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ORIGINAL RESEARCH ARTICLE

Reference values for interleukin-6 in the amniotic fluid of asymptomatic pregnant women

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Abstract

Introduction: Nowadays, proinflammatory factors are considered to play an important role in the pathophysiology of threatened preterm labor or chorioamnionitis. The aim of this study was to establish the normal reference range for interleukin-6 (IL-6) levels in the amniotic fluid and to identify factors which may alter this value.

Material and methods: Prospective study in a tertiary-level center including asymptomatic pregnant women undergoing amniocentesis for genetic studies from October 2016 to September 2019. IL-6 measurements in amniotic fluid were performed using a fluorescence immunoassay with microfluidic technology (ELLA Proteinsimple, Bio Techne). Maternal history and pregnancy data were also recorded.

Results: This study included 140 pregnant women. Of those, women who underwent termination of pregnancy were excluded. Therefore, a total of 98 pregnancies were included in the final statistical analysis. The mean gestational age was 21.86 weeks (range: 15–38.7) at the time of amniocentesis, and 38.6 weeks (range: 30.9–41.4) at delivery. No cases of chorioamnionitis were reported. The $\log_{10}$ IL-6 values follow a normal distribution ($W = 0.990$, $p = 0.692$).

The median, and the 5th, 10th, 90th, and 95th percentiles for IL-6 levels were 573, 105, 130, 1645, and 2260 pg/mL, respectively. The $\log_{10}$ IL-6 values were not affected by gestational age ($p = 0.395$), maternal age ($p = 0.376$), body mass index ($p = 0.551$), ethnicity ($p = 0.467$), smoking status ($p = 0.933$), parity ($p = 0.557$), method of conception ($p = 0.322$), or diabetes mellitus ($p = 0.381$).

Conclusions: The $\log_{10}$ IL-6 values follow a normal distribution. IL-6 values are independent of gestational age, maternal age, body mass index, ethnicity, smoking status, parity and method of conception. Our study provides a normal reference range for IL-6 levels in the amniotic fluid that can be used in future studies. We also observed that normal IL-6 values were higher in the amniotic fluid than in serum.

Abbreviations: BMI, body mass index; ELISA, enzyme-linked immunosorbent assays; IL-6, interleukin-6; PPROM, preterm premature rupture of membranes.

KEYWORDS
amniocentesis, amniotic fluid, chorioamnionitis, interleukin-6, intra-amniotic infection, intra-amniotic inflammation, preterm labor, spontaneous preterm delivery

1 | INTRODUCTION

Preterm birth is defined as delivery before the 37th gestational week and is the most important cause of neonatal mortality and morbidity, being intra-amniotic infection and inflammation some of its causes. Pathogenesis of preterm labor is not well understood, but is thought to be a syndrome initiated by multiple mechanisms, including infection, inflammation, ischemia, uterine overdistension and other immunologically mediated processes.[1]

Previous studies have shown that inflammatory mediators and cytokines such as interleukin-6 (IL-6) may be involved in the pathophysiology of preterm labor and premature rupture of membranes, indicating that infection and subclinical inflammation play a crucial role.[1–3] Currently, systemic inflammation is considered to be less important than increased inflammation at the maternal-fetal interface. In addition, intra-amniotic inflammation is associated with adverse perinatal outcomes, even in the absence of intra-amniotic microbes.[4]

Many maternal and fetal characteristics, including markers such as IL-6, have been associated with preterm birth. However, to date, the number of studies measuring IL-6 in asymptomatic patients is limited.[3,5–7] In this study, we aimed to establish a normal reference range for IL-6 values in amniotic fluid and to investigage if IL-6 values are affected by maternal or fetal factors.

Key message

Interleukin-6 has been linked to the pathophysiology of preterm labor and chorioamnionitis. Interleukin-6 levels in amniotic fluid remain fairly constant during the pregnancy and are not affected by maternal factors such as ethnicity, age, BMI, smoking status, parity, or method of conception.

2 | MATERIAL AND METHODS

2.1 | Study design

This prospective study included pregnant women who underwent amniocentesis for genetic studies at Vall d'Hebron Hospital, Barcelona, between October 2016 and September 2019. Women with clinical signs of chorioamnionitis, preterm premature rupture of membranes, threatened preterm labor and age below 18 years old were excluded.

Measurements of IL-6 levels in the amniotic fluid were performed using a fluorescence immunoassay with microfluidic technology (ELLA Proteinsimple, Bio Techne). Samples were stored at −20°C until their processing. Samples were run in triplicate with a 1:5 dilution using the buffer provided in the kit.

Maternal history and pregnancy data were recorded. Maternal and pregnancy factors included age, body mass index (BMI), parity, ethnicity, smoking status and method of conception. Delivery variables included gestational age at delivery, birthweight, type of birth (vaginal or cesarean section), perinatal complications and newborn gender.

2.2 | Amniocentesis

Amniocentesis was performed by an experienced obstetrician with the patient in the supine position. Skin asepsis of the abdominal wall was performed using chlorhexidine or povidone-iodine. The puncture site was selected by ultrasound, based on placental location, fetal position and movements, and area with the largest pool of amniotic fluid. Amniotic fluid was collected using a 22 gauge needle. When possible, transplacental puncture was avoided.

TABLE 1 Demographic characteristics

Demographic characteristics ($n = 98$)	
Age (years), mean (SD)	33.84 (4.55)
BMI (kg/m^2), mean (SD)	24.27 (4.41)
Ethnicity, *n* (%)	
Arab	14 (14.3%)
Asian	1 (1.0%)
Caucasian	74 (75.5%)
Indian	1 (1%)
South American	8 (8.2%)
Smoking status, *n* (%)	
Smoker	8 (8.2%)
Non-smoker	90 (91.8%)
Assisted reproductive techniques, *n* (%)	
Yes	14 (14.3%)
IVF with oocyte donor	4 (4.0%)
IVF	9 (9.2%)
AI	1 (1.0%)
No	84 (85.7%)

Abbreviations: AI, artificial insemination; BMI, body mass index; IVF, in vitro fertilization; SD, standard deviation.

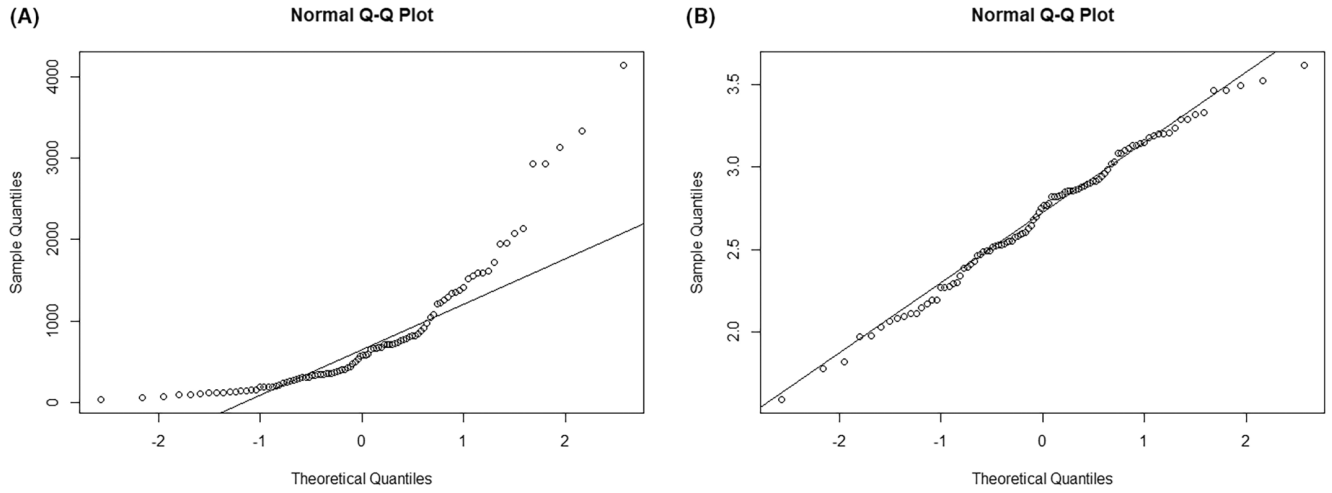

FIGURE 1 Distributions of (A) IL-6 values and (B) log10-transformed IL-6 values.

2.3 | Statistical analyses

All statistical analyses were performed using the computing environment R (version 4.0.0).[8] The Shapiro test was used to assess normality of distribution. The $\log_{10}$-transformed IL-6 values followed a Gaussian distribution.

The association of $\log_{10}$ IL-6 values with continuous variables was assessed using the Pearson's correlation coefficient or the Spearman's rank correlation coefficient, as appropriate, whereas association of $\log_{10}$ IL-6 values with categorical variables was assessed using the Wilcoxon test for variables with two categories and the Kruskal Wallis test for variables with more than two categories.

Results are reported as the median, mean and 5th, 10th, 90th and 95th percentile.

2.4 | Ethics statement

This study was approved by the Ethics Committee of Vall d'Hebron Research Institute (registration number PR(AMI)541-2016) on September 1, 2016. All participants provided their written informed consent.

3 | RESULTS

A total of 140 patients were recruited. Of those, 42 underwent termination of pregnancy for genetic anomalies and were excluded from the analysis. Therefore, 98 pregnancies were included in the final statistical analysis (Table 1). In one of these pregnancies, a trisomy 18 case was found; the patient decided to carry on with the pregnancy.

Mean gestational age was 21.8 weeks (range 15.0–38.7) at the time of amniocentesis and 38.6 weeks (range 30.9–41.4) at delivery. Late amniocentesis at 38 gestational weeks was performed to study seroconversion of toxoplasma in the third trimester; there was a positive PCR in amniotic fluid for toxoplasma. No cases of chorioamnionitis were reported.

TABLE 2 IL-6 levels

Gestational age at inclusion (weeks), mean (SD)	22.49 (5.21)
Gestational age at delivery (weeks), mean (SD)	38.06 (2.33)
IL-6 levels (pg/mL), mean (SD)	791.28 (782.93)
IL-6 levels ($\log_{10}$-transformed), mean (SD)	2.71 (0.43)
IL-6 levels, percentiles	
5th	105
10th	130
90th	1645
95th	2260
Log_{10}-transformed IL-6 levels, percentiles	
5th	2.022
10th	2.113
90th	3.216
95th	3.351

Abbreviations: IL-6, interleukin-6; SD, standard deviation.

Raw values of IL-6 levels in the amniotic fluid did not follow a normal distribution ($W = 0.789$, $p < 0.001$), however, log10-IL-6 values did ($W = 0.990$, $p = 0.692$) (Figure 1).

Median IL-6, and 5th, 10th, 90th, and 95th percentiles were 573, 105, 130, 1645, and 2260 pg/mL, respectively (Table 2).

Log_{10} IL-6 levels were independent of gestational age (rho = 0.086, $p = 0.395$), maternal age (rho = 0.090, $p = 0.376$), BMI (rho = −0.064, $p = 0.551$), ethnicity ($p = 0.467$), smoking status ($p = 0.933$), parity ($p = 0.557$), method of conception ($p = 0.322$) or diabetes mellitus ($p = 0.381$) (Figure 2). Log10 IL-6 levels did not show an association with gestational age at delivery (rho = −0.192, $p = 0.089$).

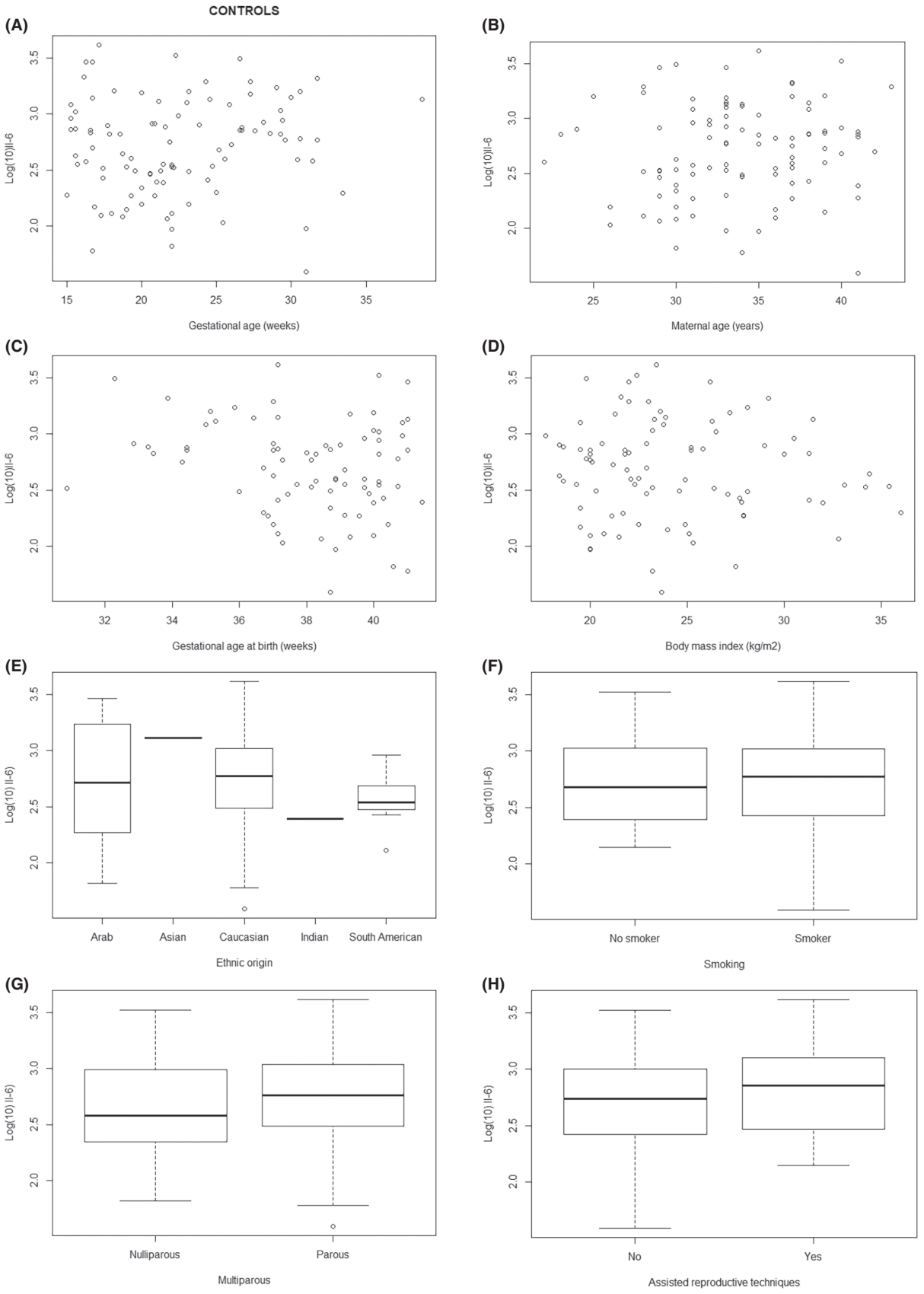

FIGURE 2 IL-6 levels according to clinical data: Gestational age (A), maternal age (B), gestational age at birth (C), body mass index (D), ethnicity (E), smoking status (F), multiparity (G) and assisted reproductive techniques (ART) (H).

4 | DISCUSSION

Our results show that there is no association between IL-6 levels and maternal factors such as gestational age, maternal age, BMI, ethnicity, smoking status, parity and method of conception. Moreover, we observed that IL-6 levels in amniotic fluid are substantially higher than in serum (0–20 pg/mL).

Many studies have examined inflammatory markers in symptomatic patients, either with premature rupture of membranes or threatened preterm labor.[3,6,9,10] These studies have shown increased inflammatory markers in patients with these pathologies and an association between intra-amniotic inflammation and adverse perinatal outcomes, even in the absence of intra-amniotic microbes. Lee et al. found that women with preterm premature rupture of membranes (PPROM) and intra-amniotic infection have lower IL-6 levels than women with intra-amniotic infection but intact membranes.[11] In our study, patients with PPROM or suspected I intra-amniotic infection were excluded.

For this reason, prediction models have been designed to predict spontaneous preterm delivery or microbial invasion of the amniotic cavity based on levels of inflammatory markers, such as IL-6.[4,9,12–14]

Studies with asymptomatic patients have also been conducted, although the literature is limited. Melekoglu et al. measured IL-6 levels in 22 asymptomatic pregnant women between 16 and 24 gestational weeks. They excluded patients with fetuses having any congenital or chromosomal abnormality, pre-gestational diabetes, hypertensive disease, obstetric cholestasis, chronic maternal disease, and use of tobacco or alcohol. Mean IL-6 values in the amniotic fluid of these asymptomatic patients were 95.8 ± 16.4 pg/mL. Following the same inclusion and exclusion criteria as in the previous study, the same group measured IL-6 levels in 20 patients diagnosed with gestational diabetes and found increased IL-6 levels in these patients as compared to pregnant women without gestational diabetes (mean IL-6 of 136.2 ± 17.3 pg/mL and 98.3 ± 11.5 pg/mL, respectively).[3,7] In our study, a total of seven patients were diagnosed with gestational diabetes and one patient had type 2 diabetes. None of the patients had type 1 diabetes. There were no differences between patients with diabetes and patients without diabetes.

Gervasi et al. measured IL-6 values between 15.6 and 16.9 gestational weeks in 652 term pregnancies without obstetric complications (i.e., placental abruption, small for gestational age, fetal death, gestational hypertension and pre-eclampsia), and their results agree with our results (the 95th percentile had mean IL-6 values of 2935 pg/mL).[5] In Gervasi's studies, IL-6 levels in the amniotic fluid were measured with commercially available enzyme-linked immunosorbent assays (ELISA), as in our study.

Although previous studies have reported increased IL-6 levels during pregnancy in asymptomatic women, measurements were performed only during the second trimester. In addition, these studies did not investigate whether IL-6 values were affected by maternal factors.

Of note, in our study we observed increased IL-6 levels in the amniotic fluid after 15 gestational weeks as compared to mean normal IL-6 values in serum throughout the duration of pregnancy.

Fu et al. measured serum IL-6 in healthy non-pregnant women and healthy pregnant women and established the following reference intervals for serum IL-6: <1.518 pg/mL for healthy non-pregnant women, <3.52 pg/mL for healthy pregnant women during the first trimester, and <4.40 pg/mL for healthy pregnant women during the second and third trimesters.[15] Our study offers the additional benefit of including asymptomatic women in the second and third trimesters of pregnancy. Additionally, we studied several maternal and fetal factors that may alter IL-6 values, and confirmed that there was no association between these factors and IL-6 values.

A study has recently been published showing that immunoassays using the Cobas system (Roche) and the traditional ELISA method are comparable for measuring IL-6 levels in amniotic fluid.[16] Clinically, the Cobas system has advantages over the ELISA method, such as being more robust and rapid, plus totally automated. The ELISA system used in our study (ELLA) gives results comparable to those obtained with the Cobas system (data not shown). Therefore, the reference values reported in our study can also be applied to evaluate results obtained in studies using the Cobas system.

Our study had a number of limitations, such as the small sample size and single-center population. Furthermore, measurements of IL-6 in the maternal serum were not performed. First, this would have allowed the direct comparison of levels in both fluids, and second to establish whether there is a correlation between IL-6 levels in serum and IL-6 levels in amniotic fluid. However, the number of patients participating was sufficient to measure IL-6 levels in the amniotic fluid and to evaluate the factors that may affect these values. To date, there were no normal reference ranges for IL-6 levels in amniotic fluid. Moreover, we have investigated this parameter in asymptomatic pregnant women and in a wide range of gestational ages.

This study provides a normal reference range for IL-6 values in the amniotic fluid of asymptomatic pregnant women. In addition, since IL-6 values are not affected by gestational age or other maternal factors, IL-6 values can be used without having to convert them to MoMs or without performing other forms of standardization.

5 | CONCLUSION

In asymptomatic women, IL-6 levels remain fairly constant throughout the duration of pregnancy, and are not affected by maternal factors such as ethnicity, age, body mass index, smoking status, parity, or method of conception. Our study provides a normal reference range for IL-6 values in the amniotic fluid of asymptomatic pregnant women. These normal reference range for IL-6 values can be used as a reference in future studies.

AUTHOR CONTRIBUTIONS

EB was the principal investigator at Hospital Vall d'Hebron, contributed to the study design, patient follow-up and data collection,

and wrote the first draft of both the protocol and manuscript. EB is the guarantor for the manuscript. MG was the study coordinator, contributed to the study design, patient follow-up and data collection, and wrote the protocol and manuscript with EB. MV, MM and CF-J contributed to the study design and data collection. SA, MÁS, MA and CR contributed to data collection. MH-G and EC contributed to the study design. All authors had complete access to the data at the end of the study and the decision to submit the manuscript for publication was taken at a team joint meeting where everyone reviewed and approved the final version of the manuscript. This was subsequently submitted by the corresponding author.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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