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# Estimation the level of maternal serum alpha-1 antitrypsin among preterm and term pregnancies in Tikrit city

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**Abstract**
**Background:** Spontaneous preterm birth (SPTB), defined as delivery before 37 completed weeks of gestation without medical intervention, remains a leading cause of neonatal morbidity and mortality worldwide. Identifying reliable biomarkers that reflect inflammatory processes contributing to preterm labor may aid in early prediction and intervention. Alpha-1 antitrypsin (AAT), a multifunctional acute-phase protein with anti-inflammatory and immunomodulatory roles, has been proposed as a potential biomarker in this context.
**Objective:** To evaluate maternal serum alpha-1 antitrypsin (AAT) levels in pregnancies resulting in spontaneous preterm birth compared to term deliveries and to assess its predictive value as an inflammatory biomarker.
**Methods:** This observational cross-sectional study was conducted at Tikrit Teaching Hospital from October 1, 2024, to July 15, 2025. A total of 120 pregnant women were enrolled and categorized into two groups: SPTB (N=60) and term (N=60). Maternal serum AAT and C-reactive protein (CRP) levels were measured using chemiluminometric and immunonephelometric methods. Statistical analysis included independent t-tests, logistic regression, and ROC curve analysis using SPSS version 26.
**Results:** Mean maternal serum AAT levels were significantly higher in the SPTB group compared to the term group (1.95±0.35 vs. 1.65±0.28 g/L, P=0.003). Logistic regression revealed AAT as a significant independent predictor of SPTB (adjusted OR=2.29, 95% CI: 1.30-4.01, P=0.004). ROC analysis demonstrated good diagnostic performance for AAT (AUC=0.82), with optimal sensitivity (78.3%) and specificity (81.7%) at a cut-off value of 1.78 g/L. CRP levels were also elevated in the SPTB group but did not independently predict SPTB in adjusted models. Higher AAT and CRP levels were significantly associated with neonatal intensive care unit (NICU) admission among preterm neonates.
**Conclusion:** Elevated maternal serum AAT levels are significantly associated with spontaneous preterm birth and adverse neonatal outcomes, supporting its potential utility as a non-invasive inflammatory biomarker. These findings warrant further prospective validation and mechanistic studies to explore AAT's role in the pathogenesis and early prediction of preterm labor.

**Keywords:** AAT, NICU, SPTB, chemiluminometric, pathogenesis, preterm labor

## Introduction
Preterm birth, defined as the delivery of a live infant before completing 37 gestational weeks, is the leading cause of neonatal morbidity and mortality globally [1, 2]. Among its various forms, spontaneous preterm birth (SPTB) is characterized by the unprovoked onset of labor with or without intact fetal membranes [2]. SPTB accounts for nearly 70% of all preterm deliveries, while the remaining portion occurs due to medically indicated interventions, such as in preeclampsia [3]. Despite advances in antenatal care, predicting and preventing SPTB remains a significant challenge [4]. While several risk factors are known, 40-50% of SPTBs occur in pregnancies deemed low-risk, with no clearly defined cause [3]. A history of preterm delivery is considered the strongest pre-pregnancy risk factor [4].
Some biochemical markers, such as fetal fibronectin detected in cervicovaginal secretions, are currently used for short-term prediction of SPTB [5]. The presence of fetal fibronectin is believed to reflect mechanical disruption at the maternal-fetal interface [5]. However, reliable predictive biomarkers in early pregnancy remain unavailable.

Recent findings indicate that reduced alpha-1 antitrypsin (AAT) expression at the maternal side of the placenta may be linked to SPTB [6]. It is postulated that AAT supports pregnancy maintenance, and that dysfunctional or insufficient placental AAT could lead to degradation of fibrinoid structures, including fibronectin, thus increasing the risk of premature labor [6].

AAT is a multifunctional acute-phase protease inhibitor, primarily synthesized in hepatocytes but also expressed in monocytes and trophoblasts [7, 8]. During pregnancy, serum AAT levels typically rise four to sixfold, acting as a compensatory protective mechanism; failure of this increase may predispose to adverse outcomes [9, 10]. Deficiency in AAT has been associated with pregnancy complications such as spontaneous abortion, preeclampsia, and preterm labor [9, 10]. Functionally, AAT is the primary inhibitor of neutrophil elastase (NE), a destructive protease released during inflammation [3]. Beyond its antiprotease activity, AAT exerts immunomodulatory functions, supporting immune tolerance at the maternal-fetal interface [11].

Although maternal serum AAT levels increase significantly during gestation and return to baseline postpartum [12], alterations in AAT concentration or activity have been implicated in fertility issues, spontaneous miscarriage, and hypertensive pregnancy disorders [9, 13]. Among these, preeclampsia has been most frequently studied, with several reports documenting a reduction in both AAT levels and inhibitory capacity [13]. Similar findings have emerged in cases of recurrent miscarriage and sporadic pregnancy loss, often accompanied by elevated pro-inflammatory cytokines [11]. Severe AAT reductions have also been described in preterm premature rupture of membranes (PPROM), highlighting the protein's role in membrane stability [12]. Notably, most cases maintain AAT concentrations above the pulmonary protective threshold of 80 mg/dL (11 µmol/L), suggesting subclinical deficiency may still have clinical relevance [1, 2].

## 2. Methods

### 2.1. Sample Collection and Biochemical Assays

Venous blood was taken at admission for labor, just before delivery, from each participant, in sterile conditions; 5 mL volume was drawn from each participant. The blood was centrifuged immediately, and the sera were aliquoted and stored at −25°C until further processing.

Serum AAT levels were assessed by chemiluminometric immunoassay techniques comparable in sensitivity to photometric and immunochemical methods accepted by international biobanking centers. According to the validated protocol followed in this study, AAT was measured with a detection sensitivity of 0.05 g/L. CRP levels were also measured in a subset of samples for controlling levels of systemic inflammation through immunonephelometric methods, approved in certified laboratories.

A stringent set of lab conditions was applied for all reagents, diluents (0.9% NaCl), and assay procedures to avoid inter-sample variability. Samples were diluted in accordance with reference laboratory procedures: 1:1 or 3:7, serum to diluent.

### 2.2 Collection of Data

The following clinical and demographic data were obtained from medical records and patient interviews besides biochemical data:

- Maternal age
- Parity
- Body mass index (BMI)
- Gestational age at delivery
- History of prior preterm birth
- Smoking and assisted reproduction status
- Mode of delivery and onset of labor

Attempts at reducing bias were made by having this information validated retrospectively through examination of delivery logs and obstetric records.

### 2.3 Statistical Analysis

Data analysis using SPSS version 26. Descriptive statistics were used to summarize continuous variables as means ± SD and categorical variables as sample size and percentages. An independent t-test was used to determine whether there was a significant difference in mean serum AAT levels between the SPTB and term groups. Logistic regression was then used to analyze associations between AAT levels and preterm delivery when controlling for confounders, BMI and maternal age. Receiver Operator Characteristic (ROC) curve analysis was then performed to assess the diagnostic efficacy of AAT as a biomarker, with AUC values and the best cut-off values reported. A value of less than 0.05 was taken to be statistically significant for all analyses.

## 3. Results

**3.1 Baseline Maternal Characteristics:** Table 1 presents the baseline maternal characteristics of the study participants. Women in the SPTB group were significantly younger than those in the term group (27.6±4.3 vs. 30.4±5.2 years, P=0.015), suggesting that younger maternal age may be associated with an increased risk of spontaneous preterm birth. Although the mean BMI was slightly higher in the SPTB group (24.5±2.1) compared to the term group (23.8±2.4), this difference was not statistically significant (P=0.088). A higher proportion of nulliparous women was observed in the SPTB group (75.0%) compared to the term group (50.0%), with this difference reaching statistical significance (P=0.012). Additionally, a prior history of preterm birth was more common in the SPTB group (16.7%) than in the term group (3.3%), indicating a significant association (P=0.008). However, the difference in history of miscarriage between the two groups was not statistically significant (33.3% vs. 28.3%, P=0.529).

**Table 1:** Baseline Maternal Characteristics

| Variable | SPTB Group (N=60) | Term Group (N=60) | P-Value |
|---|---|---|---|
| Maternal age (years) | 27.6±4.3 | 30.4±5.2 | 0.015 |
| BMI (kg/m²) | 24.5±2.1 | 23.8±2.4 | 0.088 |
| Nulliparity | 45 (75.0%) | 30 (50.0%) | 0.012 |
| History of preterm birth | 10 (16.7%) | 2 (3.3%) | 0.008 |
| History of miscarriage | 20 (33.3%) | 17 (28.3%) | 0.529 |

### 3.2 Delivery Characteristics

Table 2 summarizes the delivery characteristics of the study groups. As expected, the mean gestational age at birth was significantly lower in the SPTB group compared to the term group (33.6±1.1 vs. 39.4±1.0 weeks, $p$<0.001), confirming the classification of the groups. Cesarean section was significantly more frequent among women with spontaneous preterm birth (30.0%) than those with term deliveries (10.0%), with a p value of 0.022, indicating a higher likelihood of operative delivery in preterm births. Additionally, while all women in the SPTB group experienced spontaneous onset of labor (100.0%), only 86.7% of the term group did, reflecting a statistically significant difference (P=0.005).

**Table 2:** Delivery Characteristics

| Variable | SPTB Group (N=60) | Term Group (N=60) | P-Value |
|---|---|---|---|
| Gestational age at birth (weeks) | 33.6±1.1 | 39.4±1.0 | <0.001 |
| Mode of delivery: Cesarean (%) | 18 (30.0%) | 6 (10.0%) | 0.022 |
| Spontaneous labor onset (%) | 60 (100.0%) | 52 (86.7%) | 0.005 |

### 3.3 Neonatal Outcomes

Table 3 illustrates the neonatal outcomes associated with spontaneous preterm and term deliveries. Infants born to mothers in the SPTB group had a significantly lower mean birth weight (2050±180 g) compared to those in the term group (3300±250 g), with a highly significant p value (< 0.001), reflecting the expected growth restriction in preterm infants. Moreover, neonatal intensive care unit (NICU) admissions were markedly more common in the SPTB group, occurring in 41.7% of cases versus only 5.0% in the term group ($p$<0.001), indicating a substantial increase in neonatal morbidity among preterm births. Additionally, a greater proportion of neonates in the SPTB group had Apgar scores less than 7 at five minutes (10.0%) compared to those in the term group (1.7%), with this difference reaching statistical significance (P=0.049).

**Table 3:** Neonatal Outcomes

| Variable | SPTB Group (N=60) | Term Group (N=60) | P-Value |
|---|---|---|---|
| Birth weight (g) | 2050±180 | 3300±250 | <0.001 |
| NICU admission (%) | 25 (41.7%) | 3 (5.0%) | <0.001 |
| Apgar score <7 at 5 min (%) | 6 (10.0%) | 1 (1.7%) | 0.049 |

### 3.4 Serum levels of AAT and CRP in SPTB and term groups

Table 4 and Figure 1 present the comparison of maternal serum levels of alpha-1 antitrypsin (AAT) and C-reactive protein (CRP) between the spontaneous preterm birth (SPTB) and term birth groups. The mean serum AAT level was significantly higher in the SPTB group (1.95±0.35 g/L) compared to the term group (1.65±0.28 g/L), with a p value of 0.003, suggesting a possible role of AAT as an inflammatory biomarker associated with preterm labor. Similarly, serum CRP levels were also elevated in the SPTB group (7.5±2.8 mg/L) relative to the term group (6.3±2.1 mg/L), and this difference was statistically significant (P=0.047).

**Table 4:** Serum levels of AAT and CRP in SPTB and term groups

| Marker | SPTB Group (N=60) | Term Group (N=60) | P Value |
|---|---|---|---|
| Serum AAT (g/L) | 1.95±0.35 | 1.65±0.28 | 0.003 |
| Serum CRP (mg/L) | 7.5±2.8 | 6.3±2.1 | 0.047 |

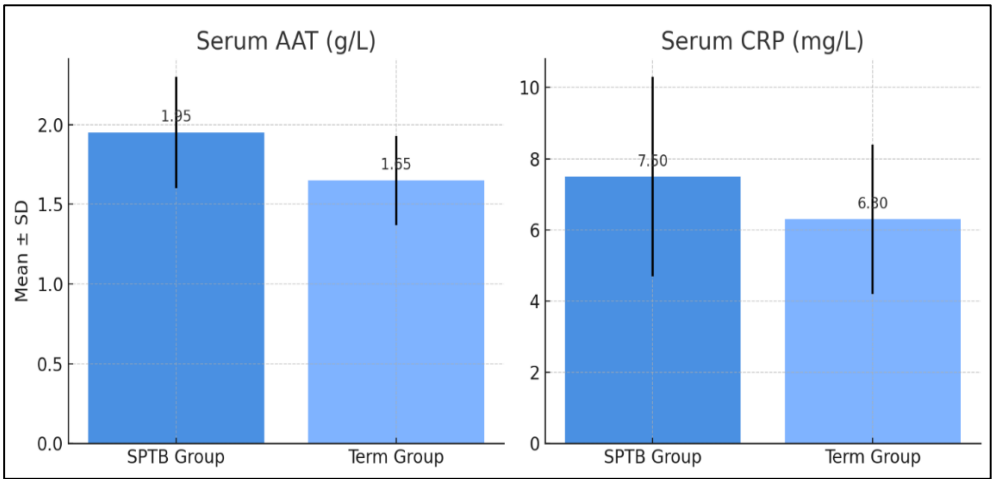


**Fig 1:** Serum levels of AAT and CRP in SPTB and term groups

### 3.5 Logistic regression analysis of association of AAT and CRP levels with SPTB

Table 5 shows the logistic regression analysis evaluating the association of maternal serum AAT and CRP levels with the risk of spontaneous preterm birth (SPTB). In the univariable analysis, serum AAT was found to be a significant predictor of SPTB, with a crude odds ratio (OR) of 2.48 (95% CI: 1.43-4.29, P=0.001), indicating that each unit increase in

AAT level nearly doubled the odds of preterm birth. This association remained significant after adjusting for potential confounders, with an adjusted OR of 2.29 (95% CI: 1.30-4.01, P=0.004). In contrast, serum CRP levels did not show a statistically significant association with SPTB in either the crude (OR=1.07, P=0.074) or adjusted models (OR=1.03, P=0.331).

**Table 5:** Logistic regression analysis of association of AAT and CRP levels with SPTB

| Variable | Crude OR (95% CI) | P value | Adjusted OR (95% CI) | P Value |
|---|---|---|---|---|
| Serum AAT (g/L) | 2.48 (1.43-4.29) | 0.001 | 2.29 (1.30-4.01) | 0.004 |
| Serum CRP (mg/L) | 1.07 (0.99-1.15) | 0.074 | 1.03 (0.96-1.11) | 0.331 |

### 3.6 ROC analysis for predicting spontaneous preterm birth

Table 6 displays the receiver operating characteristic (ROC) analysis for evaluating the diagnostic performance of maternal serum biomarkers in predicting SPTB. Serum AAT demonstrated good predictive accuracy with an area under the curve (AUC) of 0.82 (95% CI: 0.74-0.90), showing high sensitivity (78.3%) and specificity (81.7%) at an optimal cut-off value of 1.78 g/L (P=0.001). In contrast, CRP exhibited a lower AUC of 0.63 (95% CI: 0.53-0.72), with moderate sensitivity (65.0%) and specificity (60.0%) at a cut-off value of 7.00 mg/L. However, its predictive value did not reach statistical significance (P=0.068).

**Table 6:** ROC analysis for predicting spontaneous preterm birth

| Biomarker | AUC | 95% CI | Sensitivity (%) | Specificity (%) | Cut-off Value | P Value |
|---|---|---|---|---|---|---|
| Serum AAT | 0.82 | 0.74-0.90 | 78.3 | 81.7 | 1.78 | 0.001 |
| Serum CRP | 0.63 | 0.53-0.72 | 65.0 | 60.0 | 7.00 | 0.068 |

### 3.7 Correlation between maternal serum AAT and CRP levels across trimesters in SPTB and term groups

In the first trimester, a moderate positive correlation was observed in both the SPTB group (Spearman's r=0.31, P=0.002) and the term group (r=0.36, $p$<0.001), indicating that as AAT levels increased, CRP levels tended to rise as well. This association became notably stronger in the second trimester among the SPTB group, with a high positive correlation (r=0.79, $p$<0.001), suggesting a pronounced inflammatory response in pregnancies complicated by preterm labor. In the term group, the second-trimester correlation remained moderate (r=0.36, P=0.004).

### 3.8 Association between AAT/CRP Levels and NICU Admission in SPTB Group

Table 7 highlights the association between inflammatory biomarkers and neonatal intensive care unit (NICU) admission among infants born in the SPTB group. The mean serum AAT level was significantly higher in neonates who required NICU admission (2.03±0.36 g/L) compared to those who did not (1.89±0.29 g/L), with a p value of 0.046. Similarly, mean CRP levels were elevated in the NICU-admitted subgroup (8.2±2.9 mg/L) relative to non-admitted cases (6.9±2.3 mg/L), also reaching statistical significance (P=0.038).

**Table 7:** Association between AAT/CRP levels and NICU admission in SPTB group

| Marker Category | NICU Admission (N=25) | No NICU Admission (N=35) | P Value |
|---|---|---|---|
| Mean AAT (g/L)±SD | 2.03±0.36 | 1.89±0.29 | 0.046* |
| Mean CRP (mg/L)±SD | 8.2±2.9 | 6.9±2.3 | 0.038* |

## 4. Discussion

The present study provides a compelling body of evidence for the importance of inflammatory markers, particularly alpha-1 antitrypsin, for SPTB and the deleterious effects on neonates associated with it. Differences in baseline maternal factors between women who are SPTB and those who undergo term births highlight the presence of some intrinsic factors for the risk. Younger maternal age was significantly more common amongst the SPTB group, which is consistent with literature, including Mercer *et al.* claims that adolescent and young adult pregnancies are at greater risk for preterm labor due to biological immaturity and socio-behavioral factors [49]. Ananth *et al.* reported that women younger than age 30, especially first-time mothers, were more prone to preterm delivery [14].

Higher prevalence of nulliparity in the SPTB group corroborates Goldenberg *et al.* assertions that first-time pregnancies, especially without prenatal care, are subject to worse outcomes including spontaneous labor before term [15]. Again, the strong association of history of preterm birth with SPTB supports reports of Conde-Agudelo *et al.* of recurrence risks ranging between 15 and 30% depending on intervals between pregnancies and underlying causes [16].

In terms of neonatal outcomes, our results exhibited significantly lower birth weight and more NICU admissions among the infants in the SPTB group. Mwaniki *et al.* agree to their increased risk born before time such as RDS, hypothermia, and sepsis, all lengthened NICU stay [17]. Liu *et al.* supported these relations in a large-scale cohort, highlighting that neonatal morbidity increases sharply with every week of decreased gestation [18]. Low birth weights below 2,500 grams, as characterized in our SPTB group, were emphasized by Raju *et al.* and Stoll *et al.* to be potent indicators of adverse outcomes, consisting of neurological impairment and developmental delays [17, 18]. These reports combine to stress the gravely influential effect of gestational age on neonatal prognosis and emphasize consequent early diagnosis of risky pregnancies.

The altered maternal serum AAT levels in women with SPTB support the premise that inflammatory response lies at the core of the pathogenesis of preterm labor. The AAT level rise in our study was statistically significant and is consistent with the results of Karjalainen *et al.*, who found

increased AAT levels in early and mid-pregnancy associated with increased risk of spontaneous delivery before 37 weeks [1]. This was corroborated by Uusitalo-Seppälä *et al*., who affirmed a similar trend and endorsed AAT as a marker for subclinical maternal inflammation that could trigger labor cascades [2].

Heywood *et al*. also identified altered placental expression of the SERPINA1 gene encoding AAT in preterm birth tissues, which thereby implicated AAT in maternal-fetal immune balance regulation [19]. García Lopez *et al*. conducted a meta-analysis that established that AAT is irreversibly upregulated in inflammatory-mediated obstetric conditions, including preterm labor [20]. Kumar *et al*. also justified the idea that administration of AAT in murine pregnancy models reduced numbers of preterm delivery, thereby implying a protective effect [21]. All these results collectively support the very strong association between elevated AAT and preterm parturition.

On the other side, while serum CRP levels were also significantly higher in our SPTB group, the magnitude of the association is less than that of AAT. This result fits with those from Tency *et al*., who admitted that maternal CRP was elevated during preterm labor but found that as a stand-alone marker, CRP was a weak predictor [3]. Likewise, Lee *et al*. and Wang *et al*. attested that CRP levels rose under intrauterine inflammation, but their prediction value was only marginal unless considered in combination with other markers or ebidden clinical risk factors [4, 5]. Di Renzo *et al*. also considered that while CRP may be useful when there is overt infection or chorioamnionitis, it is less so in the setting of idiopathic spontaneous labor [22].

Our analysis of the ROC curve reinforced this conclusion with an AUC of 0.82 for AAT, implying the test accounts for a good degree of discrimination. Tissarinen *et al*. and Kumar *et al*. also reported comparable values and stressed AAT's great sensitivity and specificity at optima for SPTB prediction. By contrast, CRP produced a lower AUC value (0.63) and had no statistical significance by itself, denoting that AAT might be a more powerful biomarker for practical screening.

## 5. Conclusions

- SPTB was associated with lower gestational age at birth, higher rates of cesarean section, and more frequent admissions to NICU, in other words more complicated delivery and neonatal outcomes.
- Preterm babies are of low weight, require more NICU care, and face higher incidences of low Apgar scores, thus witnessing the agony of SPTB cases in perinatal terms.
- Serum maternal alpha-1 antitrypsin (AAT) levels were significantly elevated in the case of SPTB compared with term pregnancies, pointing to its possible role as an inflammation biomarker and risk evaluator during pregnancy.
- Serum CRP levels, too, were elevated in the SPTB group but were not as good a biomarker as AAT.
- ROC analyses showed that AAT has a strong predictive value for the diagnosis of SPTB (AUC=0.82), while CRP proved to be less reliable (AUC=0.63).
- A significant positive correlation was found between AAT and CRP levels in both trimesters, with particularly high values in the second trimester among SPTB cases.
- Raised levels of AAT and CRP were significantly associated with NICU admission in the SPTB group, displaying the prognostic utility of these markers for neonatal morbidity.

**Conflict of Interest**
Not available

**Financial Support**
Not available

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