross-sectional study in mothers and their newborns: the Monebo study (STROBE-compliant article). Medicine (Baltimore) 2016;95:e4098.
[3] Mărginean C, Mărginean CO, Iancu M, et al. The role of TGF-β1 869 T>C and PPAR γ2 34 C>G polymorphisms, fat mass, and anthropometric characteristics in predicting childhood obesity at birth: a cross-sectional study according the parental characteristics and newborn's risk for child obesity (the newborns obesity's risk) NOR study. Medicine (Baltimore) 2016;95:e4265.
[4] Mărginean C, Bănescu CV, Mărginean CO, et al. Glutathione S-transferase (GSTM1, GSTT1) gene polymorphisms, maternal gestational weight gain, bioimpedance factors and their relationship with birth weight: a cross-sectional study in Romanian mothers and their newborns. Rom J Morphol Embryol 2017;58:1285–93.
[5] Mărginean CO, Mărginean C, Bănescu C, et al. The relationship between MMP9 and ADRA2A gene polymorphisms and mothers-newborns' nutritional status: an exploratory path model (STROBE compliant article). Pediatr Res 2019;85:822–9.
[6] Mărginean C, Mărginean CO, Iancu M, et al. The FTO rs9939609 and LEPR rs1137101 mothers-newborns gene polymorphisms and maternal fat mass index effects on anthropometric characteristics in newborns: a cross-sectional study on mothers-newborns gene polymorphisms-The FTO-LEPR Study (STROBE-compliant article). Medicine (Baltimore) 2016;95:e5551.
[7] Mărginean C, Mărginean CO, Bănescu C, et al. The relationship among GNB3 rs5443, PNPLA3 rs738409, GCKR rs780094 gene polymorphisms, type of maternal gestational weight gain and neonatal outcomes (STROBE-compliant article). Medicine (Baltimore) 2019;98:e16414.
[8] Andrews NC. Forging a field: the golden age of iron biology. Blood 2008;112:219–30.
[9] Koenig MD, Tussing-Humphreys L, Day J, et al. Hepcidin and iron homeostasis during pregnancy. Nutrients 2014;6:3062–83.
[10] Anelli GM, Cardellicchio M, Novielli C, et al. Mitochondrial content and hepcidin are increased in obese pregnant mothers. J Matern Fetal Neonatal Med 2018;31:2388–95.
[11] Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood 2003;102:783–8.
[12] Garcia-Valdes L, Campoy C, Hayes H, et al. The impact of maternal obesity on iron status, placental transferrin receptor expression and hepcidin expression in human pregnancy. Int J Obes (Lond) 2015; 39:571–8.
[13] Cao C, Pressman EK, Cooper EM, et al. Prepregnancy body mass index and gestational weight gain have no negative impact on maternal or neonatal iron status. Reprod Sci 2016;23:613–22.
[14] Dosch NC, Guslits EF, Weber MB, et al. Maternal obesity affects inflammatory and iron indices in umbilical cord blood. J Pediatr 2016;172:20–8.
[15] Mărginean CO, Meliţ LE, Ghiga DV, et al. Early inflammatory status related to pediatric obesity. Front Pediatr 2019;7:241.
[16] Mărginean CO, Meliţ LE, Huţanu A, et al. The adipokines and inflammatory status in the era of pediatric obesity. Cytokine 2020; 126:154925.
[17] Hrolfsdottir L, Schalkwijk CG, Birgisdottir BE, et al. Maternal diet, gestational weight gain, and inflammatory markers during pregnancy. Obesity (Silver Spring) 2016;24:2133–9.
[18] Kucukgul S, Ozkan ZS, Yavuzkir S, et al. Investigation of the maternal and cord plasma levels of IL-1 beta, TNF-alpha and VEGF in early membrane rupture. J Matern Fetal Neonatal Med 2016;29: 2157–60.
[19] Žák P, Souček M. Correlation of tumor necrosis factor alpha, interleukin 6 and interleukin 10 with blood pressure, risk of preeclampsia and low birth weight in gestational diabetes. Physiol Res 2019;68:395–408.
[20] Perng W, Rifas-Shiman SL, Rich-Edwards JW, et al. Inflammation and weight gain in reproductive-aged women. Ann Hum Biol 2016; 43:91–5.
[21] Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines. Weight Gain During Pregnancy: Reexamining the Guidelines. (Rasmussen KM, Yaktine AL, editors). Washington (DC): National Academies Press (US); 2009 [cited 2020 Oct 27]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK32813/
[22] Growth Charts - WHO Child Growth Standards [Internet]. 2019 [cited 2020 Oct 27]. Available from: https://www.cdc.gov/growthcharts/who_charts.htm
[23] Pocobelli G, Dublin S, Enquobahrie DA, et al. Birth weight and birth weight for gestational age in relation to risk of hospitalization with primary hypertension in children and young adults. Matern Child Health J 2016;20:1415–23.
[24] Meliţ LE, Mărginean MO, Mocan S, et al. The usefulness of inflammatory biomarkers in diagnosing child and adolescent's gastritis: STROBE compliant article. Medicine (Baltimore) 2019;98: e16188.
[25] Catalano PM, Shankar K. Obesity and pregnancy: mechanisms of short term and long term adverse consequences for mother and child. BMJ 2017;356:j1.
[26] Heerwagen MJR, Miller MR, Barbour LA, et al. Maternal obesity and fetal metabolic programming: a fertile epigenetic soil. Am J Physiol Regul Integr Comp Physiol 2010;299:R711–722.
[27] Overcash RT, Hull AD, Moore TR, et al. Early second trimester weight gain in obese women predicts excessive gestational weight gain in pregnancy. Matern Child Health J 2015;19:2412–8.

[28] Gibson KS, Waters TP, Catalano PM. Maternal weight gain in women who develop gestational diabetes mellitus. Obstet Gynecol 2012;119:560–5.
[29] Walter JR, Perng W, Kleinman KP, et al. Associations of trimester-specific gestational weight gain with maternal adiposity and systolic blood pressure at 3 and 7 years postpartum. Am J Obstet Gynecol 2015;212:499e1-12.
[30] Mărginean C, Bănescu C, Duicu C, et al. The role of IL-6 572C/G, 190 C/T, and 174 G/C gene polymorphisms in children's obesity. Eur J Pediatr 2014;173:1285–96.
[31] Qu D, Liu J, Lau CW, et al. IL-6 in diabetes and cardiovascular complications. Br J Pharmacol 2014;171:3595–603.
[32] Hedengran KK, Nelson D, Andersen MR, et al. Hepcidin levels are low during pregnancy and increase around delivery in women without iron deficiency - a prospective cohort study. J Matern Fetal Neonatal Med 2016;29:1506–8.
[33] Kulik-Rechberger B, Kosciesza A, Szponar E, et al. Hepcidin and iron status in pregnant women and full-term newborns in first days of life. Ginekol Pol 2016;87:288–92.
[34] Lopez A, Cacoub P, Macdougall IC, et al. Iron deficiency anaemia. Lancet 2016;387:907–16.
[35] Jones AD, Zhao G, Jiang Y-P, et al. Maternal obesity during pregnancy is negatively associated with maternal and neonatal iron status. Eur J Clin Nutr 2016;70:918–24.
[36] Calder PC, Ahluwalia N, Brouns F, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr 2011;106 (Suppl 3):S5–78.
[37] Sun L, Mao D, Cai Y, et al. Association between higher expression of interleukin-8 (IL-8) and haplotype -353A/-251A/+678T of IL-8 gene with preeclampsia: a case-control study. Medicine (Baltimore) 2016;95: e5537.