Association between serum alkaline phosphatase levels in late pregnancy and the incidence of venous thromboembolism postpartum: a retrospective cohort study

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Summary

Background Two previous studies found alkaline phosphatase (ALP) levels were related with the development of venous thromboembolism (VTE) in hospitalised patients. VTE is a leading cause of death during pregnancy and postpartum. No prior study has investigated the associations of ALP levels and VTE postpartum, and the related mechanisms remain unclear. This study aimed to investigate the associations between ALP levels and VTE postpartum, and to reveal the potential mechanisms.

Methods In this retrospective cohort study, we included pregnant women who planned to deliver at the Department of Obstetrics and Gynecology in the three designated hospitals in a multicentre cohort of pregnant women in Wuhan, China, during two recruitment periods of January 1, 2018 to December 31, 2019, and May 14, 2020 to March 25, 2022. A total of 10,044 participants with serum ALP and whole blood hemoglobin measurements in late pregnancy (median, 37 (35, 39) weeks) were enrolled. The participants' incidences of VTE (deep venous thrombosis and/or pulmonary embolism) postpartum were confirmed from the medical records. Pregnant women with new-onset VTE postpartum (within 6 weeks after delivery) were confirmed as VTE cases.

Findings Approximately 0.8% (79/10,044) of the pregnant women were diagnosed with VTE postpartum. In the unadjusted model, pregnant women with the lowest quintile of serum ALP levels (≤116 U/L) in late pregnancy had higher risk of VTE postpartum compared with those with the highest quintile (≥199 U/L) (OR, 2.83 [1.32, 6.05]). After adjusting for covariates of demographic, life style, birth outcomes, and other liver enzymes, pregnant women with the lowest quintile of serum ALP levels (≤116 U/L) in late pregnancy had increased risk of VTE postpartum compared with those with the highest quintile (≥199 U/L) (OR, 2.48 [1.14, 5.40]). A one standard deviation decrease of ln-transformed ALP levels were associated with elevated risk of VTE postpartum (OR, 1.29 [1.02, 1.62]). Significant negative associations of ALP with VTE were found in the unadjusted and adjusted models. The negative associations between ALP and VTE remained consistent in sensitivity analyses among participants with non-GDM, single pregnancy, non-preeclampsia, non-postpartum hemorrhage, non-extremely/very preterm and cesarean delivery. Decreased serum ALP levels significantly ($P < 0.05$) related to decreased hemoglobin, which was significantly ($P < 0.05$) related to increased risk of VTE postpartum. Decreased hemoglobin significantly ($P < 0.05$) mediated 7.59% of ALP-associated VTE postpartum.

Interpretation This study suggested that low serum ALP levels in late pregnancy were associated with increased risk of VTE postpartum, and the ALP-associated VTE risk may be partially mediated by hemoglobin, suggesting that serum ALP in late pregnancy could be a promising biomarker for the prediction of VTE postpartum.

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Keywords: Alkaline phosphatase; Venous thromboembolism; Hemoglobin; Pregnant women; Postpartum

Research in context

Evidence before this study

We searched PubMed for papers published until 9 April, 2023, we found very few studies were carried out on relationships of alkaline phosphatase (ALP) levels and venous thromboembolism (VTE). Only two studies in hospitalised patients in the United States have found associations between ALP levels and VTE. The incidence of VTE in pregnant women is higher than that before pregnancy. VTE is one of the important causes of maternal death. However, no prior study focusing on associations of ALP levels and VTE has been performed in pregnant women. The associations between ALP and VTE postpartum are worth exploring. Whether serum ALP could be used as a potential new biomarker to predict VTE is unknown, and mechanistic studies should also be carried out.

Added value of this study

In this multi-center cohort of pregnant women, approximately 0.8% (79/10,044) of the pregnant women were diagnosed with VTE postpartum. Pregnant women with the lowest quintile of serum ALP levels (≤116 U/L) in late pregnancy had increased risk of VTE postpartum compared with those with the highest quintile (≥199 U/L). The negative associations between ALP and VTE remained consistent in sensitivity analyses among participants with non-GDM, single pregnancy, non-preeclampsia, non-postpartum hemorrhage, non-extremely/very preterm and cesarean delivery. Decreased hemoglobin significantly mediated 7.59% of ALP-associated VTE postpartum.

Implications of all the available evidence

To our knowledge, this is the first report on the associations of ALP levels and VTE postpartum. This study suggests that low serum ALP levels in late pregnancy was associated with increased risk of VTE postpartum, and the ALP-associated VTE risk may be partially mediated by hemoglobin, suggesting that serum ALP in late pregnancy could be a promising biomarker for the prediction of VTE postpartum. This study provides important evidence for the prevention of VTE in pregnant women.

Introduction

Venous thromboembolism (VTE) includes deep venous thrombosis (DVT) and pulmonary embolism (PE). Pregnant women had 5 to 10-fold increased risk of VTE compared with non-pregnant women of the same age due to hypercoagulability during pregnancy or after delivery. The incidence of VTE in pregnant women ranged from 0.82 to 19.97 per 10,000 deliveries.[1,2] Although the overall incidence is lower in Asian populations than in western populations, the disease burden of VTE is not low in Asia because of the large population base.[3] There is some evidence demonstrating that VTE among pregnant women is associated with substantial adverse outcomes (such as maternal mortality, VTE recurrence, bleeding, and lower infant birth weight),[4] which may be related to maternal environment exposure including nutritional/metabolic milieu.[5] Concerning trends in maternal risk factors were observed over the past three decades,[6] identifying more maternal risk factors and strengthen prevention is of great public health importance in the VTE prevention worldwide.

Alkaline phosphatase (ALP) is a glycoprotein found on the cell membrane. It mainly exists in liver, bone, placenta, kidney, small intestine, and other tissues of human. To support fetal growth and development, high ALP levels from placental production are normal variant of pregnancy. Serum ALP is generally known as an indicator of cholestasis and bone turnover. Unfortunately, although it appears frequently in routine clinical practice, people's understanding of its physiological function is still limited. During pregnancy, serum ALP levels increase progressively with the highest levels reached in late pregnancy.[7] Only the normal reference intervals of ALP levels in non-pregnant women are established, the normal reference intervals of ALP levels for pregnant women in different stages of pregnancy remain unclear.

Prior studies had reported that cholestasis, liver disease, multiple pregnancies, smoking habit, and low weight for gestational age were associated with higher ALP levels.[8] Conversely, gestational diabetes mellitus (GDM), preterm, stillbirths, fetal distress in utero, intrauterine growth retardation, and preeclampsia were associated with a decrease in ALP.[8,9] Studies exploring the associations between ALP and VTE are sparse. Higher[10] and abnormal (higher + lower)[11] ALP levels were found to be related with the development of VTE and DVT in hospitalised patients in the United States. So far, no prior study has been conducted among

pregnant women in investigating the associations of ALP and VTE. Considering that the late pregnancy is a period with rapid increase in ALP activity in pregnant women, and the postpartum is a period with high incidence of VTE.

Therefore, this study aims to explore the associations of ALP levels in late pregnancy with VTE postpartum in a multi-centre cohort, investigating whether serum ALP could be used as a potential new biomarker in the prediction of VTE postpartum. Given that previous study had found that ALP levels were decreased in pregnant women with hemoglobin SC (Hb S and Hb C gene) disease, and sickle cell anaemia,[12] therefore, another aim of this study was to investigate the potential mediating effect of Hb in revealing the mechanisms of the associations between ALP and VTE postpartum.

Methods

Study design and population

The study population were from a multi-centre cohort of pregnant women. The cohort included pregnant women who planned to deliver in the Department of Obstetrics and Gynecology in the three designated hospitals in Union Hospital (the Main Campus, West Campus, and Cancer Centre), Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, during two recruitment periods of January 1, 2018 to December 31, 2019, and May 14, 2020 to March 25, 2022. The pregnant women were enrolled in the cohort in late pregnancy (median, 37 (35, 39) weeks) in the designated hospitals by doctors, and the health and birth outcomes of the mother–infant pairs were followed at delivery and puerperium (6 weeks postpartum) from medical records. This study was approved by the ethics committee of Tongji Medical College affiliated with Huazhong University of Science and Technology (number: [2015] S014). Informed consent was not required, as the information was retrieved from the medical records retrospectively.

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies.

The current study included pregnant women from the cohort, exclusion criteria are: 1) previous VTE at erollment, medication thromboprophylaxis (within 2 weeks at erollment), intrahepatic cholestasis of pregnancy, chronic hepatitis or serious acute infections, and history of tumor; 2) missing ALP/Hb levels in late pregnancy.

Data collection

We retrieved the information on demographic, history of reproduction, history of disease, history of medicine use, lifestyle, laboratory findings, delivery, and admission/discharge diagnoses from medical records. Data on diagnoses was based on International Classification of Diseases, 10th Revision (ICD-10), coding system. The accuracy of ICD-10 in detecting VTE has been previously validated to have high specificity and acceptable sensitivity.[13] The diagnoses of VTE were checked by two trained researchers (Qian Li and Hongfei Wang), and any difference in the interpretation was adjudicated by another researcher (Liang V Tang).

Laboratory findings

At enrollment in late pregnancy, the pregnant women were tested for whole Hb, and serum liver function indicators (including ALP, γ-glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST)). Whole Hb was measured using the Beckman Coulter's DxH 800. Serum liver function indicators was determined by enzymatic method using an automatic analyser (ANL AU5800, supplied by Beckman Coulter).

Diagnosis of VTE

Participants were asked to take physical examinations in the designated hospitals by their doctors. Pregnant women with new-onset VTE postpartum (within 6 weeks after delivery) were confirmed as VTE cases. VTE includes DVT and PE. The diagnosis of DVT was made when there were standard clinical symptoms and the findings of venography or ultrasonography (in the medical records) were in agreement with DVT.[14] PE was diagnosed when there were clinical symptoms of PE coupled with support of pulmonary angiography, a computed tomography, or a ventilation-perfusion lung scan.[15] Only pregnant women with confirmed DVT and/or PE were verified as a VTE case in this study, those with superficial phlebitis were not considered as VTE.

Assessment of covariates

This study adjusted covariates of maternal age, multiple pregnancy, primipara, *in vitro* fertilisation (IVF) pregnancy, smoking habit, alcohol drinking habit, history of diabetes, GDM, body mass index (BMI) at enrollment, preeclampsia, extremely/very preterm, delivery mode, postpartum hemorrhage, GGT, ALT and AST. Extremely/very preterm was defined as birth before 32 weeks of gestation.[16] Preeclampsia was diagnosed among pregnant women with new-onset gestational hypertension (blood pressure ≥140/90 mm Hg after 20 weeks of gestation) plus proteinuria (>300 mg/24 h) after 20 weeks of gestation.[17] The participants' height and weight were measured at enrollment. BMI at enrollment was calculated by dividing weight (kg) at enrollment by the square of height (m) measured at enrollment. GDM was diagnosed if any of the following criteria were met at oral glucose tolerance test: fasting blood glucose ≥5.1 mmol/L, 1 h post-load blood glucose ≥10.0 mmol/L, and 2 h post-load blood glucose ≥8.5 mmol/L.[18]

Statistical analysis

Continuous variables of maternal age, height, wight, BMI at enrollment, and laboratory findings were expressed as median (interquartile range, IQR). The remaining categorical variables were expressed as n (%). Logistic regression models were used to investigate the odds ratios (ORs) and 95% confidence intervals (CIs) for VTE postpartum according to serum ALP levels (quintiles and continuous natural log-transformed) in late pregnancy. In adjusted model 1, the adjusted covariates included ALP, maternal age, multiple pregnancy, primipara and IVF pregnancy. In adjusted model 2, the adjusted covariates included smoking habit, alcohol drinking habit, history of diabetes, GDM, BMI at enrollment, preeclampsia, extremely/very preterm, delivery mode, postpartum hemorrhage, GGT, ALT, AST, and covariates in model 1. Sensitivity analyses using Logistic regression models were performed among pregnant women with non-GDM, single pregnancy, non-preeclampsia, non-postpartum hemorrhage, non-extremely/very preterm and cesarean delivery. Multivariate linear regression models were utilized to explore the associations of ALP levels and Hb levels. Logistic regression models were applied in analysing the relationships of Hb levels (quintiles and continuous natural log-transformed) in late pregnancy and VTE postpartum. Correlation coefficients (Spearman's rho and Kendall's tau) were calculated to investigate the ALP-Hb and ALP- platelet counts associations. This study examined the proportion of mediation through Hb in the associations of ALP levels and VTE risk using the *Process* SPSS macro tool based on the mediation method recommended by Hayes.[19] The data were analysed with SAS, version 9.4 (SAS institute, Cary, NC) and SPSS version 21.0 (IBM Corp., Armonk, NY, USA). The statistically significant cutoff of two-sided *P* value was 0.05.

Role of the funding source

The funder of this study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Enrollment of the study population

A total of 11,523 pregnant women were enrolled in the three designated hospitals during two recruitment periods of January 1, 2018 to December 31, 2019, and May 14, 2020 to March 25, 2022. This study excluded pregnant women with previous VTE at erollment or medication thromboprophylaxis (within 2 weeks at erollment) (n = 113), intrahepatic cholestasis of pregnancy (n = 170), chronic hepatitis or serious acute infections (n = 89), history of tumor (n = 55), and 1052 pregnant women missing ALP/Hb levels in late pregnancy. Finally, 10,044 pregnant women were eligible for this study (Fig. 1).

Characteristics of the pregnant women

Table 1 describes the social-demographic, anthropometric, reproductive, life style and laboratory findings of the participants. Approximately 0.8% (79/10 044) of the pregnant women were diagnosed with VTE postpartum. Approximate 83.5% (66/79) of the VTE patients were diagnosed with DVT only, 8.9% (7/79) with PE only, and 7.6% (6/79) with DVT and PE. The mean time of diagnoses for VTE in the participants with VTE was 2 ± 1 weeks postpartum. The median age of the pregnant women was 31.0 (28.0, 34.0) years. A total of 431 (4.3%) pregnant women had multiple pregnancy, and 6125 (61.0%) pregnant women were primipara. The characteristics of pregnant women involved for analyses were similar to those who were excluded (due to missing ALP/Hb levels or other reasons) from this study (Table S1).

Relationships of ALP and VTE

VTE risk in associations with ALP levels categorized into quintiles among pregnant women are presented in Table 2. In the unadjusted models, increased risk of VTE

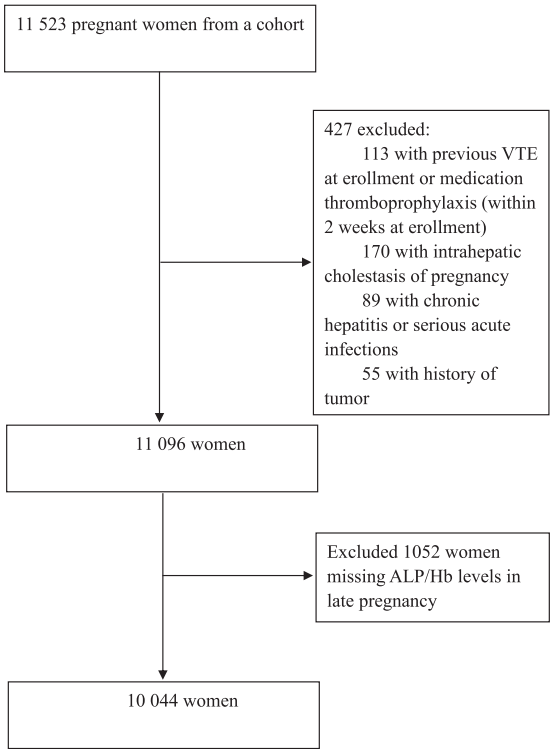

***Fig. 1:* Flow diagram of this study.** Abbreviations: ALP: Alkaline phosphatase; Hb: Hemoglobin; VTE: Venous thromboembolism.

Characteristics	Total	ALP levels (U/L)				
		Q1 (Lowest) (≤116)	Q2 (117–140)	Q3 (141–164)	Q4 (165–198)	Q5 (Highest) (≥199)
n	10 044	2075	2006	1988	1961	2014
Maternal age (years)	31.0 (28.0, 34.0)	32.0 (29.0, 35.0)	31.0 (28.0, 34.0)	31.0 (28.0, 33.0)	30.0 (28.0, 33.0)	30.0 (28.0, 33.0)
Multiple pregnancy (%)	431 (4.3)	44 (2.1)	66 (3.3)	68 (3.4)	83 (4.2)	170 (8.4)
Primipara (%)	6125 (61.0)	1071 (51.6)	1161 (57.9)	1230 (61.9)	1272 (64.9)	1391 (69.1)
IVF pregnancy (%)	834 (8.3)	147 (7.1)	157 (7.8)	161 (8.1)	153 (7.8)	216 (10.7)
Smoking habit (%)	1 (0.01)	1 (0.05)	0 (0)	0 (0)	0 (0)	0 (0)
Alcohol drinking habit (%)	3 (0.02)	0 (0)	1 (0.05)	1 (0.05)	1 (0.05)	1 (0.05)
History of diabetes (%)	43 (0.4)	13 (0.6)	8 (0.4)	8 (0.4)	5 (0.3)	9 (0.4)
GDM (%)	2221 (22.1)	507 (24.4)	463 (23.1)	427 (21.5)	441 (22.5)	383 (19.0)
Height at enrollment (cm)	161.0 (158.0, 165.0)	161.0 (158.0, 165.0)	161.0 (158.0, 165.0)	162.0 (159.0, 165.0)	161.0 (158.0, 165.0)	160.0 (158.0, 165.0)
Weight at enrollment (kg)	69.0 (63.0, 75.0)	70.0 (63.0, 77.0)	70.0 (64.0, 76.0)	70.0 (65.0, 75.4)	68.0 (63.0, 74.0)	66.0 (61.0, 72.5)
BMI at enrollment (kg/m^2)	26.4 (24.3, 28.7)	26.9 (24.6, 29.1)	27.0 (24.6, 29.1)	26.7 (24.8, 28.7)	26.2 (24.2, 28.4)	25.4 (23.7, 27.9)
Preeclampsia (%)	540 (5.4)	144 (6.9)	115 (5.7)	72 (3.6)	89 (4.5)	120 (6.0)
Extremely/very preterm (%)	129 (1.3)	76 (3.7)	33 (1.6)	7 (0.4)	5 (0.3)	8 (0.4)
Delivery mode (cesarean, %)	7290 (72.6)	1601 (77.2)	1508 (75.2)	1432 (72.0)	1377 (70.2)	1372 (68.1)
Postpartum hemorrhage (%)	220 (2.2)	66 (3.2)	42 (2.1)	22 (1.1)	41 (2.1)	49 (2.4)
VTE (%)	79 (0.79%)	26 (1.25%)	18 (0.90%)	10 (0.50%)	16 (0.82%)	9 (0.45%)
Laboratory findings						
Hb (g/L)	119.0 (110.0, 127.0)	117.0 (108.0, 125.0)	119.0 (110.0, 127.0)	119.0 (110.0, 127.0)	119.0 (110.0, 128.0)	119.0 (109.0, 128.0)
GGT (U/L)	12.0 (10.0, 16.0)	11.0 (10.0, 16.0)	12.0 (10.0, 16.0)	12.0 (10.0, 16.0)	12.0 (10.0, 16.0)	12.0 (10.0, 18.0)
ALT (U/L)	16.0 (11.0, 22.0)	16.0 (11.0, 22.0)	16.0 (11.0, 21.0)	16.0 (11.0, 22.0)	16.0 (12.0, 22.0)	17.0 (12.0, 23.0)
AST (U/L)	19.0 (17.0, 23.0)	18.0 (16.0, 22.0)	19.0 (16.0, 22.0)	19.0 (17.0, 22.0)	19.0 (17.0, 23.0)	20.0 (17.0, 24.0)
Platelet counts (10^9/L)	198.0 (164.0, 234.0)	198.0 (163.0, 232.0)	198.0 (167.0, 234.0)	198.0 (164.0, 234.0)	200.0 (166.0, 237.0)	195.0 (158.0, 235.0)

Abbreviations: ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; GDM: Gestational diabetes mellitus; GGT: γ-glutamyl transferase; Hb: Hemoglobin; IVF: *in vitro* fertilisation.

Table 1: **Characteristics and laboratory findings of the participants according to ALP levels (n = 10,044).**

was observed in pregnant women with the lowest quintile of ALP levels (≤116 U/L) compared with those with the highest quintile (≥199 U/L) with OR 2.83 [1.32, 6.05]. In models 1, and 2, after adjusting for potential confounders, the ALP–VTE associations were still significant with OR 2.67 [1.24, 5.75], and 2.48 [1.14, 5.40], respectively. This study conducted sensitivity analyses among pregnant women with non-GDM (Table S2), single pregnancy (Table S3), non-preeclampsia (Table S4), non-postpartum hemorrhage (Table S5), non-extremely/very preterm (Table S6) and cesarean delivery (Table S7), all the models observed consistent findings.

In model 2, a one SD decrease of ln-transformed ALP levels were associated with elevated risk of VTE (OR, 1.29 [1.02, 1.62]) (Table 2).

Relationships of ALP and Hb

Compared with pregnant women with highest quintile of ALP levels, pregnant women with lowest quintile of ALP levels had lower Hb levels. βs were –2.60, –2.68, and –2.27 in the unadjusted model, model 1, and model 2, respectively (Table 3). This study found that ALP levels were positively related to Hb levels in all models (Table 3). A one SD decrease of ln-transformed ALP

	ALP levels (U/L)					Per-SD decrease of ln (ALP levels)
	Q1 (Lowest) (≤116)	Q2 (117–140)	Q3 (141–164)	Q4 (165–198)	Q5 (Highest) (≥199)	
No. of VTE, (%)	26 (1.25%)	18 (0.90%)	10 (0.50%)	16 (0.82%)	9 (0.45%)	
Unadjusted	2.83 (1.32, 6.05)	2.02 (0.90, 4.50)	1.13 (0.46, 2.78)	1.83 (0.81, 4.16)	1	1.32 (1.07, 1.65)
Model 1	2.67 (1.24, 5.75)	1.95 (0.87, 4.37)	1.10 (0.44, 2.71)	1.82 (0.80, 4.15)	1	1.29 (1.04, 1.61)
Model 2	2.48 (1.14, 5.40)	1.83 (0.81, 4.13)	1.07 (0.43, 2.65)	1.76 (0.77, 4.01)	1	1.29 (1.02, 1.62)

Abbreviations: ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CI: Confidence interval; GGT: γ-glutamyl transferase; OR: Odds ratio; SD: Standard deviation; VTE: Venous thromboembolism. Logistic regression model 1 included covariates of ALP, maternal age, multiple pregnancy, primipara, and *in vitro* fertilisation pregnancy. Logistic regression model 2 included covariates of smoking habit, alcohol drinking habit, history of diabetes, gestational diabetes mellitus, body mass index at enrollment, preeclampsia, extremely/very preterm, delivery mode, postpartum hemorrhage, γ-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, and covariates included in model 1.

Table 2: **Adjusted ORs and 95% CIs for ALP levels and VTE risk (n = 10,044).**

levels were associated with a 1.07%, 1.09%, and 0.92% decrease in Hb levels in the unadjusted model, model 1, and model 2, respectively. Correlation analyses found significant positive correlations of ALP levels with Hb levels in late pregnancy (Spearman's rho: r = 0.067, $P < 0.0001$; Kendall's tau: r = 0.046, $P < 0.0001$). Correlation analyses observed non-significant correlations of ALP levels with platelet counts (Spearman's rho: r = –0.0030, $P = 0.74$; Kendall's tau: r = –0.0020, $P = 0.75$).

Relationships of Hb and VTE

This study observed a negative association between Hb and VTE. As shown in Table 4, a one SD decrease of ln-transformed Hb levels was significantly linked to higher risk of VTE with OR 1.41 [1.21, 1.64], 1.40 [1.19, 1.64], 1.37 [1.17, 1.61] and 1.36 [1.15, 1.59] in the unadjusted model, model 1, model 2 and model 3, respectively.

Mediation analyses

As shown in Table 5, significant mediated effects by Hb were observed on the relationships between ALP levels and VTE. Decreased Hb levels significantly mediated 7.59% of the ALP-associated elevated VTE risk. Interaction effect was not significantly observed between ALP and Hb on VTE ($P > 0.10$).

Discussion

To the best of our knowledge, this is the first study to investigate the associations between ALP and the risk of VTE among the special population——pregnant women. This study found pregnant women with the lowest quintile of serum ALP levels (≤116 U/L) in late pregnancy had increased risk of VTE postpartum compared with those in the highest quintile group (≥199 U/L). Sensitivity analyses among participants with non-GDM, single pregnancy, non-preeclampsia, non-postpartum hemorrhage, non-extremely/very preterm and cesarean delivery observed consistent findings. Decreased ALP levels were found to be related to the decrease of Hb levels, and Hb significantly mediated ALP-associated VTE risk. The results demonstrates that serum ALP in late pregnancy could be a promising biomarker for the prediction of VTE postpartum.

Prior to this study, only two studies have explored the relationships of ALP levels and VTE in the patients of the United States. A study by Motaganahalli et al. found that elevated ALP levels were associated with a slightly increased risk of DVT (one type of VTE) among novel coronavirus disease 2019 positive patients (n = 71) with OR 1.03 [1.00, 1.05].[10] The median age of the participants was 63 years, and more than half of them were males. Another study by Li et al. observed that patients with an abnormal ALP (<44 IU/L or >147 IU/L) were significantly more likely to develop VTE within one year after discharge compared with those who had normal ALP (44–147 IU/L) (OR: 1.91 [1.81–2.01].[11] In that study, participants with ALP levels above or below the reference ranges were categorized into the same group, therefore, it is not possible to determine the associations of high or low ALP levels with VTE risk separately. Unlike these two studies, the current study is the first to explore the associations of ALP with VTE in pregnant women, suggesting that low ALP levels in late pregnancy were related to increased risk of VTE postpartum, and Hb may involve in mediating the ALP-related VTE risk.

With the increase of gestational weeks, the burden on the liver of pregnant women increased, fetal bone development accelerated, and ALP levels in pregnant women also changed physiologically. In pregnant women, ALP levels were found to be almost unchanged or decreased slightly in early pregnancy, increasing slightly in middle pregnancy, peaking in late pregnancy, and declining postpartum.[8,20,21] In the past, more attention has been paid to the disease risk associated with increased ALP (e.g., cholestasis), although a few studies had reported that decreased ALP was associated with premature birth, stillbirth, and intrauterine growth restriction.[8,9] It is easier to overlook the worse maternal and infantile outcomes caused by decreased ALP levels. Moreover, there is no uniform international consistent reference intervals for ALP levels in late pregnancy. Therefore, it is very difficult to define the cut-off value of low ALP levels among pregnant women in late pregnancy.

Jin et al. recommended reference intervals of 85–322 U/L for ALP in late pregnancy among pregnant women of Hangzhou, China (enrolled from 2013 to 2014).[22] Abbassi-Ghanavati et al. summarized a comprehensive electronic database review (published from 1975 to 2008), and found that the recommended reference intervals of ALP in late pregnancy were 38–229 U/L.[23] Our study found that ALP ≤116 U/L was a risk factor for VTE postpartum, and the cutoff value in our study (116 U/L) was slightly higher than that reported by Jin et al. (85 U/L),[22] and much higher than that in study by Abbassi-Ghanavati et al. (38 U/L).[23] We speculate this may be due to the differences in races, and the years of enrollment of the study population. The normal reference intervals for ALP in late pregnancy remain to be investigated in subsequent larger multi-centre studies.

GDM was found to be a risk factor of VTE,[24] possibly related to the effect of high blood glucose on vascular inflammation leading to increased risk of a cardiovascular event. Multiple pregnancy was also proposed to be an important risk factor for VTE.[24,25] Age, cesarean delivery, IVF pregnancy, parity, history of diabetes, smoking, gestational hypertension, preterm delivery, and postpartum hemorrhage were found to be significant risk factors for VTE during pregnancy and in the postpartum period.[24–26] In this study, we conducted sensitivity analyses, and found the relationships

	ALP levels (U/L)					Per-SD decrease of ln (ALP levels)
	Q1 (Lowest) (≤116)	Q2 (117–140)	Q3 (141–164)	Q4 (165–198)	Q5 (Highest) (≥199)	
Median (IQR)	117.0 (108.0, 125.0)	119.0 (110.0, 127.0)	119.0 (110.0, 127.0)	119.0 (110.0, 128.0)	119.0 (109.0, 128.0)	
Unadjusted	−2.60 (−3.44, −1.76)	−0.10 (−0.95, 0.74)	0.06 (−0.78, 0.90)	0.73 (−0.12, 1.58)	0	−1.07 (−1.34, −0.81)
Model 1	−2.68 (−3.51, −1.84)	−0.22 (−1.06, 0.62)	−0.03 (−0.87, 0.81)	0.59 (−0.26, 1.43)	0	−1.09 (−1.36, −0.83)
Model 2	−2.27 (−3.11, −1.44)	−0.003 (−0.84, 0.83)	−0.02 (−0.85, 0.81)	0.59 (−0.25, 1.42)	0	−0.92 (−1.19, −0.66)

Abbreviations: ALP: Alkaline phosphatase; CI: Confidence interval; Hb: Hemoglobin; IQR: Interquartile range; SD: Standard deviation. Multivariate linear regression models 1 included covariates of ALP, maternal age, multiple pregnancy, primipara, and *in vitro* fertilisation pregnancy. Multivariate linear regression models 2 included covariates of smoking habit, alcohol drinking habit, history of diabetes, gestational diabetes mellitus, body mass index at enrollment, preeclampsia, extremely/very preterm, delivery mode, postpartum hemorrhage, γ-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, and covariates included in model 1.

Table 3: **Adjusted βs and 95% CIs for ALP levels and Hb levels (n = 10,044).**

	Hb levels (g/L)					Per-SD decrease of ln (Hb levels)
	Q1 (Lowest) (<107)	Q2 (107–117)	Q3 (118–125)	Q3 (118–125)	Q5 (Highest) (≥126)	
No. of VTE (%)	29 (1.41%)	21 (1.05%)	8 (0.42%)	13 (0.66%)	8 (0.38%)	
Unadjusted	3.75 (1.71, 8.21)	2.78 (1.23, 6.30)	1.11 (0.42, 2.97)	1.73 (0.72, 4.19)	1	1.41 (1.21, 1.64)
Model 1	3.67 (1.67, 8.09)	2.75 (1.21, 6.21)	1.10 (0.41, 2.94)	1.72 (0.71, 4.17)	1	1.40 (1.19, 1.64)
Model 2	3.44 (1.55, 7.65)	2.61 (1.14, 5.95)	1.10 (0.41, 2.96)	1.72 (0.71, 4.17)	1	1.37 (1.17, 1.61)
Model 3	3.33 (1.50, 7.41)	2.50 (1.10, 5.72)	1.07 (0.40, 2.88)	1.68 (0.69, 4.08)	1	1.36 (1.15, 1.59)

Abbreviations: CI: Confidence interval; Hb: Hemoglobin; OR: Odds ratio; SD: Standard deviation; VTE: Venous thromboembolism. Logistic regression model 1 included covariates of Hb, maternal age, multiple pregnancy, primipara, and *in vitro* fertilisation pregnancy. Logistic regression model 2 included covariates of smoking habit, alcohol drinking habit, history of diabetes, gestational diabetes mellitus, body mass index at enrollment, preeclampsia, extremely/very preterm, delivery mode, postpartum hemorrhage, and covariates included in model 1. Logistic regression model 3 included covariates of ALP, and covariates included in model 1 and model 2.

Table 4: **Adjusted ORs and 95% CIs for Hb levels and VTE risk (n = 10,044).**

between ALP and VTE postpartum were still significant. In the adjusted models, this study adjusted covariates of GDM, multiple pregnancy, and other comprehensive covariates (demographic, history of reproduction, history of disease, life style, birth outcomes, and other liver enzymes). The main results remained robust, indicating that the associations between ALP and VTE was independent of these adjusted covariates.

A study found that ALP levels were significantly decreased in patients with Hb SC (Hb S and Hb C gene) disease and sickle cell anaemia compared with controls,[12] which backed up our findings of significant positive relationships of ALP and Hb levels. Hb measurement is a routine test for pregnant women during antenatal care, and there has been a strong interest in studying Hb worldwide. One recent study had reported an association between Hb and VTE postpartum in pregnant women.[27] It found that the risk of VTE postpartum increased continuously across the Hb categories when the Chinese pregnant women had anaemia (Hb <110 g/L) at the admission for delivery, which was consistent with our findings. Scholars speculated that the links of lower Hb levels and increased risk of VTE postpartum, were possibly related to the increased frequency of thrombocytosis in part.[27] There were also studies reporting that pregnant women with sickle cell disease had elevated pregnancy-related VTE risk.[28] In addition to Hb, the associations between ALP and VTE may also be affected by some nutrients such as zinc and calcium. It had been found that the activity of ALP decreases with the decrease of serum zinc. Zinc played a role in increasing bone formation through increasing osteoblast cell growth, ALP activity and collagen synthesis in MC3T3-E1 cells.[29] It had been found that calcium uptake correlated well with ALP activity in animal[30] and population-based studies.[31] There is also hypothesis that birth outcomes affect the associations between ALP and VTE, although potential confounders (e.g., preeclampsia) were adjusted, and results of sensitivity analyses remain robust. Researches of mechanism should be carried out in the future.

Categories	Quintiles of ALP levels and VTE	*P*
Mediation: Hb levels		
Total effects (95% CI)	−0.224 (−0.385, −0.063)	0.0064
Direct effects (95% CI)	−0.202 (−0.362, −0.042)	0.014
Mediated effects (95% CI)	−0.017 (−0.028, −0.009)	0.0009
Proportion mediated by Hb	7.59%	

Abbreviations: ALP: Alkaline phosphatase; CI: Confidence interval; Hb: Hemoglobin; VTE: Venous thromboembolism. Mediation analyses were conducted using the *Process* SPSS macro.

Table 5: **Mediated effects by Hb on the associations of ALP levels with VTE (n = 10 044).**

This study has several crucial strengths that worth mentioning. Firstly, this study demonstrated the first evidence of significant associations between low ALP levels and increased risk of VTE postpartum based on a cohort design. Secondly, for the first time, this study further investigated the potential mechanism underlying ALP-associated VTE risk. The significant mediating role of Hb in the relationships between low ALP levels and increased risk of VTE postpartum provided important clues for further mechanism researches. Thirdly, the information of medical records of the participants is relatively complete, all the disease diagnoses were double-checked by two researchers separately, the third researcher would join the discussion when there was discordance, which made the information on disease diagnoses reliable. Lastly, comprehensive potential confounders including demographic, history of reproduction, history of disease, life style, birth outcomes, and other liver enzymes were taken into consideration, allowing for relevant adjustments.

Inevitably, this study has limitations. Firstly, pregnant women with hereditary antithrombin, protein C or protein S deficiencies were found to have high risk of VTE.[32] These factors were not considered in this study, although women with a history of VTE at enrolllment were excluded. Secondly, some nutrients, such as zinc and calcium, have been found to potentially affect the activity of ALP, and whether these nutrients are involved in mediating the ALP-associated VTE risk should be further investigated. Thirdly, the retrospective data collection which may prone to missing data, measurement errors and inaccuracy. Obesity pre-pregnancy was found to be associated with VTE,[26] but pre-pregnancy BMI could not be adjusted because information on weight pre-pregnancy was missing in this study. The effect of pre-pregnancy BMI on the ALP-VTE associations still needs to be studied. In this study, 1052 participants who missed ALP/Hb levels in late pregnancy were exluded, though the characteristics of the participants included and excluded were similar, future prospective cohort studies with comprehensive follow-up visits are wanrranted. Lastly, there may also be other confounders (e.g., status of immobilisation) that were not considered in this study. Therefore, we should be cautious when extrapolating the current findings to other populations.

In conclusion, this is the first study which demonstrated that low serum ALP levels in late pregnancy were associated with increased risk of VTE postpartum, and the ALP-associated VTE risk may be partially mediated by Hb. This study suggests that serum ALP in late pregnancy could be a promising biomarker for the prediction of VTE postpartum, providing more scientific basis for the prevention of VTE. The normal reference intervals of serum ALP in late pregnancy should be further studied. More researches are needed to elucidate additional mechanisms.

Contributors

YH, VLT, QL and HoW designed this study. QL, VLT, HoW, HuW, JD, ZPC, WYL, RQZ, SC, JRG analysed the data. QL, YH, VLT, and HoW drafted the manuscript. All authors had full access to the data, and verified the results in this study. All authors revised the manuscript and gave final approval for the version to be published.

Data sharing statement

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declaration of interests

All authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102088.

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