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Fetal lung maturity assessment with lamellar body count in women with obesity and excessive gestational weight gain

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Abstract

The aim of this study was to evaluate fetal lung maturation in term pregnant women with obesity and excessive gestational weight gain using the lamellar body count (LBC) test in amniotic fluid samples obtained during cesarean section. This retrospective study included women experiencing term pregnancies and undergoing cesarean delivery, categorized as normal weight (n=37), overweight (n=59), or obese (n=48) due to body mass index values of 18.5 to 24.9; 25 to 29.9; and ≥30, respectively, as well as presence of excessive weight gain during pregnancy. During cesarean delivery, amniotic fluid samples were obtained without contamination of blood or meconium. Lamellar bodies in these samples were quantified using a standard hematologic counter. No significant difference was found among the LBC of the normal weight, overweight, and obese groups [42 (32-60), 40 (26-63), and 43 (24-52) x103/μL; p=0.307], although there was a subtle decrease in the LBC of the obese group with excessive weight gain. Although it is noteworthy that the LBC in the amniotic fluid of mothers with obesity and excessive weight gain during pregnancy showed a partial decrease, this decrease did not reach statistical significance, likely due to the relatively low number of cases in the study groups. Additional research is warranted to elucidate the relationship between LBC alterations and the severity of obesity and weight gain during pregnancy.

Keywords: Fetal lung maturity, gestational weight gain, lamellar body count, obesity, pregnancy

Introduction

In the practice of antenatal care, the progressively increasing prevalence of obesity and excessive weight gain during pregnancy continues to be a pivotal concern. This intricate interplay between maternal weight status and its implications for both maternal and fetal well-being has spurred a growing body of investigations aimed at elucidating the underlying mechanisms and optimizing antenatal care strategies [1,2]. Maternal obesity and excessive weight gain have been linked to a spectrum of adverse outcomes, ranging from gestational diabetes, hypertensive disorders, and increased cesarean section rates in mothers, to preterm birth, macrosomia, childhood obesity, and potentially delayed fetal lung maturation in their neonates [3,4].

Pulmonary surfactant, a complex amalgamation of proteins and lipids discharged by alveolar type 2 (AT2) epithelial cells, plays a pivotal role in facilitating respiration and preventing the collapse of alveoli. Within the alveoli, surfactant establishes a fine extracellular stratum that dramatically mitigates surface tension, permitting the lungs to expand, increasing the ability to regulate alveolar dimensions, and safeguarding against fluid accumulation in the air passages. The insufficiency activities of surfactant are also observed in conjunction with acute respiratory distress syndrome. Surfactant is initially stockpiled within distinctive organelles specific to AT2 cells, termed lamellar bodies (LBs), seen as concentric arrays of membranous layers. Consequently, LBs assume a critical role as the fundamental reservoir of extracellular surfactant. Irregularities in their biogenesis yield dire consequences [5]. Apart from lipids, surfactant comprises four distinct proteins, namely surfactant proteins (SP) -A, -B, -C, and -D. Among these, SP-B stands as the sole essential component responsible for the formation of LBs, postnatal

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viability, and the mechanics of respiration. In instances where SP-B is absent, AT2 cells exhibit multi-vesicular bodies (MVBs), characterized by vesicles within their lumens. Hence, MVBs are postulated to serve as the precursors to LBs. The process of SP-B biosynthesis commences with its translocation into the lumen of the endoplasmic reticulum. Subsequent to translocation, the signal sequence is excised, yielding the luminal proSP-B protein. This proSP-B entity is transported within vesicles, initially to the Golgi apparatus, followed by transit through endosomal compartments/MVBs, culminating in its delivery to LBs. Along its journey to the LBs, proSP-B undergoes a sequential series of proteolytic cleavages orchestrated by various proteases, resulting in the generation of the three SP-B proteins, namely SP-BN, SP-BM, and SP-BC. However, the specific roles played by these individual SP-B proteins remain relatively obscure. Of note, SP-BM is regarded as the fully mature protein. This status is attributed to its secretion in conjunction with the internal membranes of LBs and its presence within the extracellular surfactant milieu [5].

Various methods have been developed to assess lung maturation, with one such method being the lamellar body count (LBC) test, which aims to estimate fetal lung maturation by determining the quantity of LBs present in amniotic fluid. LBs serve as a reservoir for surfactant within type II pneumocytes and are actively released into the alveolar space, subsequently entering the amniotic fluid. Comparable in size to platelets, LBs can be quantified in amniotic fluid samples using standard hematologic counters. This approach facilitates a quantitative appraisal of surfactant production, enabling the anticipation of fetal lung maturation. However, standardized methodologies and definitive threshold values for LBC that can reliably predict the occurrence of respiratory distress syndrome (RDS) are presently lacking. The variability observed in determining LB maturity thresholds can be attributed to multiple factors, including centrifugation-induced loss of LBs; potential contamination of samples including instances of misidentification of meconium or platelets as LBs; and disparities in sample types such as vaginal pool specimens containing mucus, which may artificially elevate LBC. Depending on the specific protocols employed, the designated cutoff values for LBC that signify the absence of neonatal RDS exhibit a substantial range, spanning from 15,000 to elevated levels as high as 80,000 LBs per microliter (μL) [6-8]. As a result, the LBC test emerges as a potential tool for evaluating lung maturation.

The objective of this study was to investigate fetal lung maturation in pregnant women with obesity and excessive gestational weight gain at term pregnancy and to assess the clinical utility of the LBC test. Our investigation aims to elucidate a more comprehensive assessment regarding the plausible ramifications stemming from excessive weight gain in pregnant individuals afflicted with obesity, particularly in relation to lung maturation. Moreover, this inquiry holds the potential to represent a notable advancement in the exploration of the prospective applicability of the LBC test as a precision tool for gauging the optimal timing of delivery in pregnant women with . The aim of this study was to evaluate fetal lung maturation in term pregnant women with obesity and excessive gestational weight gain using the LBC test in amniotic fluid samples obtained during cesarean section.

Material and Methods

This retrospective study was conducted with women experiencing term pregnancy and undergoing cesarean delivery; being normal, overweight or obese; and gaining excessive weight during pregnancy or not. The research adhered to the latest Helsinki Declaration and was conducted following approval from the Institutional Review Board for Human Research at our institution (Registry no 139/2023), with participants providing informed consent. Delivered in last two years, the study participants were categorized into research groups based on their body mass index (BMI) values at the time of cesarean delivery and the weight gained during pregnancy. A rigorous set of inclusion and exclusion criteria were adhered to for participant selection. Pregnant individuals with singleton pregnancies at or beyond 37 weeks of gestation were eligible for participation. Both elective and medically indicated cesarean deliveries were included in the study. Pregnant individuals with polyhydramnios, oligohydramnios, multiple gestations, chorioamnionitis, fetal growth restriction, and receipt of antenatal steroids before delivery were excluded due to potential confounding effects. Pregnant individuals with preexisting medical conditions affecting amniotic fluid composition were excluded. Pregnant women with additional systemic diseases such as diabetes mellitus were excluded. Participants with a history of substance abuse or smoking during pregnancy were not be included. Pregnant individuals with known fetal anomalies or genetic disorders impacting pregnancy outcomes were excluded. In this current study, the gestational age was confirmed based on the ultrasonographic biometry results obtained in the first trimester of pregnancy.

Eligible participants were be categorized into specific groups based on BMI: normal weight group, participants with a BMI within the range of 18.5 to 24.9; overweight group, participants with a BMI between 25 and 29.9; obese group, participants with a BMI equal to or exceeding 30. Within each BMI group, participants were be further divided based on the extent of pregnancy weight gain: normal pregnancy weight gain subgroup, participants adhering to recommended weight gain levels; and excessive pregnancy weight gain subgroup, participants experiencing weight gain beyond recommended levels.

A baseline range of demographic, obstetric, and perinatal variables was collected to present clinical characteristics of the study population: age of the participant; socioeconomic status; gestational age at the time of cesarean delivery; gravidity, parity; history of previous pregnancy complications (e.g., gestational diabetes, preeclampsia); maternal BMI at the initiation of pregnancy; pregnancy weight gain up to the point of delivery;

Amniotic fluid samples were collected aseptically during cesarean delivery. In the presence of gross contamination specimen with meconium or blood [9,10], the patient was excluded. The LBC test, a recognized indicator of fetal lung maturity, was conducted on the amniotic fluid samples due to LBs being comparable in size to platelets and LBs were quantified in amniotic fluid samples using a standard hematologic counter.

Statistical analysis

Gpower software (www.psychologie.hhu.de/arbeitsgruppen/

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allgemeine-psychologie-und-arbeitspsychologie/gpower) was used to calculate the sample size. It was deemed sufficient to have 132 participants in the study groups. However, considering that 10% of the participants may drop out of the study, it was found appropriate to continue the study with 144 participants.

When calculating the post hoc power value, the effect size found according to the LBC values obtained from the normal weight, overweight and obese groups was 0.27 and the total sample size was 144 and the power value was found to be sufficient as 0.83.

A statistical software was used to perform statistical procedures (IBM SPSS v27, IBM SPSS, USA). Descriptive statistics of demographic, obstetric, and perinatal characteristics were presented. Kolmogorov-Smirnov test was used to examine the distribution of numeric variables. Statistical methods, including ANOVA with Tukey test or Kruskal-Wallis ANOVA with Mann Whitney for numeric data with or without normal distribution. and chi-square tests for proportions, were utilized to explore the differences of study variables. A significance level of $p<0.05$ was denoted statistical significance.

Results

Within the scope of this study, hospital records were reviewed and data of 144 mothers and newborns who had term cesarean delivery and whose data could be collected adequately and who met the research criteria were analyzed. Table 1 presents selected baseline maternal and neonatal clinical variables. There was no significant difference among the normal weight-normal weight gain (NW-NWG); normal weight-excessive weight gain (NW-EWG); overweight-normal weight gain (OW-NWG); overweight-excessive weight gain (OW-EWG); obese-normal weight gain (O-NWG); and obese-excessive weight gain (O-EWG) with regard to the age, gravidity, parity, socioeconomic status, and gestational age at delivery ($p>0.05$).

Table 1. Baseline clinical characteristics of study population

	Normal weight (n=37)		Overweight (n=59)		Obese (n=48)		Significance
	Normal WG (n=20)	Excessive WG (n=17)	Normal WG (n=26)	Excessive WG (n=33)	Normal WG (n=23)	Excessive WG (n=25)	
Age (year)	31 (21-41)	27 (22-42)	27 (20-41)	31 (21-42)	30 (22-42)	28 (22-37)	p=0.833
Gravidity	3 (1-5)	3 (1-8)	3 (1-5)	3 (1-6)	3 (1-5)	3 (1-6)	p=0.896
Parity	1 (0-3)	1 (0-3)	1 (0-3)	2 (0-3)	1 (0-3)	2 (0-3)	p=0.551
Socioeconomic status							
Low	14 (70%)	14 (82.4%)	20 (77%)	27 (82%)	18 (78%)	20 (80%)	p=0.950
Normal	6 (30%)	3 (17.6%)	6 (23%)	6 (18%)	5 (22%)	5 (20%)	
Gestational age (week)	38 (37-39)	38 (37-40)	39 (37-40)	38 (37-40)	38 (37-41)	38 (37-39)	p=0.092
BMI (kg/m²)	22.9 (19.8-24.2)	24 (21-24.2)	26.2 (25-28.7)	26.8 (25-29.4)	36.5 (30.8-42.4)	34.5 (30-45)	p=0.001
Gestational WG (kg)	9.5 (1-14)	17 (16-22)	9 (5-11)	15 (12-20)	7 (5-9)	12 (10-23)	p=0.001
OGTT							
Fasting (mg/dL)	70 (63-82)[a]	69 (56-78)[a]	77 (61-85)[b]	75 (61-90)[b]	82 (67-92)[c]	88 (65-92)[c]	p=0.001
1-hour (mg/dL)	132 (110-145)[a]	128 (110-165)[a]	145 (124-170)[b]	146 (123-178)[b]	166 (143-179)[c]	166(125-177)[c]	p=0.001
2-hour (mg/dL)	90 (76-127)[a]	98 (86-124[a]	103 (85-135)[b]	120 (87-137)[b]	120 (98-149)[c]	122 (88-140)[c]	p=0.001
C/S indications							
Previous C/S	16 (80%)	13 (76.4%)	19 (73.1%)	29 (87.9%)	15 (62.5%)	19 (76%)	
Prolonged labor	2 (10%)	4 (23.6%)	6 (23.1%)	4 (12.1%)	2 (8.7%)	2 (8%)	
Breech presentation	2 (10%)	0 (0%)	1 (3.8%)	0 (0%)	0 (0%)	1 (4%)	
CPD	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (26.1%)	3 (12%)	
Blood glucose							
Fasting (mg/dL)	76 (63-88)[a]	75 (65-87)[a]	73 (67-86)[a]	78 (65-91)[a]	85 (78-92)[b]	91 (78-93)[b]	p=0.009
Postprandial (mg/dL)	128 (120-135)[a]	134 (125-135)[a]	116 (87-134)[a]	125 (87-138)[b]	137 (103-145)[b]	138 (114-145)[b]	p=0.016
Neonatal gender							
Female	8 (40%)	11 (64.7%)	13 (50%)	15 (45.5%)	15 (65.2%)	12 (48%)	p=0.441
Male	12 (60%)	6 (35.3%)	13 (50%)	18 (54.5%)	8 (34.8%)	13 (52%)	
Birthweight	3120 (2870-3560)	3330 (2890-4020)	3280 (2610-3980)	3370 (2870-4040)	3390 (2955-4440)	3210 (2670-4730)	p=0.054
Cord blood pH	7.40 (7.30-7.50)[a]	7.45 (7.30-7.50)[a]	7.40 (7.30-7.50)[a]	7.40 (7.30-7.56)[a]	7.30 (7.23-7.40)[b]	7.33 (7.23-7.50)[b]	p=0.001
NICU admission							
No	19 (95%)	17 (100%)	24 (92.3%)	31 (93.9%)	16 (69.6%)	20 (80%)	p=0.019*
Yes	1 (5%)	0 (0%)	2 (7.7%)	2 (6.1%)	7 (30.4%)	5 (20%)	
NICU stay (day)	0 (0-3)	0 (0-0)	0 (0-5)	0 (0-3)	0 (0-7)	0 (0-7)	p=0.015*

WG: weight gain, BMI: body mass index, OGTT: oral glucose tolerance test, C/S: cesarean section, CPD: cephalo-pelvic disproportion, NICU: neonatal intensive care unit. *After Bonferroni correction there was no signification correction among the study groups $p>0.05$
When presenting the results of post hoc analyses, the study groups with significant differences were coded with different letters (a, b, and c) ($p<0.05$) and similar groups were coded with the same letter (a and b) ($p>0.05$).

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When the 75-g OGTT results were analyzed, the values of the NW-NWG and NW-EWG groups were smaller than those of the other groups and the values of the OW-NWG and OW-EWG groups were smaller than those of the O-NWG and O-EWG groups ($p<0.05$). The indication for cesarean section in the majority (at least 62.5%) of the patients included in the study was previous cesarean section. Fasting and postprandial blood glucose values of NW-NWG, NW-EWG, and OW-NWG groups were smaller than those of OW-EWG, O-NWG, and O-EWG groups ($p<0.05$). The study groups were similar in terms of neonatal gender and birthweight parameters ($p>0.05$). When cord blood pH obtained from newborns after birth was evaluated, the values of O-NWG, and O-EWG groups were significantly lower than those of NW-NWG, NW-EWG, OW-NWG, and OW-EWG groups ($p<0.05$). The NICU admission rate and NICU stay of the study groups were found similar ($p>0.05$).

Figure 1 shows median LBC of the study groups.

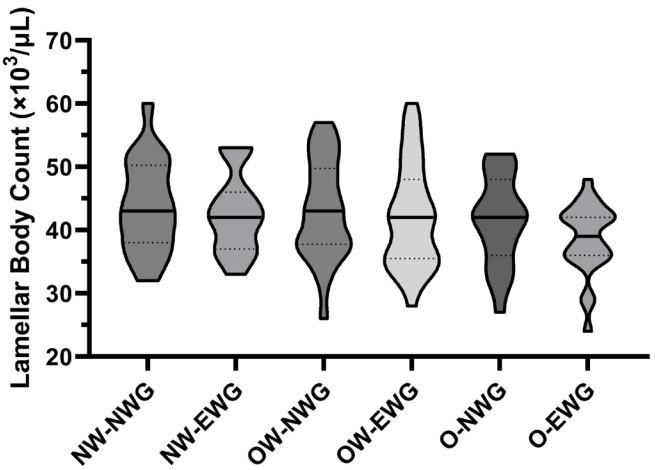

Figure 1. Median lamellar body count of study groups. Data are presented as median with inter quartile range (25-75%). Kruskal-Wallis ANOVA reveals no significant difference among the lamellar body count of the study groups ($p=0.307$). NW-NWG, normal weight-normal weight gain; NW-EWG, normal weight-excessive weight gain; OW-NWG, overweight-normal weight gain; OW-EWG, overweight-excessive weight gain; O-NWG, obese-normal weight gain; and O-EWG, obese-excessive weight gain

Kruskal-Wallis ANOVA reveals no significant difference among the LBC of the study groups ($p=0.307$), although there was a subtle decrease in the LBC of obese-excessive weight gain group.

Discussion

In this retrospective study, whether there was a delay in fetal lung maturation in infants of mothers with obesity and excessive weight gain during pregnancy at term delivery by cesarean section was investigated. According to the findings of this study considering both obesity status and weight gain during pregnancy, it was found that obesity caused a partial decrease in the LBC value, especially when obesity was associated with excessive weight gain, although the differences obtained did not reach statistical significance. The fact that the basic maternal and neonatal data of the study groups were largely similar increases the reliability of the results obtained by comparing the LBC test results evaluating fetal lung maturation.

The incidence of obesity has increased worldwide, especially in developed countries, over the last few decades, and it has proportionally affected women of reproductive age [11,12]. Obesity is currently the most common issue of antenatal care that can affect both the mother and the newborn during pregnancy and later [4]. Pregnant women with obesity may experience short-term and long-term consequences; particularly related to the excessive weight gain during pregnancy, they are more likely to develop gestational diabetes, pregnancy-related hypertensive disorders, preterm birth, venous thromboembolism, and have a higher need for the instrumental assistance as well as cesarean section during childbirth [3,13,14]. Many of these maternal conditions having a degree of association with obesity may have negative influences on fetal health by creating an obesogenic intrauterine environment that delays lung maturity and puts the newborn at a higher risk of respiratory distress syndrome (RDS) due to transient tachypnea of the newborn (TTN) or surfactant deficiency [15]. This phenomenon may potentially arise due to glucose intolerance, including gestational diabetes which frequently accompanies maternal obesity. This, in turn, augments the likelihood of fetal hyperinsulinemia and perturbed glucose homeostasis in the neonatal period [8].

In a study by DeRoche et al. [16], the utility of amniotic fluid LBCs in predicting fetal lung maturity in pregnancies complicated by maternal diabetes mellitus was assessed. The study findings demonstrated a significant correlation between amniotic fluid LBCs and the lecithin/sphingomyelin ratio as well as phosphatidylglycerol values. Moreover, an LBC of 37,000/µL was identified to exhibit an 80% sensitivity and 100% specificity in predicting fetal lung maturity. Those results underscored the potential of LBCs as a reliable screening test for amniotic fluid analysis in diabetic pregnancies. This approach has the potential to reduce the need for more laborious and costly phospholipid profiles.

Tsuda et al. [17] investigated whether twin fetuses achieve fetal lung maturity and lung fluid absorption earlier than singletons. Their study involved singleton and twin pregnancies without congenital respiratory abnormalities or neonatal deaths. They performed cesarean deliveries occurred between 24 to 38 gestational weeks. Amniotic fluid samples were directly analyzed, and logistic regression revealed that twin pregnancies significantly reduced the risk of neonatal RDS and TTN. Singleton pregnancies had higher RDS and TTN rates and lower LBCs compared to twin pregnancies. The findings suggest that twin fetuses experience quicker lung maturation and fluid absorption, supported by higher LBC values.

Walker et al. [18] examined the link between amniotic fluid LBC and risk factors for respiratory distress in term cesarean births. They stated that LBs reflect fetal lung maturity and surfactant production. LBC increased significantly with gestational age,

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particularly in the 38th, 39th, and 40th weeks compared to the 37th week. Female fetuses had higher values of LBC. Low LBC (<100 x 109 mL−1) correlated with a 5.6-fold increased risk of neonatal respiratory distress requiring NICU admission. They noted that LBC provides an accessible and cost-effective measure of fetal lung maturity, especially in the absence of widely available phospholipid-based tests. They suggested that while it has limitations, further investigation is needed to determine its value in managing moderate prematurity, delivery timing, or predicting postnatal respiratory outcomes.

In a study by Visnjevac et al. [19], the test of LBC for scheduling elective caesarean delivery at ≥39 weeks was studied. Their research involved study groups with or without transient tachypnea. The participants with transient tachypnea exhibited significantly lower LBC levels compared to the controls. They concluded that considering its favorable accuracy, using LBC as a marker for scheduling elective cesarean delivery timing, especially in cases of transient tachypnea prediction, might be considered.

Since the study was retrospective, the study groups could not be formed more homogeneously and this can be considered as a limitation. The fact that the study examined the change in LBC values according to obesity and weight gain during pregnancy provided results that contributed to the literature as a first-time study. The fact that the basic sociodemographic and obstetric characteristics of the study groups were similar increases the value of the study results. The potential impact of obesity and excessive weight gain during pregnancy on fetal lung maturation has been indirectly explored through the utilization of a straightforward and accessible laboratory test known as LBC. By specifically selecting cases with term pregnancies according to gestational weeks, the research aims to ascertain the extent of any adverse effects observed during the term pregnancy phase in terms of obesity and excessive weight gain during pregnancy and to assess whether these have a retarding effect on fetal lung maturation. The categorization of participants in the study based on their obesity status and the occurrence of excessive weight gain during pregnancy is envisioned to contribute to a more comprehensive interpretation of the underlying relationship. In addition to the important outcomes of our study, the fact that the minor differences obtained in pregnant women with different degrees of obesity and weight gain in pregnancy did not reach significance may be due to the fact that the sample size of the study was partially insufficient. In addition, the inability to collect clinical data indicating ultrasonographic fetal lung maturation may also be considered as a limitation.

Conclusion

When the results of the aforementioned studies are evaluated together with the findings of the current study, it seems that the LBC test is one of the tools that should be included in the toolbox of obstetricians. Considering that it can be performed with a blood counting device in cases where amniotic fluid can be obtained during cesarean delivery and is not contaminated with meconium and blood, the LBC test has significant ease of use. Considering the lack of easy-to-use and practical tests for fetal lung maturation, the application of the LBC test can be expected to increase. Further studies are needed to investigate how the presence of LBs in the amniotic fluid changes in pregnant women with obesity and excessive weight gain.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
The Human Research Ethics Committee of Haseki Training and Research Hospital was approved the study protocol (Registry no 139/2023)

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