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AMNIOTIC FLUID FETAL HEMOGLOBIN IN NORMAL PREGNANCIES AND PREGNANCIES COMPLICATED WITH PRETERM LABOR OR PRELABOR RUPTURE OF MEMBRANES

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Abstract

Objective—Hemoglobin and its catabolic products have been associated with amniotic fluid (AF) discoloration and intra-amniotic infection/inflammation (IAI). However, the origin of AF hemoglobin (maternal or fetal) has not been determined. The aims of this study were to determine if fetal hemoglobin can be detected in AF obtained from normal pregnancies, and whether there is an association between AF fetal hemoglobin concentrations and gestational age, spontaneous labor (term and preterm), preterm prelabor rupture of membranes (PPROM) and IAI.

Study design—This cross-sectional study included pregnant women in the following groups: 1) mid-trimester (n=60); 2) term not in labor (n=21); 3) term in labor (n=47); 4) spontaneous preterm labor with intact membranes (PTL) without IAI who delivered at term (n=89); 5) PTL without IAI who delivered preterm (n=74); 6) PTL with IAI (n=78); 7) PPROM with (n=48) and 8) without IAI (n=48). AF fetal hemoglobin concentrations were determined by ELISA. Non-parametric statistics were used for analyses.

Results—1) Fetal hemoglobin was detected in 80.4% of all AF samples; 2) women at term not in labor had a higher median AF fetal hemoglobin concentration than those at mid-trimester (p=0.008); 3) labor at term was not associated with a significant difference in the median AF fetal hemoglobin concentration; 4) the median AF fetal hemoglobin concentration was not significantly different among the 3 PTL groups, between the PPROM groups; 5) women with PTL and IAI had a lower AF fetal hemoglobin percentage of the total hemoglobin than those without IAI who delivered preterm (p=0.03) or at term (p<0.001); (6) the median AF fetal hemoglobin

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concentration was higher in pregnancies complicated with PTL or PPROM than in women at term ($p<0.001$ for all comparison).

Conclusions—1) The concentration of immunoreactive AF fetal hemoglobin increases with gestational age; 2) the median AF fetal hemoglobin concentration is higher in pregnancies complicated with PTL or PPROM than in term pregnancies; 3) among women with PTL or PPROM, the AF fetal hemoglobin concentrations were not associated with IAI; 4) however, women with PTL and IAI had a lower percentage of AF fetal hemoglobin of the total hemoglobin than those without IAI, suggesting different mechanisms of disease.

Keywords

Hemoglobin; fetus; preterm delivery; PPROM; pregnancy; amniocentesis; intra-amniotic infection/inflammation

INTRODUCTION

Hemoglobin and its catabolic products have been associated with discolored amniotic fluid,[1,2] which is considered a risk factor for intra-amniotic infection and/or inflammation (IAI).[3] A putative explanation is that even small amounts of intra-amniotic bleeding can serve as a medium for bacterial growth,[4,5] and may increase the risk for intra-amniotic infection and subsequent preterm parturition. Indeed, we recently reported that IAI is associated with elevated median amniotic fluid total hemoglobin concentration in patients with spontaneous preterm labor and intact membranes (PTL), as well as in those with preterm prelabor rupture of membranes (preterm PROM).[6] However, it is unclear if the origin of the detected hemoglobin is maternal or fetal.

Gomez et al[7] proposed that a sub-clinical intra-uterine infection can cause deciduitis and decidual bleeding, leading to the clinical manifestation of vaginal bleeding and subsequently to preterm PROM and early preterm delivery. Thus, we hypothesized that the main origin of amniotic fluid hemoglobin detected in pregnancies complicated with IAI, as well as in those with spontaneous labor at term is of maternal origin.[6] Indeed, less than 1% of the hemoglobin in the normal adult corresponds to the fraction of hemoglobin F ($\alpha_2\gamma_2$), also known as fetal hemoglobin,[8,9] whereas in the fetal circulation the majority of hemoglobin is hemoglobin F.[9] Therefore, determination of fetal hemoglobin concentrations in amniotic fluid can assist in determining the origin of the total hemoglobin in the amniotic cavity.[6]

The aims of this study were to determine: 1) if fetal hemoglobin can be detected in normal pregnancies; and 2) whether there is an association between the fetal hemoglobin concentrations and advancing gestational age, spontaneous labor (term and preterm), preterm PROM, and the presence of IAI.

MATERIALS AND METHODS

Study design and population

This cross-sectional study was conducted by searching our clinical database and bank of biological specimens, and included 465 pregnant women in the following groups: 1) women in the mid-trimester of pregnancy (14–18 weeks) who underwent amniocentesis for genetic indications and delivered a healthy neonate at term (*n*=60); 2) normal pregnant women at term not in labor (*n*=21) and 3) at term in spontaneous labor (*n*=47); 4) women with an episode of PTL without IAI who delivered at term (*n*=89); 5) PTL without IAI who delivered preterm (*n*=74); and 6) PTL with IAI (*n*=78); 7) women with preterm PROM without IAI (*n*=48); and 8) preterm PROM with IAI (n=48).

All women provided written informed consent prior to the collection of amniotic fluid. The collection of amniotic fluid and its utilization for research purposes was approved by the Institutional Review Boards of the participating institutes and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, (NICHD/NIH/DHHS). Many of these samples have been used previously to study the biology of inflammation, hemostasis, angiogenesis regulation, and growth factor as well as for measurement of the total hemoglobin concentrations, in normal pregnant women and those with pregnancy complications.

Clinical definitions

Pregnant women were considered to have a normal pregnancy if they did not have obstetrical complications, and delivered a term (≥37 weeks) neonate with appropriate for gestational age birthweight[10,11] without complications. Spontaneous preterm labor was defined by the presence of regular uterine contractions occurring at a frequency of at least two every 10 minutes associated with cervical changes before 37 completed weeks of gestation that required hospitalization. Preterm PROM was diagnosed by sterile speculum examination confirming pooling of amniotic fluid in the vagina in association with nitrazine and ferning tests when necessary, before 37 weeks of gestation and in the absence of labor. Women at term in labor consisted of women who were suspected to have preterm labor because of uncertain dates and had an amniocentesis for the assessment of fetal lung maturity and microbial invasion of the amniotic cavity. If analysis of amniotic fluid was consistent with maturity, tocolysis was not used. However, if they delivered a baby larger than 2500 grams without complications of prematurity, they were considered to represent patients in spontaneous labor at term. Intra-amniotic infection was defined as a positive amniotic fluid culture for micro-organisms. Intra-amniotic inflammation was diagnosed by an amniotic fluid interleukin (IL)-6 concentration >2.6 ng/mL.[12]

Amniotic fluid sample collection

Ultrasound guided trans-abdominal amniocenteses were performed for evaluation of microbial status of the amniotic cavity and/or assessment of fetal lung maturity. Amniotic fluid white blood cell (WBC) count, glucose concentration and Gram-stain were performed shortly after collection, and amniotic fluid was cultured for aerobic/anaerobic bacteria and genital mycoplasmas (*Ureaplasma urealyticum* and *Mycoplasma hominis*). The results of these tests were used for subsequent clinical management. Amniotic fluid not required for clinical assessment was centrifuged for 10 minutes at 4°C and the supernatant was aliquoted and stored at −70°C until analysis. Mid-trimester samples were not evaluated for infection, but intra-amniotic inflammation was assessed by measuring IL-6 concentration, and all mid-trimester samples included in this study had an amniotic fluid IL-6 concentration <2.6 ng/mL.[12]

Determination of fetal hemoglobin concentration in amniotic fluid

Amniotic fluid concentration of human fetal hemoglobin was determined by sensitive enzyme-linked immunoassays (Bethyl Laboratories, Inc., Montgomery, TX USA). The fetal hemoglobin immunoassay was validated for human amniotic fluid in our laboratory prior to the conduction of this study, including spike and recovery experiments which produced parallel curves indicating that amniotic fluid constituents did not interfere with antigen-antibody binding in this assay. Immunoassays were carried out according to manufacturer recommendations. Amniotic fluid samples were incubated in duplicate wells of the micro titer plates pre-coated with an antibody specific for the fetal hemoglobin. During this incubation, the fetal hemoglobin present in the standards or amniotic fluid samples was bound by the immobilized antibodies in the respective assay plates. After repeated washing and aspiration to remove all unbound substances, an enzyme-linked polyclonal antibody

specific for the analyte was added to the wells of the assay plates. Unbound enzyme conjugate was removed by repeated washing and a substrate solution was added to the wells of the assay plates and color developed in proportion to the amount of the fetal hemoglobin bound in the initial step. Color development was stopped with the addition of an acid solution and the intensity of color was read using a programmable spectrophotometer (SpectraMax M2, Molecular Devices, Sunnyvale, CA USA). The concentrations of fetal hemoglobin in amniotic fluid samples were determined by interpolation from individual standard curves. The calculated inter- and intra-assay coefficients of variation for fetal hemoglobin immunoassays in our laboratory were 2.5% and 2%, respectively. The sensitivity for the fetal hemoglobin assay was calculated to be 3.48 ng/mL.

Statistical analysis

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to test for normal distribution of the data. Since the amniotic fluid fetal hemoglobin concentrations were not normally distributed, non parametric tests were used for analyses. Correlation between continuous variables was assessed by the Spearman's rank test. Comparisons between proportions were performed with Chi-square test. Kruskal-Wallis with post-hoc tests (Mann-Whitney U) were used for continuous variables. Analysis of covariance (ANCOVA) was performed to investigate the association between the subgroups of PTL and preterm PROM and the amniotic fluid fetal hemoglobin concentration, gestational age at amniocentesis and sample storage time. A p-value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS package version 14 (SPSS Inc, Chicago, IL, USA).

RESULTS

Demographic and clinical characteristics

Table I displays the median gestational age at amniocentesis among the study groups and the percentage of samples with measurable fetal hemoglobin, which was detected in 80.4% (374/465) of all amniotic fluid samples, but in only 31.7% (19/60) of mid-trimester samples. Tables II and III display the demographic and clinical characteristics of patients with spontaneous PTL and women with preterm PROM, respectively. The median gestational age at amniocentesis differ significantly among the subgroups of PTL (Kruskal-Wallis, p=0.002; Table II). Patients with PTL and IAI had a lower median gestational age at amniocentesis than those who delivered preterm without IAI (p=0.001) and than those who delivered at term (p=0.005). Similarly, among patients with preterm PROM, those with IAI had a lower median gestational age at amniocentesis than those without IAI (p=0.001, Table III). Among patients with PTL, those who delivered preterm (with or without IAI) were more likely to have a history of vaginal bleeding during pregnancy that those who delivered at term (p=0.04; Table II).

Amniotic fluid fetal hemoglobin concentrations in mid-trimester and term patients

Women at term not in labor had a higher median amniotic fluid fetal hemoglobin concentration than patients at mid-trimester [term not in labor: 19.5 ng/ml, interquartile range (IQR) 0–49 vs. mid-trimester: 0.0 ng/ml, IQR 0–19.9, p=<0.001; Figure 1]. Among patients at term, the median amniotic fluid fetal hemoglobin concentration did not differ significantly between women not in labor and those in labor (19.5 ng/ml, IQR 0–49 vs. 19.1 ng/ml, IQR 0–36.2, respectively; p=0.4)

Changes in amniotic fluid fetal hemoglobin concentrations in patients with spontaneous preterm labor or preterm PROM

Among women with PTL, the median amniotic fluid fetal hemoglobin concentration did not differ significantly between the 3 subgroups (PTL without IAI who delivered at term: 134.2 ng/ml, IQR 56.3–255.7 vs. PTL without IAI who delivered preterm: 109.6 ng/ml, IQR 42.0–247.1 vs. PTL with IAI: 114.6 ng/ml, IQR 33.2–283.9; Kruskal-Wallis, p=0.7; Figure 2). Similarly, among women with preterm PROM, no significant differences were found among patients without IAI and those with IAI (without IAI: 109.7 ng/ml, IQR 51.4–293.1 and with IAI: 148.4 ng/ml, IQR 82.8–811.1; p=0.07, Figure 3). However, the median amniotic fluid fetal hemoglobin concentration was significantly higher in pregnancies complicated by either PTL or preterm PROM (with or without IAI) than in pregnant women at term, either in labor or not in labor (p<0.001 for all comparisons).

Fetal hemoglobin percentage of the total hemoglobin

Recently, we reported the amniotic fluid concentrations of total hemoglobin in these patients.[6] In order to study the origin of the hemoglobin detected,[6] the percentage of fetal hemoglobin of the total hemoglobin was calculate in each sample, and the following results were found: 1) the median amniotic fluid fetal hemoglobin percentage of the total hemoglobin concentration was lower in women at term in labor than those not in labor (0.2%, IQR 0–1.1 vs. 2.2%, IQR 0–7.1; p=0.03); 2) the median amniotic fluid fetal hemoglobin percentage of the total hemoglobin differed significantly among the 3 groups of patients with spontaneous PTL (Kruskal-Wallis, p=0.001). Women with PTL and IAI had a lower median amniotic fluid fetal hemoglobin percentage of the total hemoglobin than those without IAI who delivered preterm (2.8%, IQR 1.2–7.4 vs. 5.6%, IQR 1.8–13.5; p=0.03) and than those with PTL who delivered at term (7.3%, IQR 3–15.4; p<0.001) (Figure 4). There was no significant difference in the median amniotic fluid fetal hemoglobin percentage of the total hemoglobin in patients with PTL without IAI who delivered preterm or at term (p=0.3); 3) among women with preterm PROM, the median amniotic fluid fetal percentage of the total hemoglobin did not differ significantly between patients with and without IAI (1.8%, IQR 0.5–8.4 vs. 4.3%, IQR 0.7–9, respectively; p=0.07, Figure 5).

DISCUSSION

Principal findings of the study

1) Fetal hemoglobin was detected in 80.4% of all amniotic fluid samples, but in only 31.7% of mid-trimester samples; 2) the median amniotic fluid fetal hemoglobin concentration was higher in pregnancies complicated by PTL or preterm PROM than in term pregnancies; 3) there were no significant differences in the median amniotic fluid fetal hemoglobin concentrations among patients with and without IAI (regardless of the membrane status); 4) women with PTL and IAI had a lower amniotic fluid fetal hemoglobin percentage of the total hemoglobin when compared to those without IAI; and 5) similarly, women at term in labor had a lower median fetal hemoglobin percentage than those at term not in labor.

Potential origins of the detectable fetal hemoglobin in the amniotic fluid

Normal adult hemoglobin consists of approximately 96–98% of hemoglobin A ($\alpha_2\beta_2$), less than 2% of hemoglobin A2 ($\alpha_2\delta_2$) and less than 1% of fetal hemoglobin (hemoglobin F, $\alpha_2\gamma_2$).[13] However, up to 2% of hemoglobin F in the adult blood is considered normal, [8,13] whereas in the fetus the majority is fetal hemoglobin.[9] At birth, 60 to 80% of the neonatal hemoglobin is of the fetal type,[8,14] and after birth, most of the fetal hemoglobin is replaced by the adult type, being less than 10% by 4 months of age.[14]

In the study presented herein, we identified fetal hemoglobin in 80.4% of the amniotic fluid samples by using an immunoassay specific for fetal hemoglobin. Because hemoglobin F is present in both maternal and fetal circulations, the origin of amniotic fluid fetal hemoglobin could not be ascertained.

Sources for the detected hemoglobin in amniotic fluid include contamination during amniocentesis[15] (maternal or fetal); however, maternal or placental injury at the time of amniocentesis is relatively rare, ranging from 0.3% to 10.8%, whereas fetal injury rates range from 0.6% to 2%.[16] While we cannot completely rule out the possibility of contamination during amniocentesis, the detection of fetal hemoglobin in most of our amniotic fluid samples makes the possibility unlikely.

Another source for hemoglobin in amniotic fluid can be intra-amniotic bleeding prior to amniocentesis that originated either from the maternal[17–19] or fetal[20] circulation. Maternal intra-amniotic bleeding has been reported in cases of placental abruption,[21,22] circumvallate placenta,[17] as well as preterm labor[17,18] with clinical[23] or histological chorioamnionitis.[18] Intra-amniotic bleeding of fetal origin has been associated with a sacrococcygeal teratoma[20] and fetal transfusion.[24] Of note, all the case reports above mentioned were acute events with a noticeable amount of intra-amniotic maternal or fetal bleeding, leading to maternal or fetal compromise. A more subtle or chronic intra-amniotic bleed (maternal/fetal) may remain sub-clinical.[15]

Hyperechogenic fetal bowel can be associated with an asymptomatic intra-amniotic bleeding.[24–26] Indeed, an association between fetal small bowel hyperechogenicity and the presence of heme pigments in amniotic fluid retrieved during the second trimester was described;[24–26] and heme pigments were also detected in 5% of the fetuses without echogenic bowel.[26] Interestingly, echogenic bowel was diagnosed in 25% (7/28) of fetuses within the first 12 hours after an episode of intra-amniotic fetal bleeding (diagnosed by ultrasound follow up after fetal transfusion), but in only three fetuses the persisted 4 weeks.[24] These studies indicate that intra-amniotic bleeding could be subclinical.

Fetal hemoglobin concentration in amniotic fluid is not associated with intra-amniotic infection/inflammation

In the study presented herein, women with spontaneous PTL or preterm PROM, with or without IAI, had a significantly higher median amniotic fluid fetal hemoglobin concentration than normal pregnant women at term. However, among patients with PTL or preterm PROM, there were no significant differences in the median amniotic fluid fetal hemoglobin concentration between those with or without IAI. This is in contrast to the increased amniotic fluid total hemoglobin concentrations in pregnancies complicated with IAI.[6] This observation suggests that the main source of fetal hemoglobin in cases of PTL with IAI is of maternal origin, and the detected fetal hemoglobin represents the normal percentage of hemoglobin F in the maternal blood. Indeed, Gomez et al[7] proposed that a sub-clinical intrauterine infection can cause deciduitis and decidual bleeding leading to the clinical manifestation of vaginal bleeding and subsequent adverse pregnancy outcomes. Moreover, among patients with PTL, we found a lower median fetal hemoglobin percentage of the total hemoglobin in women with IAI compared to those without IAI and a similar trend, although not statistically significant, was found among patients with preterm PROM. Collectively, these findings support the hypothesis that maternal origin is the major contributor to the detected hemoglobin in patients with IAI.

Fetal hemoglobin in preterm labor or preterm PROM

Preterm labor and preterm PROM are syndromic in nature, resulting from IAI as well as other underlying mechanisms.[27–29] Among patients with spontaneous PTL, the median amniotic fluid fetal hemoglobin percentage of the total hemoglobin was significantly higher in women with PTL who delivered preterm or at term without IAI than those with IAI. Furthermore, the median percentage of fetal hemoglobin of the total hemoglobin detected in amniotic fluid of women with PTL without IAI who delivered preterm (5.6%) or at term (7.3%), and in women with preterm PROM without IAI (4.3%) was higher than the normal percentage in normal adult blood.[8] Taken together, these findings suggest that in a subset of patients, a sub-clinical intra-amniotic bleeding of fetal origin can be associated with spontaneous PTL or preterm PROM. Our findings suggest that conditions other than IAI can lead to intra-amniotic bleed. Indeed, Hankins et al[30] detected total and fetal hemoglobin in brown, green and clear second trimester amniotic fluid samples, with higher concentrations in the brown amniotic fluid, and the fetal hemoglobin was determined to be 20% to 100% of the total hemoglobin.[30] However, different insults may result in intra-amniotic fetal bleeding that can potentially originate from the immature skin, gastrointestinal tract, urinary or respiratory systems, and lead to the initiation of the common pathway of parturition, or, teleologically, fetal intra-amniotic bleeding may be a “stress signal” that the fetus uses to initiate labor.

Fetal hemoglobin and term pregnancy

Among women with a normal pregnancy at term, spontaneous labor was not associated with a significant change in the median amniotic fluid fetal hemoglobin concentration. However, the fetal hemoglobin percentage of the total hemoglobin was significantly lower among women in labor than that of those not in labor. This suggests that the finding of a significantly higher total hemoglobin concentration found in women at term in labor compared to those not in labor[6] is predominantly of maternal origin. Possible explanations for the maternal intra-amniotic bleeding during spontaneous term labor are: 1) uterine contractions or active labor may cause vessel damage and minute intra-amniotic bleeding; and 2) the counteracting changes in the hemostatic system (bleeding/thrombosis) associated with labor[31–34] may result in some degree of intra-amniotic bleeding.

Conclusions

The concentration of fetal hemoglobin in amniotic fluid increases with gestational age. Among women with PTL or preterm PROM, the amniotic fluid fetal hemoglobin concentrations were not associated with IAI. However, the percentage of fetal hemoglobin of the total hemoglobin in amniotic fluid was lower in pregnancies complicated by PTL with IAI than those without IAI, suggesting a different source of bleeding and, thus, a different mechanism of disease. While in PTL with IAI the hemoglobin detected in amniotic fluid can be attributed to originate mainly from the maternal circulations, in PTL without IAI the fetus contributes at least for some of the detected hemoglobin.

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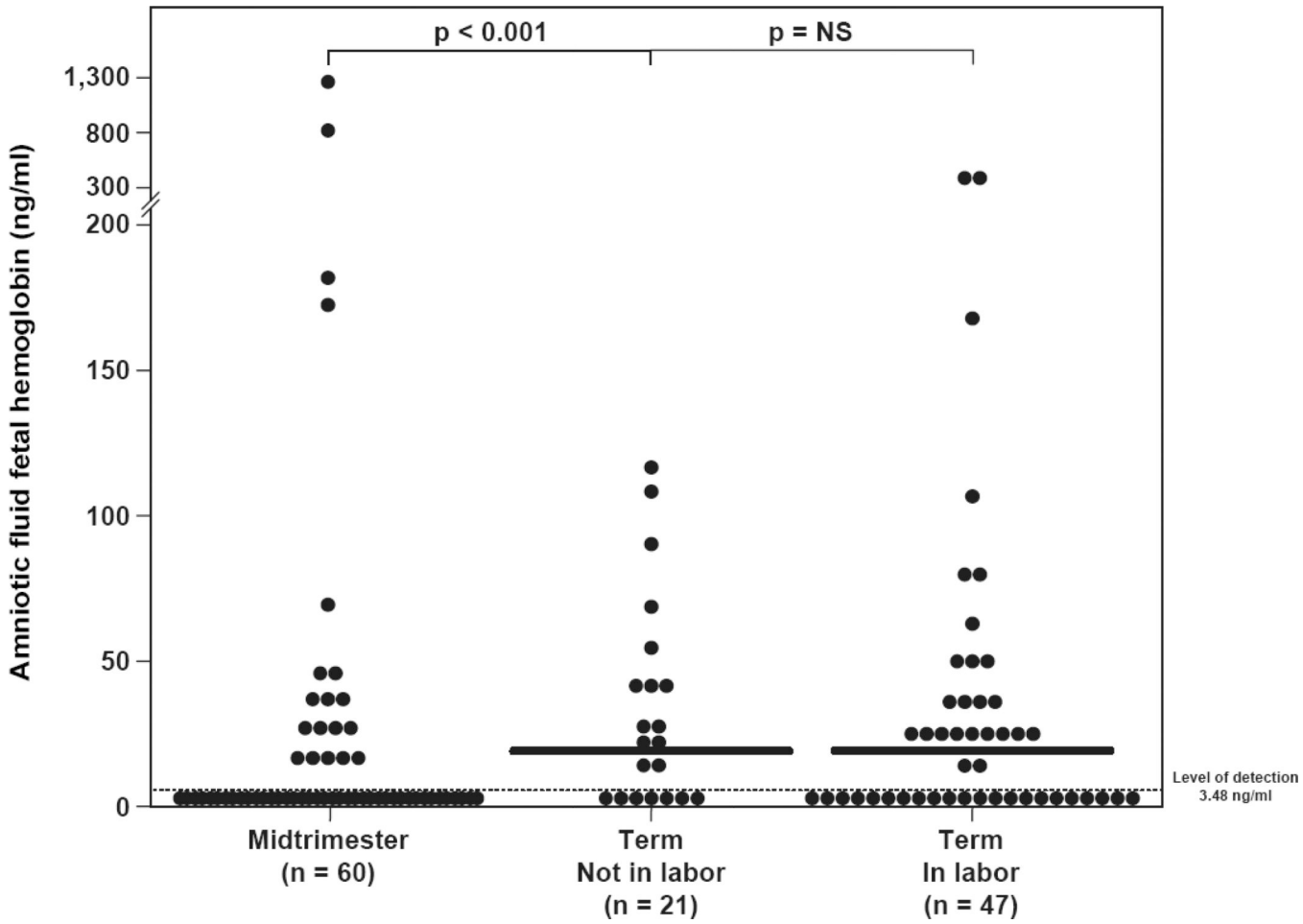

Figure 1.

Amniotic fluid concentration of fetal hemoglobin in normal pregnancies in the mid-trimester and at term, in labor and not in labor. The median amniotic fluid fetal hemoglobin concentration was significantly higher in women at term not in labor than in women in the mid-trimester [19.5 ng/ml, interquartile range (IQR) 0–49 vs. 0.0 ng/ml, IQR 0–19.9; *p=<0.001*]. Women at term, whether in labor or not in labor, had a similar median amniotic fluid fetal hemoglobin concentration (term in labor; 19.1 ng/ml, IQR 0–36.2, *p*=0.4).

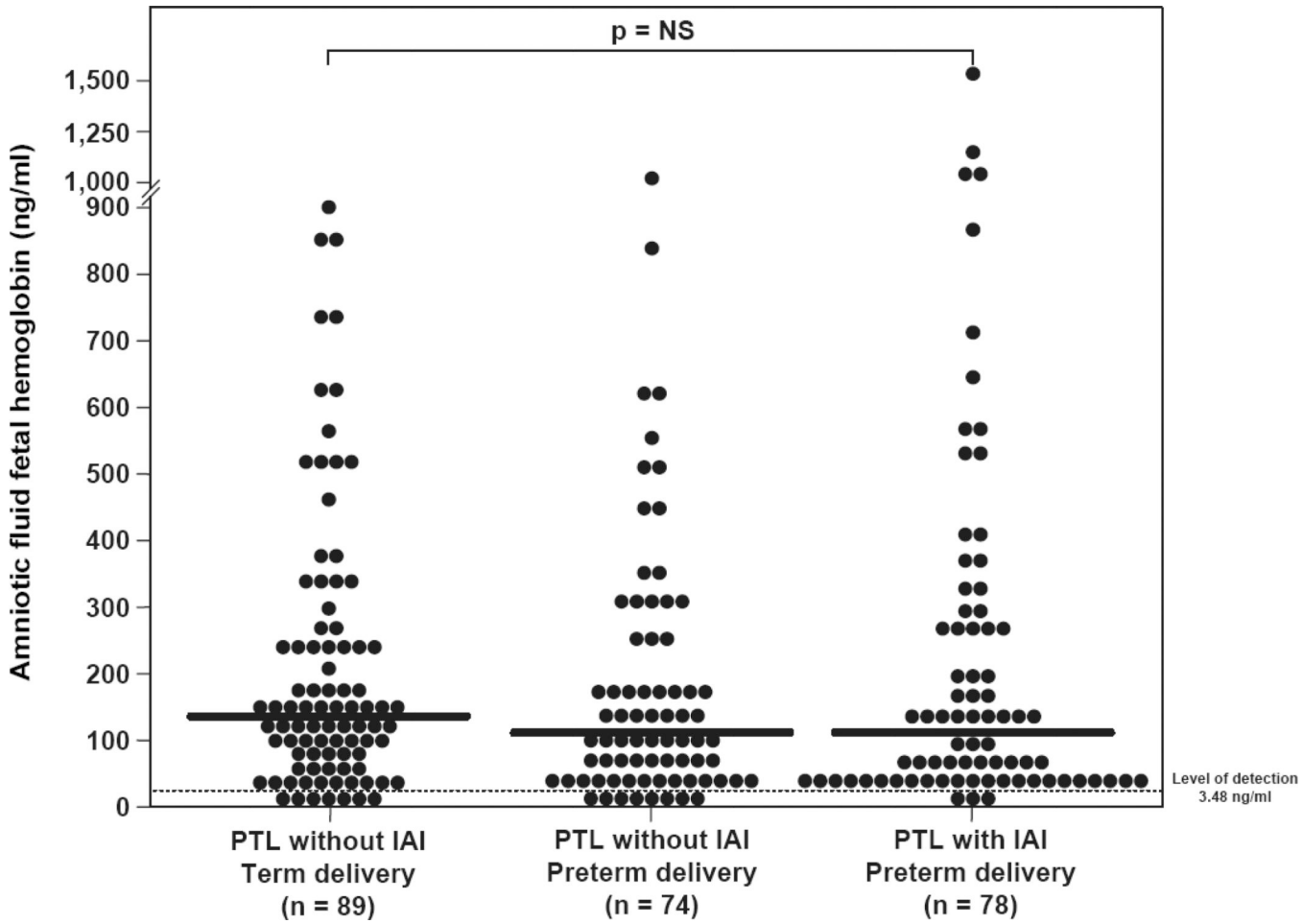

Figure 2.

Amniotic fluid concentration of fetal hemoglobin among women with spontaneous preterm labor and intact membranes (PTL). The median amniotic fluid concentration of fetal hemoglobin was not significantly different in patients with intra-amniotic infection/ inflammation (IAI) or without IAI who delivered preterm or at term [with IAI: 114.6 ng/ml, interquartile range (IQR) 33.2–283.9, without IAI delivered preterm: 109.6 ng/ml, IQR 42–247.1, delivered at term: 134.2 ng/ml, IQR 56.3–255.7, p=0.7].

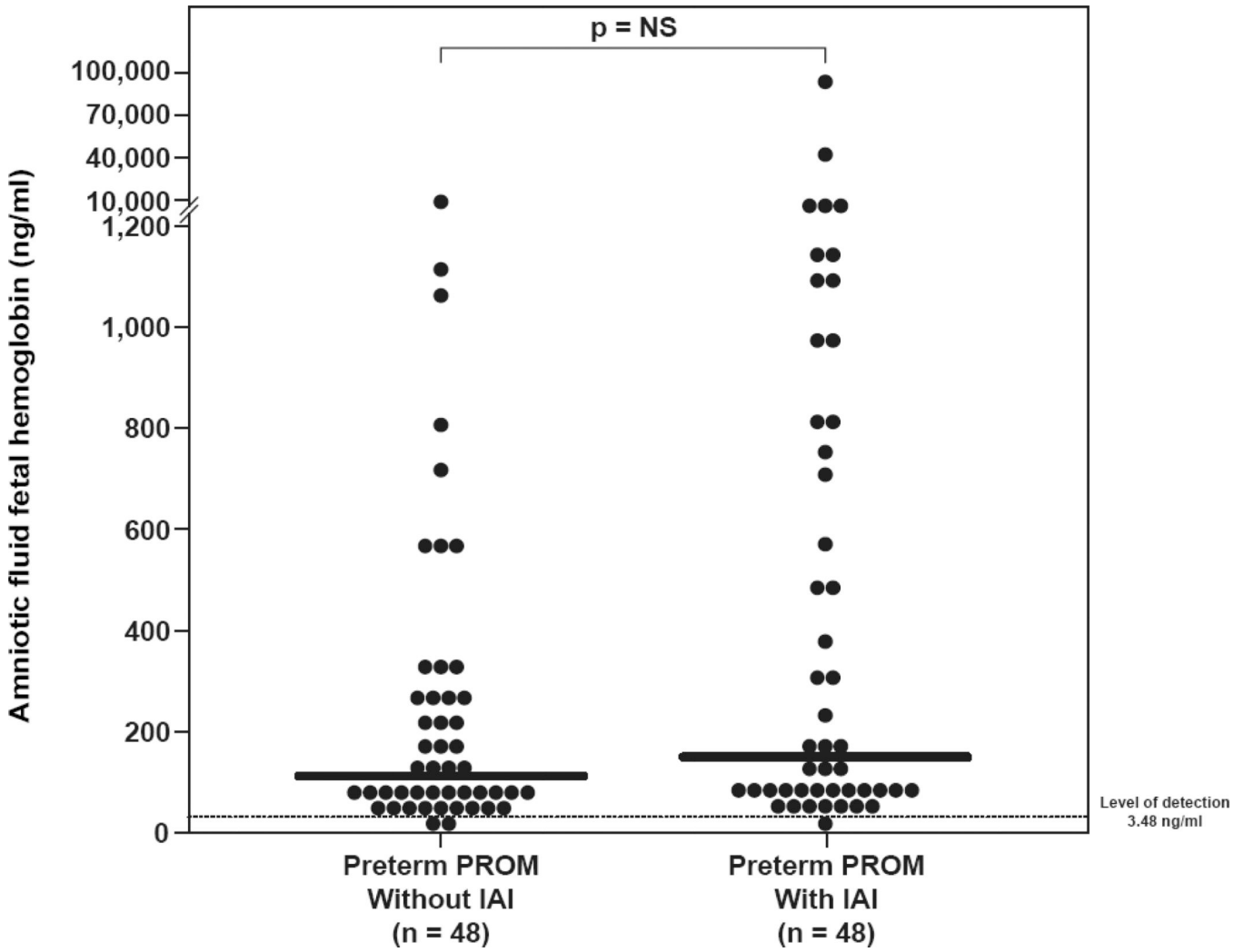

Figure 3.

Amniotic fluid concentration of fetal hemoglobin in women with preterm prelabor rupture of the membranes (preterm PROM). The median amniotic fluid concentration of fetal hemoglobin was not significantly different among patients with preterm PROM, with and without intra-amniotic infection/inflammation (IAI) [preterm PROM with IAI: 148.4 ng/ml, interquartile range (IQR) 82.8–811.1 vs. without IAI: 109.7 ng/ml, IQR; 51.4–293.1; p=0.07].

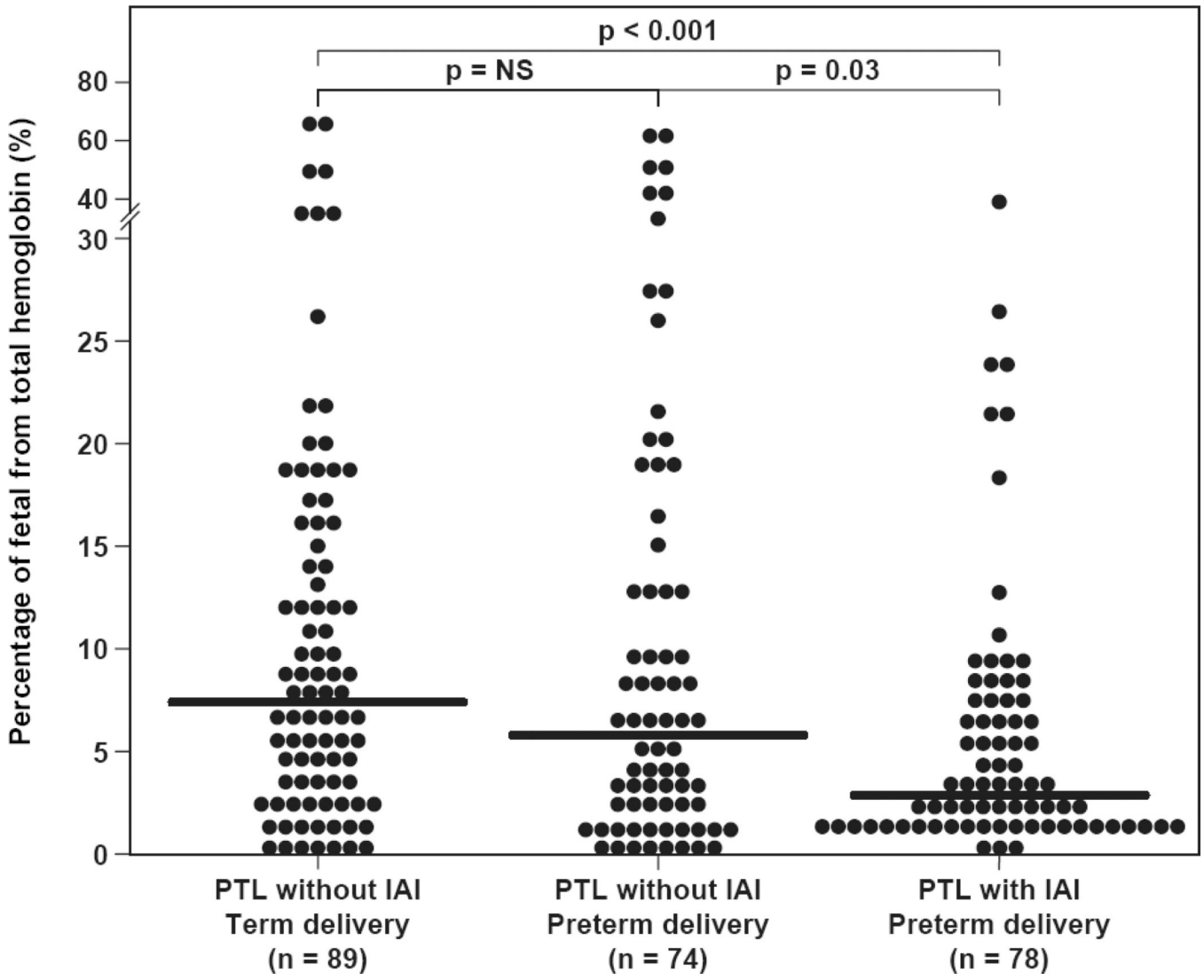

Figure 4.

The fetal hemoglobin percentage of the total hemoglobin detected in amniotic fluid among women with spontaneous preterm labor and intact membranes (PTL). The median amniotic fluid fetal hemoglobin percentage of the total hemoglobin was lower in women with IAI than those without IAI who delivered preterm (2.78%, interquartile range (IQR) 1.18–7.44 vs 5.61%, IQR 1.76–13.52; *p*=0.03) or at term (7.33%, IQR 3–15.4; p<0.001). There was no significant difference in the median amniotic fluid fetal hemoglobin percentage of the total hemoglobin in those without IAI who delivered preterm or at term (p=0.3).

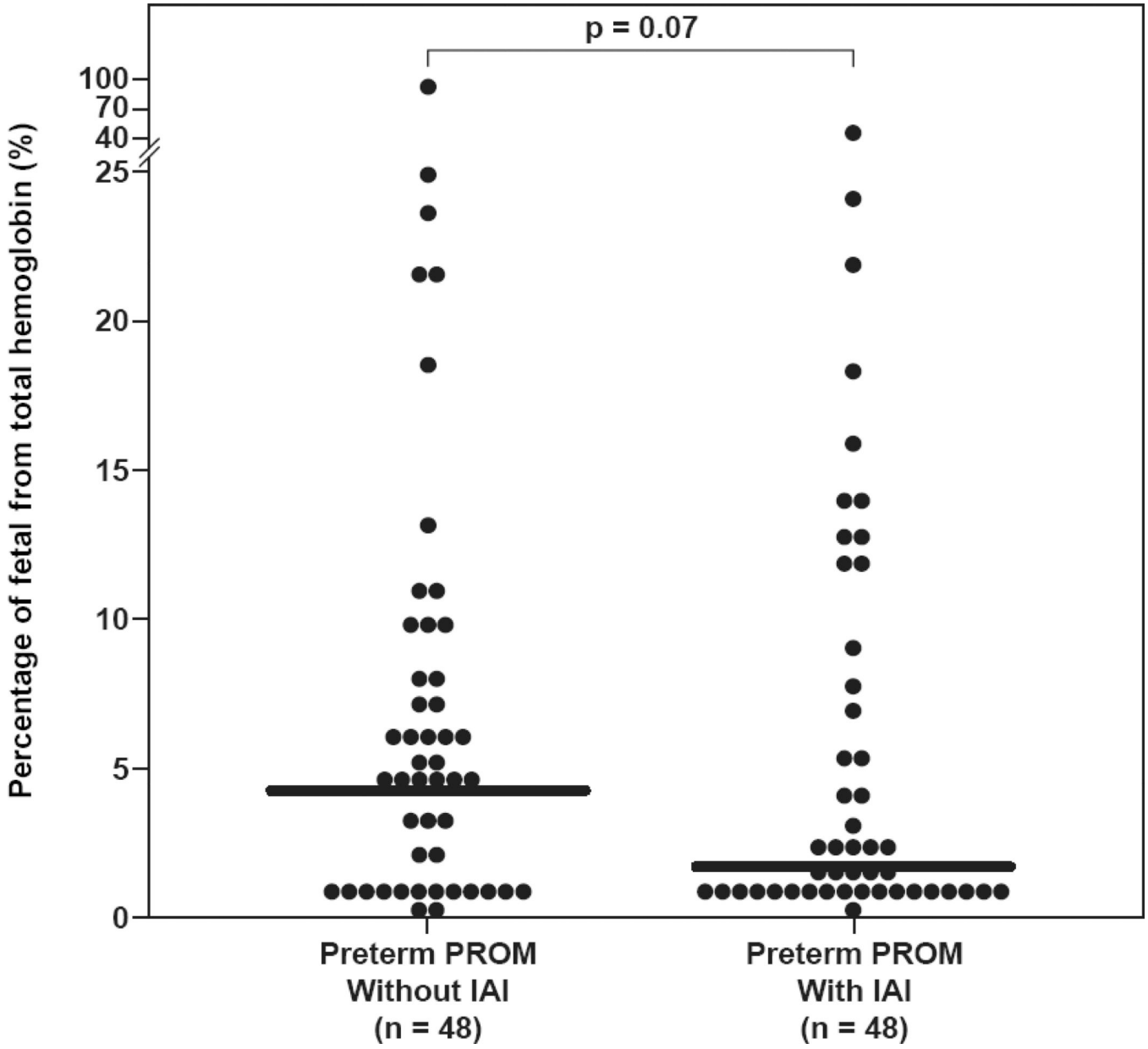

Figure 5.

The fetal hemoglobin percentage of the total hemoglobin detected in amniotic fluid among women with preterm prelabor rupture of the membranes (PPROM). The median amniotic fluid fetal percentage of the total hemoglobin did not differ significantly in women with and without IAI (1.76%, interquartile range (IQR) 0.46–8.37 vs 4.31%, IQR 0.72–8.96, respectively; p=0.07).

Table I

Gestational age at amniocentesis and the proportion of samples that had detectable amniotic fluid fetal hemoglobin concentration in each group.

	Gestational age at amniocentesis (weeks)	Fetal hemoglobin detectable
	Median (IQR)	% (n)
Mid-trimester normal pregnancy	16 (16–17)	31.7 (19/60)
PTL and delivery at term	30.6 (26.6–32.7)	92.1 (82/89)
PTL without IAI and delivery preterm	31.1 (27.5–32.2)	89.2 (66/74)
PTL with IAI	26.1 (24.1–32.1)	96.2 (75/78)
Preterm PROM without IAI	32.2 (28.7–33.7)	95.8 (46/48)
Preterm PROM with IAI	29.2 (25.4–31.3)	97.9 (47/48)
Term not in labor	39.0 (38.2–40)	66.7 (14/21)
Term and in labor	39.0 (38–40)	53.2 (25/47)

IQR, interquartile range; PTL, Preterm labor; IAI, intra-amniotic infection/inflammation; PROM, prelabor rupture of membranes

Table II

Clinical and demographic characteristics of women presenting with spontaneous preterm labor.

	Spontaneous PTL and intact membranes			
	delivered at term (*n*=89)	**without IAI (*n*=74)**	**with IAI (*n*=78)**	***p****
Maternal age (years)	23 (20–27)	21 (19–29)	24 (20–28)	*NS*
History of vaginal bleeding	9 (8/89)	20 (14/70)†	19.2 (15/78)†	0.04†
Gestational age at amniocentesis (weeks)	30.6 (26.6–32.7)	31.1 (27.5–32.2)	26.1 (24.1–32.1)	0.002
Amniotic fluid sample storage time (years)	5.6 (4.9–7.5)	5.8 (5.0–7.2)	5.9 (5.5–6.8)	*NS*
Gestational age at delivery (weeks)	38.6 (37.9–39.5)	34.6 (32.7–35.3)	28 (24.5–32.9)	<.001
Birthweight (grams)	3040 (2772–3370)	2195 (1755–2440)	1060 (647–1800)	<.001

Values expressed as median (interquartile range) or percent (number)

PTL: preterm labor; IAI: intra-amniotic infection/inflammation; NS: not significant.

*Kruskal-Wallis test with Bonferroni correction

†Chi-Square test in comparison to PTL who delivered at term

Table III

Clinical and demographic characteristics of women presenting with preterm PROM.

	Preterm PROM		
	without IAI (*n*=48)	**with IAI (*n*=48)**	***p****
Maternal age (years)	25 (20–34.5)	28.5 (22–35.5)	*NS*
History of vaginal bleeding	14.9 (7/47)	22.9 (11/48)	*NS*†
Gestational age at amniocentesis (weeks)	32.2 (28.7–33.1)	29.2 (25.4–31.3)	<.001
Amniotic fluid sample storage time (years)	4.9 (3.7–5.8)	5.0 (4.4–5.8)	*NS*
Gestational age at delivery (weeks)	33.2 (31.2–34.4)	30 (27.8–31.9)	<.001
Birthweight (grams)	2020 (1677–2292)	1520 (1060–1820)	<.001

Values expressed as median (interquartile range) or percent (number)

GA: gestational age; IAI: intra-amniotic infection/inflammation; PROM: prelabor rupture of the membranes; NS: not significant

*Mann-Whitney U-test

†Chi-Square test