The role of salivary progesterone and cervical length measurement in predicting risk of spontaneous preterm birth

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Background: Neonatal mortality and neonatal morbidity are increased by preterm birth. Researchers have assessed various biochemical and biophysical markers to predict women at risk for preterm birth in order to decrease its incidence, prevent complications and improve survival rate for infants. *Objective*: To evaluate the prediction of spontaneous preterm birth by estimation of salivary progesterone levels and by serial measurements of cervical length. *Patients and Methods*: A prospective study was carried out at the Maternity and Child Hospital, Basrah, Iraq. Symptomless women (n = 40) with a single fetus, and with a preterm birth, preterm rupture of membranes, or late spontaneous miscarriage (at 20--28 weeks of pregnancy), were recruited from the outpatient clinic at 24--28 weeks of gestation and were retested after 4 weeks. At each visit, three salivary samples were collected and assessed for their progesterone level using ELISA. Transvaginal sonography was used for cervical length estimation. *Results*: Of the 40 women, 9 were delivered at term (i.e., after 37 weeks). Their mean pregnancy duration at time of parturition was (38 ± 2) weeks. The other 31 women underwent preterm birth, 13 cases with early preterm ($\geq$24--34 weeks) and 18 cases with late preterm ($>$34--37 weeks). The mean levels of salivary progesterone for the term group at the first visit (24--28 weeks) and the 2nd visit (28--34 weeks) were 797.2 pg/mL and 899.4 pg/mL respectively. The mean levels of salivary progesterone in the preterm group at the first visit and 2nd visit were 344.2 pg/mL and 257.3 pg/mL respectively. The difference between the term and preterm birth groups was statistically significant (P = 0.04). There were significant differences regarding cervical length measurement between the preterm delivery groups and the term delivery group. *Conclusion*: Both low salivary progesterone concentrations and low cervical length can be applied for predicting preterm birth in asymptomatic at-risk women.

Keywords

Cervix; Pregnancy; Preterm birth; Salivary progesterone

1. Introduction

Preterm birth begins with contractions of the uterus, before 37 weeks of pregnancy, that lead to cervical effacement and dilatation [1]. Many developed and developing countries officially record preterm birth as all births with birth weight less than 500 gm [2].

Globally, about 15 million babies are born preterm each year. The rate of preterm birth is less than 10% in many developed countries. Nations with a preterm birth rate more than 15% include Malawi, Congo, Comoros, Zimbabwe, Equatorial Guinea, Mozambique, Goban, Pakistan, Indonesia, Mauritania, and Botswana. The highest number of preterm births are recorded in India, China, Nigeria, Pakistan and Indonesia [3].

The specific cause of preterm birth is unclear. 30–35% of preterm births are iatrogenic due to medico-obstetrical complications, 40–45% are related to spontaneous early labor, and 25–30% to early rupture of membranes [4].

In addition, symptoms of uterine contractions, pelvic pressure, menstrual like cramps, watery vaginal discharge and lower back pain have been empirically associated with impending preterm births [5].

Progesterone promotes uterine preparation for embryo implantation as well as lowered immunity in women during pregnancy. Progesterone production is started by the corpus luteum and after around 8 weeks is maintained by the placenta. Low progesterone concentrations help in fetal birth and induction of milk. Preterm birth takes place when progesterone levels become lower than normal [3, 5] which can be treated by giving progesterone.

The determination of progesterone in saliva can be a more acceptable method than in blood because repeated blood sampling is too stressful and invasive. It has been shown that the salivary steroid hormone reflects the free, unbound part in blood [6–8]. This free part is considered the biologically active element of the total amount [9–11]. Therefore, progesterone in the saliva is a good marker of progesterone function.

There is strong evidence that transvaginal sonographic estimation of cervical length can be used to diagnose patients at risk for preterm birth in both symptomatic and asymptomatic women [12]. It has been observed that a cervical length less than 20 mm has a 100% predictive value for preterm birth before 28 weeks gestation [13]. In addition, Goldenberg *et al.* [14] found a cervical length of less than 25 mm at 24 weeks gestation to be a strong indicator of preterm birth. It has been

Clinical and Experimental Obstetrics & Gynecology

Table 1. Demographic features of both groups.

Characteristic features	Group 1 Preterm birth n = 31 77.5%	Group 2 Term birth n = 9 22.5%	P value
Age (years)			0.0034
<20	0	0	
20–30	25 (62.5%)	5 (12.5%)	
30–40	6 (15%)	4 (10%)	
Socioeconomic state			0.4
upper	4 (10%)	1 (2.5%)	
middle	22 (55%)	5 (12.5%)	
lower	5 (12.5%)	3 (7.5%)	
Residency			0.2
urban	20 (50%)	6 (15%)	
rural	11 (27.5%)	3 (7.5%)	
Parity			0.005
1–4	24 (60%)	7 (17.5%)	
>4	7 (17.5%)	2 (5%)	
Gestational age at time of labor (weeks)	late preterm: 34 ± 3 early preterm: 30 ± 1	38 ± 2	0.05
Previous miscarriage			0.1
0	28	9	
1	3	0	
2 and more	0	0	

demonstrated that cervical length can be considered as predictive for preterm birth in women with triplet pregnancies [15]. Furthermore, transvaginal sonography is more convenient and efficient for cervical length estimation than transabdominal sonography [16].

Thus, the aim of the present study was to evaluate a marker for spontaneous preterm birth by analyzing salivary progesterone and transvaginal cervical length measurement after 24 weeks of pregnancy.

2. Patients and methods

2.1 Setting

This study was conducted from January 2019 until May 2020 at the Department of Gynecology and Obstetrics in the Basrah Maternity and Child Hospital, Iraq. All women gave informed consent to be involved in the study. Also, the work has been approved by the Ethical Committee of the College of Medicine, University of Basrah, Basrah, Iraq.

2.2 Subjects

Symptomless women (n = 40) with a single fetus at 24–28 weeks of pregnancy, and with a preterm birth, preterm rupture of membranes, or late spontaneous miscarriage (at 20–28 weeks of pregnancy), attending the outpatient clinic were involved in this work. The limitation in sample size was due to failure in follow up for enrolled women especially during quarantine for COVID-19.

Exclusion criteria included cases of multiple pregnancy, congenital disorders of the fetus or uterus, antepartum hem-

orrhage, women with iatrogenic preterm birth, fetal growth retardation, women with a history of cervical cerclage and patients with vaginal progesterone suppositories. Hypertension, diabetes, nephrotic disease, heart disease, hematological defects, chronic hepatic disease and vaginosis were included. Antidepressants, corticosteroids, progesterone or tocolytic therapy, drug addiction, smoking and any oral abnormality like bleeding gum were excluded.

2.3 Sample collection

Women made their first visit at 24–28 weeks of gestation and they were retested after 4 weeks. Saliva specimens were obtained from women after overnight fasting and washing the oral cavity with water for 10 min. Passive specimens were collected in sterile containers over 2 hours, at 30 min intervals and then were added together. The specimens were kept in a fridge for 30 min and then frozen at -20 °C until needed.

2.4 Estimation of salivary progesterone

Specimens were thawed and centrifuged at 1500 g for 5 min. The upper part of the sample was used for salivary progesterone estimation by ELISA according to the manufacturer's procedures (De Meditec, GmbH, Lise-Meitner-Strasse 2, 24145 Kiel, Germany). The progesterone concentration that was detected in the zero calibrator was low (5 pg/mL) at the 2SD confidence interval. The assay range was 0–5000 pg/mL. Twenty replicate records of 3 saliva specimens within a single run was depended for the intra assay difference. The within assay coefficient of variance was 5.96%, 6.44% and 9.63%. The inter assay difference was assessed by estimations

Table 2. The value of salivary progesterone concentrations in both delivery groups.

	Mean salivary progesterone for preterm group	Mean salivary progesterone for term group	P value
	n = 31	n = 9	
1st visit: (24–28 weeks)	344.2 pg/mL	747.2 pg/mL	0.04
2nd visit: (28–34 weeks)	257.3 pg/mL	899.46 pg/mL	<0.05

Table 3. Comparison of salivary progesterone levels and cervical length by TVS between the three delivery groups.

	Groups	No. of subjects	Mean 1st visit: (24–28 weeks)	Mean 2nd visit: (28–32 weeks)	P value
Salivary progesterone	Term	9	747.2 pg/mL	899.4 pg/mL	0.01
	Early	18	301.5 pg/mL	245.3 pg/mL	0.04
	Late	13	344.246 pg/mL	301.3 pg/mL	0.02
Cervical length	Term	9	30 mm	28 mm	0.01
	Early	18	20 mm	18 mm	0.08
	Late	13	22 mm	20 mm	0.001

of 3 saliva specimens repeated twice over 10 days.

2.5 Determination of cervical length

The transvaginal scan for cervical length was done at the first visit which was at 24–28 weeks of gestation. The cervical length was determined by a transvaginal transducer probe (LOGI Q 500MD 7 MHz) which was inserted into the anterior fornix of the vagina. The measurement from the external os to the internal os was repeated 3 times. The present study used the shortest estimate. The mean of 2 readings was considered for an angulated cervix.

2.6 Follow up

The two parameters (salivary progesterone and cervical length) were retested after 3–4 weeks. All women in the study were followed up till parturition. Progesterone therapy was avoided during the study. Women with history of pain and a suspicion of preterm delivery were admitted to the clinical ward in the hospital and were given antibiotics and corticosteroid for lung maturity.

2.7 Delivery history record

All the women in the study were followed until delivery. The information recorded included pregnancy age at time of parturition, mode of delivery, the neonatal outcome, Apgar score at 1 and 7 min and history of admission to intensive care unit.

The gestational age is classified as:
(1) Term birth ≥37 completed weeks of gestation.
(2) Early preterm birth ≤34 weeks.
(3) Late preterm birth at 34–36^{+6} weeks.

2.8 Statistical analysis

The collected data were statistically analyzed using Statistical Package for the Social Sciences (SPSS). Chi-square test (X^2) and t-students test were applied to compare clinical variables. Pearson correlation was used to assess the relationship between cervical length and salivary progesterone. $P < 0.05$ was the significance value.

3. Results

Forty women who met the inclusion criteria were included in the study.

There was a significant difference between the preterm group and the term group with regard to age ($P = 0.0034$). 62.5% of the preterm group were between 20–30 years old. Also, there was a significant difference with regard to parity. 60% of the preterm group has parity between 1–4 and 17.5% of them were multiparous (>4) ($P = 0.005$) (Table 1). There were no significant differences with regard to socioeconomic state and residence between the groups (Table 1).

Nine women out of the 40 were delivered at term (i.e., after completed 37 weeks). Their mean pregnancy age at time of parturition was (38 ± 2) weeks. The gestational age for the early preterm group (13 women) was 30 ± 1 weeks in comparison to 34 ± 3 weeks for the late preterm group (18 women) (Table 1).

The mean levels of salivary progesterone in the preterm group at the first visit (24–28 weeks) and the 2nd visit (28–34 weeks) were 344.2 pg/mL and 257.3 pg/mL respectively (Table 2). The mean levels of salivary progesterone in the term group at the first and second visits were 797.2 pg/mL and 899.4 pg/mL respectively. The mean salivary progesterone for the term group was statistically higher than the preterm group at both the first visit ($P = 0.04$) and the second visit ($P < 0.05$) (Table 2).

The relationship between salivary progesterone concentrations and serial measurements for cervical length by transvaginal ultrasound in both groups are shown in Table 3.

There was a significant difference between the level of progesterone in the preterm subgroups (early and late preterm) and the term group. Furthermore, there is significant difference in the measurement of cervical length in both subgroups of preterm birth when compared to the term birth group (Table 3).

In general, the salivary progesterone values as well as the cervical length measurements were lower among preterm

group in relation to term group. They were statistically significant (Table 3).

4. Discussion

Women at risk for preterm birth could potentially be identified by biochemical and biophysical markers so that patients can be followed and appropriate care given. Early diagnosis might keep pregnant women away from preterm birth and increase fetal lung maturity. At the same time, identifying women at low risk could minimize their hospital stay and antenatal requirements. Many markers have been investigated but a specific one for preterm birth still uncertain [17]. Assessment of salivary progesterone as a marker has value because it is non-invasive and saliva can easily be collected and stored.

In the present study, the explanation for the significant differences between the delivery groups with regard to age and parity might be because of the small sample size, especially for the term group. In this study, the preterm deliveries are mostly in the younger age group. This result would confirm a previous study which observed that preterm birth was more common in the younger age group (20–30 years) [18]. Social class, residency and history of previous abortions were not found to have any significant association.

In the present study, salivary progesterone concentrations were lower in the preterm group when compared to the term group. In the pre-term group, progesterone levels decreased significantly from the first to the second visit and the decrease was more noticeable in early preterm group compared with late preterm group. This finding has confirmed the theory that progesterone levels are significantly lower in women who have preterm labor than those delivered at term [19]. These results are in agreement with other workers [20, 21]. However, others have reported that the levels of plasma progesterone in women with preterm birth were identical to that of women with term birth [22].

The present study indicates that there is an inverse correlation between cervical length and gestational age. There was a similar correlation between progesterone concentrations and the shorten cervical length which was noticed in women who delivered before 34 weeks of pregnancy.

The mean cervical length at the first visit in the preterm group was significantly shorter ($P = 0.04$) in comparison with the term group. It was also observed that cervical length had decreased by the second visit for both groups (18–20 mm $\pm$ 2) in the preterm and 28 mm in the term group respectively. In addition, it was noticed that there was a decrease in the length of cervix for all the studied groups (early, late preterm and term) over visits.

Progesterone has anti-inflammatory properties and is important for maintaining pregnancy and enhancing uterine relaxation by reducing prostaglandin synthesis and increasing cellular calcium binding [23]. Progesterone treatment is widely prescribed as a preventive measure in women at risk for preterm birth [23]. Other workers have investigated the role of serum neopterin [24] and also neopterin/creatinine ratio, C-reactive protein and chitotriosidase in serum of pregnant women [25] but they have recommended further studies to evaluate these markers in clinical practice. Therefore, given the unavailability of these markers in the developing countries, the advantage for the present study is the availability of progesterone which can be measured in saliva as a non-invasive test among asymptomatic at-risk pregnant women.

In conclusion, low salivary progesterone concentrations and low cervical length can predict preterm birth in asymptomatic at-risk women. Therefore, measurement of saliva hormone and cervical length can be strongly recommended for use in clinical practice at 24–34 weeks of pregnancy.

Author contributions

Both authors involved in samples collection, data analysis and writing up the manuscript.

Ethics approval and consent to participate

All participants gave informed consent and agreed to be involved in the study. The work has been approved by the Ethical Committee of the College of Medicine, University of Basrah, Basrah, Iraq, code 59 dated 16th December 2018 in accordance with ethical standard of the Arab Board of the Medical Specialization.

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Conflict of interest

The authors declare no conflict of interest.

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