Original Article

Prediction of Preterm Delivery among Low-risk Indian Pregnant Women: Discriminatory Power of Cervical Length, Serum Ferritin, and Serum Alpha-fetoprotein

Abstract

Background: Around 80% of preterm births (PTBs) occur spontaneously. Various biomarkers are being evaluated to assess the possible role of chorioamniotic inflammation in PTBs. **Aim:** The aim of this study was to establish the accuracy of serum bio-markers(cut off values of ferritin and alpha-fetoprotein [AFP] at midtrimester) along with cervical length [CL] assessment to predict preterm delivery among low-risk women. **Methods:** Three hundred low-risk pregnant women attending the antenatal clinic of a tertiary health care facility were included and underwent CL measurement during mid-trimester by transvaginal ultrasonography and their serum levels of ferritin and AFP were recorded. All were followed up till delivery. **Results:** Receptor-operated characteristic curves for ferritin, AFP, and CL were constructed. Area under curves and Youden Index calculated for each marker were very low (<0.5) which is statistically considered very poor for a screening test. **Conclusion:** Serum ferritin and AFP together with CL measurement in the second trimester of pregnancy had poor discriminatory value in predicting preterm delivery among low-risk asymptomatic pregnant women.

Keywords: *Area under curve, biomarkers, cervical length measurement, premature birth*

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Introduction

Prevention of preterm birth (PTB) or more specifically delivery before 37 weeks of gestation is a major health concern because it is strongly associated with neonatal mortality and long-term neurological and developmental problems.[1] Globally, the incidence of PTB is estimated to be around 10% ranging between 5% and 18% in parts of Europe and Africa, respectively.[1,2] Around 20% of preterm deliveries might be medically indicated for maternal and fetal reasons while 80% of PTB are spontaneous in nature preceded either by preterm labor or prelabor rupture of membranes (PROM).[3,4] It is hypothesized that decidual chorioamniotic systemic inflammation plays an important role in the process of preterm labor and many inflammation-related biomarkers including C-reactive protein, interleukins (ILs), ferritin, alkaline phosphatase, and prolactin had been studied to identify asymptomatic women at risk of PTB.[5,6] Half of all spontaneous PTB happens among low-risk asymptomatic women.[7,8] Around 39% of low-risk asymptomatic women who delivered prematurely had shortening of cervix at their mid-trimester as diagnosed by transvaginal ultrasound (TVS) examination using a cutoff value <2.5 cm.[7] Again, preterm labor and preterm premature rupture of membranes may happen when deep placentation is affected in addition to preeclampsia and other placenta-mediated adverse pregnancy outcomes where alpha-fetoprotein (AFP) level is elevated.[9-11] This may happen due to the presence of small leaks on maternal–fetal barrier.[12] Hence, in search of the preventable causes, we hypothesized decidual inflammatory changes and placental dysfunction to be possible biological links apart from the shortening of cervical length (CL). Several biomarkers in maternal serum, amniotic fluid, cervical secretions, and ultrasound markers were extensively studied to predict PTB among high-risk asymptomatic women, as mentioned before, but none of these have been recommended to screen low-risk asymptomatic women during the prenatal period.[13] In this study, we

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have chosen ferritin as an acute-phase reactant and AFP as a marker of poor placentation due to their availability in public sector hospitals and the consequent possibility for feasible integration with management guidelines. We assessed these biochemical markers along with CL as a physical marker in an attempt to find out the distribution of the participants according to their serum levels of ferritin, AFP, and CL and to calculate the accuracy of second-trimester serum levels of ferritin, AFP, and CL as screening tests for PTBs in low-risk asymptomatic women.[14]

Methods

It was an observational prospective study conducted in the Department of Obstetrics and Gynecology of Calcutta National Medical College and Hospital (CNMCH), a tertiary care teaching hospital in Eastern India over a period of 1½ years from March 2021 to September 2022. The study was approved by the Institutional Ethics Committee of CNMCH vide memo no 22/1/2021-G&O.CNMC/100(2)-2021.

All low-risk asymptomatic women with singleton pregnancy attending antenatal clinics between 13 and 28 weeks period of gestation during the study period were eligible to be recruited. Exclusion criteria included Mullerian anomalies, multifetal gestations, congenital anomalies, severe anemia, thalassemia, hypertension, diabetes, previous history of cervical insufficiency, cone biopsy, history of two or more miscarriages, and spontaneous PTB or preterm PROM. Abortion and those requiring iatrogenic preterm delivery for medical morbidities were withdrawn from the study. Informed consent was sought from all participants. Consecutive recruitment was done. Participant's baseline demographic data such as age, parity, gravidity, body mass index, occupation, educational qualification, and number of previous miscarriages were recorded. Every participant had their CL measured by TVS-GE HealthCare Logiq P9 model using 12 MHz vaginal transducer. In addition to routine blood parameters, 5 mL of fasting venous blood was drawn from each participant to measure serum ferritin and AFP by enzyme-linked immunosorbent assay (ELISA) method. Blood sample was centrifuged, and serum was stored at −20°C. AFP was estimated by direct solid-phase sandwich ELISA method using Sigma-Aldrich kit.[15] Ferritin was measured using Thermo Fisher Scientific kit.[16] All women were followed till their delivery and obstetric outcomes were recorded.

Statistical analysis

Data were entered in Microsoft Excel Spreadsheet and later imported to SPSS version 19 (Statistical Package for Social Sciences (SPSS) IBM Corp. Released 2010 [IBM SPSS Statistics for Windows, version 19. 0. Armonk, NY, USA: IBM Corp.] for further analysis. Distribution of biomarker values were checked for normality by Shapiro-Wilk and Kolmogorov-Smirnov test. Measures of central tendency of the biomarker levels were calculated as mean or median; and measures of dispersion were calculated as standard deviation or percentiles depending on the type of distribution.

Among the markers included in this study, the cutoff value was available only for low serum ferritin (<15 as iron deficiency in 1st trimester of pregnancy).[17] However, there was no agreed-upon cutoff for ferritin as a biomarker of inflammation. Hence, AFP ≥90th percentile (200 ng/mL) was considered high value (possible inflammation); ferritin ≥90th percentile (180 μg/L) was considered high value (possible inflammation); and <15 μg/L was considered iron deficiency (this was about 28th percentile); CL ≤10th percentile (2.8 cm) was considered cervical shortening. Categories were initially created based on these assumptions. Frequencies and percentages were calculated for each category.

Receiver-operating characteristic (ROC) curves were made for serum levels of ferritin, AFP, and CL to check for their sensitivity and specificity in the detection of PTBs. The area under the ROC curves (ROC_AUC) was used to assess the discriminatory capacity of ferritin, AFP, and CL for preterm against term deliveries. The ROC_AUC is a widely used measure of predictive performance when modeling binary outcomes; each point represents one of the possible sensitivity and (1-specificity) pairs. The null hypothesis is set at 0.5 (meaning no discriminative power) to 1.0 (meaning perfect discriminative power). The ROC_AUC curve was used to determine the optimal cutoffs-based ROC_AUC values and Youden index.[18]

Results

Baseline data were collected from 300 women which are presented in Tables 1 and 2. Three women had abortions who were withdrawn from the inferential analyses. The mean gestational age at study entry was 21.3 weeks (±4.2) and that at delivery was 37.5 weeks (±2.9). The demographic and obstetric profile of participating women is presented in Table 1. The study sample included a higher proportion of nullipara and Muslim women. There were 23.67% of women who reported having induced abortions before this pregnancy. The proportion of preterm delivery in this study was 77 (26%).

Table 2 shows the distribution of participants according to their clinical and biochemical profiles at enrolment. The categorization for AFP, ferritin, and CL has been already discussed in the statistical analysis section. Such categorization based on percentile was chosen as all three markers had nonnormal distribution tested by Shapiro–Wilk and Kolmogorov–Smirnov tests. Table 2 also shows summary measures of these variables.

post hoc power analysis measured study power as 100% considering 11% population level preterm delivery

Table 1: Distribution of participants according to their demographic and obstetric profiles (*n*=300)

Variable	*n* (%)
Age (years), μ (±SD)	25.8 (±5.1)
Education	
< Primary	5 (1.67)
Primary	22 (7.33)
Secondary	164 (54.67)
Higher secondary or above	109 (36.33)
Religion	
Hindu	86 (28.67)
Muslim	214 (71.33)
Occupation	
Homemaker	276 (92.00)
Employed	24 (8.00)
Type of marriage	
Consanguineous	60 (20.00)
Nonconsanguineous	240 (80.00)
Gravidity	
Primi gravida	131 (43.67)
Second gravida	85 (28.33)
Third or higher gravida	84 (28.00)
Parity	
Nullipara	154 (51.33)
Primipara	87 (29.00)
Parity ≥2	59 (19.67)
Abortion	
Never	229 (76.33)
Ever	71 (23.67)
Dilatation evacuation (*n*'=71)	
Ever	43 (60.56)
Never	28 (39.44)
Last childbirth (*n*'=146) (years)	
Within 3	56 (38.36)
≥3	90 (61.64)
Gestational period at delivery (*n*'=297)	
Term	77 (25.9)
Preterm	220 (74.1)

SD: Standard deviation

and Type I error or α of 5%.[19,20] Finally, ROC curves were made for serum levels of ferritin, AFP, and CL. The area under the curve (AUC) for each of these variables was 0.603, 0.595, and 0.364, respectively [Figure 1]. The values of ROC curves were interpreted according to the classification proposed by Hosmer and Lemeshow; according to which values ≤0.5 means no discrimination; while 0.7–0.79 was considered acceptable discrimination.[21] Hence, all the markers in this study had poor discrimination. The Youden index calculated was very low for all the markers. A brief representation of these statistics is made in Table 3.

Discussion

In the present study, 26% of participants had preterm delivery as compared to 13.6% PTB rate in India as per a systematic review and modeling analysis conducted in the year 2014.[22] The ROC_AUC was <50% for serum levels of AFP and CL recorded in the second trimester. Youden index showed a low probability of correct classification by all the markers.

Due to multifactorial etiology, the pathophysiology of spontaneous PTB is complex and less understood. However, there is compelling evidence suggesting inflammation and infection play a key role in preterm labor onset.[23] Features of chorioamnitis on histological examination of placentae were found to be more common in PTB than in term deliveries.[24] A more or less similar prospective study was conducted by Tripathi *et al*. where CL was assessed together with biomarkers in serum and cervical secretion to predict PTB among low-risk populations.[25] Tripathi *et al*. found no association in their study between the onset of preterm labor and elevated serum levels of AFP, IL-6, and granulocyte colony-stimulating factors pointing towards some etiology other than inflammation. In their study, ferritin had some correlation but its value was again confounded in conditions such as anemia.[25] Singh *et al*. concluded ferritin as a promising biomarker to predict

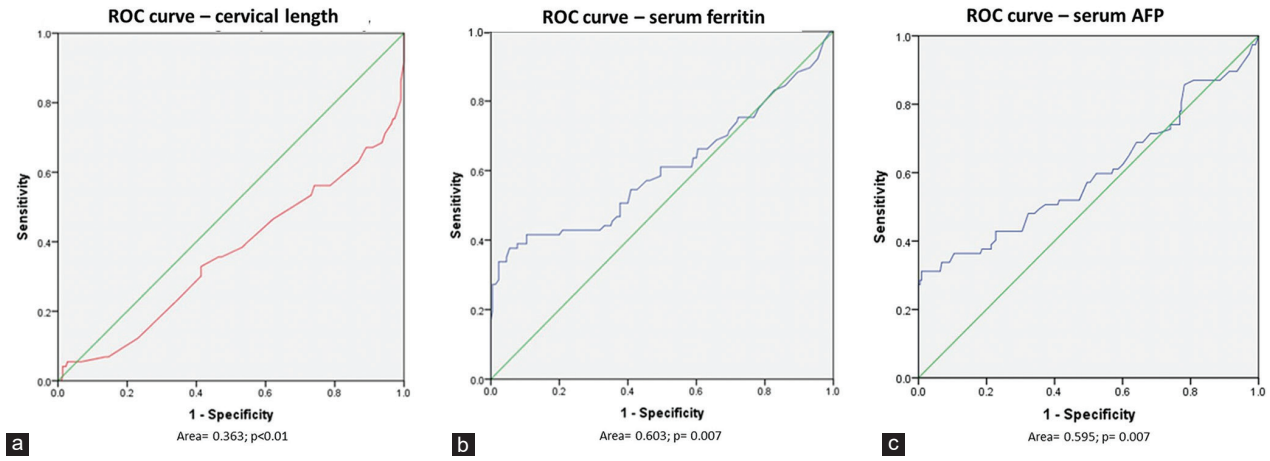

Figure 1: ROC_AUC for Cervical length (a), Serum ferritin (b) and Serum AFP(c)

Table 2: Distribution of participants according to their clinical and biomarker profiles (*n*=300)

Clinical/biochemical variables	*n* (%)
Serum ferritin (µg/L)	
µ (±SEM)	58.1 (±3.7)
Median (IQR)	39.0 (13.5–68.5)
<15	81 (27.0)
15–180	188 (60.7)
>180	31 (12.3)
Serum AFP (ng/mL)	
µ (±SEM)	69.4 (±4.2)
Median (IQR)	47.0 (25–79)
<200	270 (90.0)
≥200	30 (10.0)
TLC≥11,000 cells/mm^3	87 (29.0)
Neutrophil % >70	120 (40.0)
Median (IQR) for TLC	9000 (7800–11,000)
Positive urine cytology	31 (10.3)
Positive urine culture	8 (2.7)
Cervical length (cm)	
µ (±SEM)	3.6 (±0.04)
Median (IQR)	3.7 (3.2–4.0)
≤2.8	33 (11.0)
>2.8	267 (89.0)

SEM: Standard error of mean, IQR: Interquartile range, AFP: Alpha-fetoprotein, TLC: Total leukocyte count

Table 3: Statistics to assess accuracy of screening tests for preterm delivery

	Cervical length (cm)	AFP (ng/mL)	Ferritin (µg/L)
Sensitivity (%)	97–71	97–71	97–70
Corresponding value of the marker	1.88–2.85	4.5–28.50	5.5–17.5
Corresponding specificity (%)	29–3	3–29	3–30
Highest Youden index	3	30	32

AFP: Alpha-fetoprotein

the preterm onset of labor in their study where two cutoff values were suggested, i.e. 68.8 µg/dL and 112 µg/dL.[26] They considered the discriminative value of 68.8 µg/dL as a cutoff to predict is superior than 112 µg/dL as the former had a negative predictive value of 88.89%. However, in this study, the blood sample was collected during delivery not at mid-trimester or earlier. After excluding anemia from our study subjects, only 12.3% of them had a value of >180 µg/L which was considered high for inflammation. Although some studies showed a strong association between PTB and elevated AFP,[12,27-33] meta-analysis by Yuan *et al*. found low sensitivity of AFP in population studies.[34] They described an association between raised AFP and PTB that might be confounded by abnormalities in other biomarkers. They also suggested that 2.5 multiples of median (MOM) cutoff of AFP might improve the prediction compared to 2.0 MOM but likelihood ratios of positive and negative tests would not improve. When AFP was elevated in isolation, no association was observed with PTB (odds ratio = 1.80, 95% confidence interval: 0.92–2.68).[31] In our study, we found that more than 90% of our subjects had AFP <200 ng/dL, which was considered the cutoff value for possible inflammation. Mid-trimester CL is a good predictor for symptomatic women with spontaneous PTB.[35] A multicenter randomized clinical trial found a 45% reduction in PTB rate with the use of vaginal progesterone among low-risk asymptomatic women diagnosed to have shorter CL (≤20 mm).[36] However, ultrasound indicated cerclage in low-risk women did not seem to prevent PTB.[37,38] Hence, the role of CL as an independent predictor for PTB among low-risk populations is yet to be determined as also proposed by our study.

None of these studies have calculated the Youden index and many studies did not show ROC curves either. ROC_AUC and/or Youden index <0.5 is statistically considered extremely poor discriminative power and such tests are equally likely to give positive results for preterm as well as term deliveries. Even though some studies report associations between biomarker levels and preterm delivery, such results cannot conclusively establish cutoff values for test purposes. The limitation of our study was the lack of serial estimations of serum biomarkers that might have improved the accuracy of results.

Conclusion

A single measurement of CL, serum ferritin, and serum AFP in the second trimester of pregnancy had poor discriminatory ability to predict the risk of preterm delivery among low-risk pregnant women.

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Ethical statement

The proposal of the study was approved by Institutional Ethics Committee of CNMCH vide approval number22/1/2021-G&O.CNMC/100(2)-2021 Dated on 22.1.2021.

Authors contributions

- LJ, JB - Concept and idea of the research
- MD, DM - Study design and data analysis
- JB, AM, KK - Literature review.

The final draft was approved by all authors.

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Conflicts of interest

There are no conflicts of interest.

References

1. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, *et al.* National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: A systematic analysis and implications. Lancet 2012;379:2162-72.
2. World Health Organization. March of Dimes, Partnership for Maternal, Newborn and Child Health, Save the Children. Born Too Soon: The Global Action Report on Preterm Birth. Available from: www.who.int/maternal_child_adolescent/documents/born_too_soon/en/. [Last accessed on 2012 May 04].
3. Norman JE, Morris C, Chalmers J. The effect of changing patterns of obstetric care in Scotland (1980-2004) on rates of preterm birth and its neonatal consequences: Perinatal database study. PLoS Med 2009;6:e1000153.
4. World Health Organization. Preterm Birth; 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/preterm-birth. [Last accessed on 2023 May 30, Last updated on 2023 May 10].
5. Lockwood CJ, Kuczynski E. Markers of risk for preterm delivery. J Perinat Med 1999;27:5-20.
6. Conde-Agudelo A, Papageorghiou AT, Kennedy SH, Villar J. Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: A systematic review and meta-analysis. BJOG 2011;118:1042-54.
7. Iams JD, Goldenberg RL, Mercer BM, Moawad AH, Meis PJ, Das AF, *et al.* The preterm prediction study: Can low-risk women destined for spontaneous preterm birth be identified? Am J Obstet Gynecol 2001;184:652-5.
8. Petrini JR, Callaghan WM, Klebanoff M, Green NS, Lackritz EM, Howse JL, *et al.* Estimated effect of 17 alpha-hydroxyprogesterone caproate on preterm birth in the United States. Obstet Gynecol 2005;105:267-72.
9. Tancrède S, Bujold E, Giguère Y, Renald MH, Girouard J, Forest JC. Mid-trimester maternal serum AFP and hCG as markers of preterm and term adverse pregnancy outcomes. J Obstet Gynaecol Can 2015;37:111-6.
10. Ogge G, Chaiworapongsa T, Romero R, Hussein Y, Kusanovic JP, Yeo L, *et al.* Placental lesions associated with maternal underperfusion are more frequent in early-onset than in late-onset preeclampsia. J Perinat Med 2011;39:641-52.
11. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "great obstetrical syndromes" are associated with disorders of deep placentation. Am J Obstet Gynecol 2011;204:193-201.
12. Waller DK, Lustig LS, Cunningham GC, Feuchtbaum LB, Hook EB. The association between maternal serum alpha-fetoprotein and preterm birth, small for gestational age infants, preeclampsia, and placental complications. Obstet Gynecol 1996;88:816-22.
13. KurtulÇam E, Demircan N, Özmen Bayar Ü, Köktürk F, İnanArıkan İ. Risk factors causing preterm labor. Gynecol Obstet Reprod Med 2016;19:7-11.
14. Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: Past, present and future. Biochim Biophys Acta 2010;1800:760-9.
15. Mittal A, Sathian B, Chandrashekharan N, Farooqui SM, Hussain A. Diagnostic significance of alpha fetoprotein in carcinomas of liver and biliary tract – A comparative study from the Western region of Nepal. Asian Pac J Cancer Prev 2011;12:3475-8.
16. Forman DT, Parker SL. The measurement and interpretation of serum ferritin. Ann Clin Lab Sci 1980;10:345-50.
17. World Health Organization. WHO Guideline: Use of Ferritin Concentrations to Assess Iron Status in Individuals and Populations. Geneva. Switzerland: World Health Organization; 2017.
18. Youden WJ. Index for rating diagnostic tests. Cancer 1950;3:32-5.
19. Kane SP. Post. ClinCalc. Available from: https://clincalc.com/stats/Power.aspx. [Last accessed on 2023 Sep 10, Last updated on 2018 Nov 10].
20. Reddy KM, Ravula SR, Palakollu S, Betha K. Prevalence of preterm birth and perinatal outcome: A rural tertiary teaching hospital-based study. J Family Med Prim Care 2022;11:3909-14.
21. Hosmer DW, Lemeshow S. Applied Logistic Regression. New York, NY, USA: John Wiley and Sons; 2004.
22. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, *et al.* Global, regional, and national estimates of levels of preterm birth in 2014: A systematic review and modelling analysis. Lancet Glob Health 2019;7:e37-46.
23. Goldenberg RL, Andrews WW, Hauth JC. Choriodecidual infection and preterm birth. Nutr Rev 2002;60:S19-25.
24. Salafia CM, Vogel CA, Vintzileos AM, Bantham KF, Pezzullo J, Silberman L. Placental pathologic findings in preterm birth. Am J Obstet Gynecol 1991;165:934-8.
25. Tripathi R, Tyagi S, Singh N, Mala YM, Singh C, Bhalla P, *et al.* Can preterm labour be predicted in low risk pregnancies? Role of clinical, sonographic, and biochemical markers. J Pregnancy 2014;2014:623269.
26. Singh B, Goswami B, Gupta N, Bajaj AD, Mallika V. Potential biochemical markers for preterm labor: A pilot study in North India. Indian J Clin Biochem 2011;26:41-5.
27. Akinbiyi AA. Unexplained elevated maternal serum alpha-fetoprotein in singleton pregnancies as a predictor of fetal risk. Int J Gynaecol Obstet 1996;53:17-21.
28. Bernstein IM, Barth RA, Miller R, Capeless EL. Elevated maternal serum alpha-fetoprotein: Association with placental sonolucencies, fetomaternal hemorrhage, vaginal bleeding, and pregnancy outcome in the absence of fetal anomalies. Obstet Gynecol 1992;79:71-4.
29. Williams MA, Hickok DE, Zingheim RW, Luthy DA, Kimelman J, Nyberg DA, *et al.* Elevated maternal serum alpha-fetoprotein levels and midtrimester placental abnormalities in relation to subsequent adverse pregnancy outcomes. Am J Obstet Gynecol 1992;167:1032-7.
30. Chitayat D, Farrell SA, Huang T, Meier C, Wyatt PR, Summers AM. Double-positive maternal serum screening results for down syndrome and open neural tube defects: An indicator for fetal structural or chromosomal abnormalities and adverse obstetric outcomes. Am J Obstet Gynecol 2002;187:758-63.
31. Cho S, Durfee KK, Keel BA, Parks LH. Perinatal outcomes in a prospective matched pair study of pregnancy and unexplained elevated or low AFP screening. J Perinat Med 1997;25:476-83.
32. Cox MU, Kingdom J, Whittle M, McNay M, Fleming J, Bowman A, *et al.* Unexplained increase in maternal plasma in alpha-fetoproteins in the second trimester of pregnancy: Perinatal results. Rev Chil Obstet Ginecol 1995;60:174-80.
33. van Rijn M, van der Schouw YT, Hagenaars AM, Visser GH, Christiaens GC. Adverse obstetric outcome in low-and high- risk

pregnancies: Predictive value of maternal serum screening. Obstet Gynecol 1999;94:929-34.

34. Yuan W, Chen L, Bernal AL. Is elevated maternal serum alpha-fetoprotein in the second trimester of pregnancy associated with increased preterm birth risk? A systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol 2009;145:57-64.
35. Ness A. Prevention of preterm birth based on short cervix: Symptomatic women with preterm labor or premature prelabor rupture of membranes. Semin Perinatol 2009;33:343-51.
36. Hassan SS, Romero R, Vidyadhari D, Fusey S, Baxter JK, Khandelwal M, *et al.* Vaginal progesterone reduces the rate of preterm birth in women with a sonographic short cervix: A multicenter, randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol 2011;38:18-31.
37. To MS, Alfirevic Z, Heath VC, Cicero S, Cacho AM, Williamson PR, *et al.* Cervical cerclage for prevention of preterm delivery in women with short cervix: Randomised controlled trial. Lancet 2004;363:1849-53.
38. Berghella V, Odibo AO, To MS, Rust OA, Althuisius SM. Cerclage for short cervix on ultrasonography: Meta-analysis of trials using individual patient-level data. Obstet Gynecol 2005;106:181-9.