Research article

A prospective observational study to assess serial salivary estriol measurements during third trimester of pregnancy as a marker for predicting spontaneous preterm labor in pregnant women

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ABSTRACT

Introduction and Aim: Preterm labor is a matter of concern in developed and developing nations, rural and urban areas as well. In this context a relatively cheaper biochemical marker in a specimen which can be easily collected from pregnant women will be beneficial in rural areas. Hence, in this context salivary estriol was selected for studying its diagnostic potential.

Materials and Methods: A prospective study was conducted in two sittings (1st sitting at 26 to 29 weeks and 2nd after 2-4 weeks of gestation) among pregnant women (n= 206) who attended T.D. Medical College for antenatal check-up. The salivary samples were collected and estriol (E3) assayed by ELISA. The pregnant women were followed up till delivery to know the type, as term (n=194) or preterm (n=12). MedCalc was used for statistical analysis.

Results: Reference interval of increase in salivary E3 over a period of 2 - 4 weeks in pregnant women during 26 – 32 gestational weeks who delivered at term by nonparametric method was 16 to 1468 pg/ml. Gestational period wise reference intervals of salivary estriol at 26- 28, 29 – 30 and 31 to 32 weeks were 74-3227, 119 -3422 and 203-4346 pg/ml respectively. There was a statistically significant difference in the increase in salivary estriol among different time intervals (p=0.001). Diagnostic cut off values of single measurements of salivary E3 and increase in salivary estriol between 2-4 weeks were 492, 561 and 159 pg/ml without acceptable diagnostic accuracy.

Conclusion: Serial rise in salivary estriol at 2-4 weeks interval can be utilized but not a single measurement to monitor fetoplacental wellbeing. The high NPV of serial rise in salivary estriol (E3) suggests its usefulness to exclude chances for preterm labor.

Keywords: Salivary estriol (E3); preterm labor (PTL); diagnostic cut off value; reference interval.

INTRODUCTION

Preterm labor (PTL) can be defined as labor resulting in birth before 37 completed weeks (259 days) of gestational age (1). Preterm labor is of two types-induced due to maternal or fetal risks and spontaneous. Induced preterm labor follow medical or obstetric disorders such as maternal hypertension, diabetes mellitus, placenta previa, abruptio placentae, intrauterine growth retardation that place the fetus at risk and account for 20-30 % and the rest 70-80% constitute spontaneous preterm births (2).

Preterm labor is a matter of concern because many of the surviving preterm infants especially from the earlier gestations, suffer serious morbidities such as bronchopulmonary dysplasia, intraventricular hemorrhages, retrolental fibroplasia, neuro developmental problems, cognitive difficulties (3), mental retardation, cerebral palsy, seizure disorders, blindness, and deafness (4). The hospital based immediate neonatal health care and subsequent rearing of preterm babies involves huge financial burden to the health care systems and family. Moreover, the mental agony, sociological and economical stress created to the family is enormous.

Mechanism of spontaneous preterm labor

The exact molecular pathogenesis of PTL is still not clear. The proposed mechanism is described below. When fetal membranes and decidua are exposed to inflammatory insult consequent to infection, ischemia, trauma, or allergy (delayed type hypersensitivity) will cause generation of TNF α, IL-1 and IL -6 which in turn lead to production of uterotonic bioactive lipids – PG E_2, PG $F_{2\alpha}$, thromboxane A_2, leukotriene B_4, leukotriene C_4 and 5HETE (hydroxyperoxyeicosa tetraenoic acid). These in turn will cause production of estrogens (E1 estrone, E2 – estradiol, E3 – estriol). All these bioactive lipids stimulate myometrial contractions and elaborate proteases capable of damaging membranes and underlying decidua. The prostaglandins stimulate cervical ripening, dilation

and/ or membrane rupture. The gestational age at the time of insult is important because responsiveness of the uterus to stimulation increases with gestational age especially after 30 -32 weeks. Thus, exogenous, and endogenous factors combine to create a stimulus for labor or amniorhexis (5,6). Congenital weakness of cervix leads to cervical insufficiency and PTL is another mechanism.

Diagnosis of spontaneous preterm labor

Despite better antenatal care, the incidence of preterm labor in India is 18.4 % (7). It is 10 % in T.D. Medical College, Alappuzha, Kerala, India as per 2011 hospital records where the health care delivery system is better. The modern obstetric strategies like administration of corticosteroids to enhance fetal lung maturation and tocolysis have been found to be beneficial to the preterm infant. But the timely diagnosis of preterm labor is a prerequisite for these management strategies. The clinical diagnosis of preterm labor is mostly carried out by clinical evaluation such as ≥6 contractions/hour, ≥ 80 % effacement of cervix and advanced cervical dilatation (≥ 3 cm). But most often these will be evaluated too late for intervention. Uterine activity monitoring by tocodynamometer needs patient's cooperation and compliance. Digital assessment of cervical length and dilation is highly subjective (8). Transvaginal sonography improved the accuracy of diagnosis of PTL (8), but its availability is limited.

Identification of women at risk for preterm delivery is critical because injection of antenatal corticosteroid at least 48 hours prior to delivery dramatically reduces morbidity and mortality of premature infants (9). Simple effective predictive markers of PTL, if available, could prevent or postpone PTL, improve the fetal outcome, avert unnecessary treatment, and avoid complications of tocolysis.

Biochemical markers of preterm labor

Currently three biochemical markers are found to be useful. Fetal fibronectin and interleukin -6 (IL-6) in cervicovaginal fluid, and estriol in saliva. Out of these three, salivary estriol carries the merit of easy collection of specimens unlike the other two, which requires expertise to collect the cervical-vaginal secretions. In addition, it involves inconvenience of internal examination to the patient.

Salivary estriol estimation in predicting preterm labor

Activation of the fetal hypothalamic pituitary axis typically occurs after 32 -34 weeks. (10) Fetal adrenal gland produces dihydroepiandrosterone (DHEA) which is converted to placental estriol by placental enzymes. This estriol enters maternal circulation and secretes in saliva which is in proportion to increasing maturation of the fetal hypothalamic pituitary adrenal axis. When the HPA axis is stimulated by maternal stress, it will cause production of more estriol. The estriol will stimulate myometrial oxytocin receptors and prostaglandin synthase to produce prostaglandins which in turn cause uterine contractions, cervical effacement and dilatation culminating in preterm labor.

Of the three estrogens, estriol (E3), estradiol (E2) and estrone (E1), E3 is the most abundant during late pregnancy (10). In humans over 90% of E3 is derived from feto-placental sources (11). Maternal serum E3 first detectable at 9 weeks of gestation. Serum E3 level gradually increases during the first and second trimesters. More rapid increases occur during the 3rd trimester with a characteristic surge preceding the onset of labor by a few weeks. This increase occurs approximately 3-4 weeks before the onset of parturition, in term, preterm and post term pregnancies.
Saliva is a useful matrix for measuring E3 steroid hormones because the unbound, unconjugated (free, biologically active) fraction of E3 in saliva correlates with free serum concentrations (11).

In this study we prospectively examined and assayed salivary E3 levels in pregnant women during 26 -29 weeks (1st sitting) and after 2-4 weeks (2nd sitting). The utility of salivary E3 for identifying women at risk for PTL done by noting salivary E3 levels in two sittings and increase in E3 between two serial measurements and type of delivery.

The aim of the study was to find out reference intervals of salivary estriol at different interval between 26 to 36 weeks (26 to 28 weeks, 29 to 30 weeks, 31 to 32 weeks and 33 to 36 weeks) and to know the pattern of salivary estriol levels during 3rd trimester of pregnancy at an interval of 2-4 weeks and its diagnostic potential in predicting preterm labor.

MATERIALS AND METHODS

The minimum sample size for this study was set to be 120 as per IFCC (International Federation for Clinical Chemistry; 12,13) guidelines.

To compensate for a dropout rate of 50 %, N= n/1-(z/100), Where N = expected sample size; n= sample size; z = dropout rate (50%): N = 120/1-(50/100) = 240. Additional samples of 17 numbers were also included to compensate for outliers so that the total number enrolled became 257.This observational cohort study was conducted at the Government T. D. Medical College, Alappuzha. In this study 257 asymptomatic pregnant women attending antenatal OPD were recruited by consecutive enrolment for 26-29 weeks (1st sitting) and follow up was done on the next antenatal visit (2nd sitting) based on inclusion and exclusion criteria shown below, after getting informed consent.

Inclusion criteria

Included singleton pregnancies with 26 – 29 weeks of gestation for the 1st testing and 2nd testing 2 - 4 weeks later, with cervical dilatation <3 cm, effacement < 80 %, intact membranes and uterine contractions < 6/hour.

Exclusion criteria

Included multiple pregnancy, pre-eclampsia with fetal growth retardation (FGR), preterm rupture of membranes, and steroid therapy < 1 week.

Salivary samples were collected in the morning between 8-10 AM. The salivary estriol was tested two times, 1st between 26-29 weeks and the second at an interval of around 2-4 weeks after the 1st testing. All the pregnant women were followed up till delivery to record the type of delivery as term or preterm.

Saliva was collected 60 minutes after a major meal, acidic food, high sugar food and after tooth cleaning. All were instructed mouth rinsing to remove food residue with plain water 10 minutes prior to collection of saliva. The subject was asked to collect unstimulated passive drool of saliva up to 0.5 -1 ml into two polypropylene micro centrifuge tubes (1.5 ml) at an interval of 30 minutes. Equal volumes of each of the samples were pooled to create one sample. Within 30 minutes samples will be frozen at -20^{0}C to break down mucin. Repeated freezing – thawing was avoided to preserve the analyte E3.

On the day of assay, samples will be thawed completely and centrifuged at 3000 rpm for 15 minutes to remove mucins and other debris. Supernatant will be used for salivary estriol testing. Samples were made to attain room temperature before assay. Estimation of salivary E3 was done by competitive immuno enzymatic assay method (ELISA).

1. Increase in salivary estriol over 2-4 weeks was recorded against each subject. The values of increase in salivary estriol around 2-4 weeks' time, related to pregnant women delivered at term were used for reference interval determination.
2. Single salivary E3 values of the two visits of each pregnant woman who had delivered at term were used for determining the reference interval of SE during 26-28, 29-30 and 31-32 weeks of gestation.

Statistical analysis

Microsoft Excel was used to summarize the characteristics of pregnant women enrolled in the study. Med calc 17.9.7. a free trial version was used to assess the reference intervals of single salivary E3 levels and increase in salivary E3 during the third trimester and diagnostic accuracy of increase in salivary estriol as a marker for predicting spontaneous preterm labor.

Nonparametric method (inter percentile interval of 95 % bounded by 2.5 and 97.5 percentiles) was adopted to find out reference interval of single salivary E3 as well as increase in salivary estriol over a period between two visits (2-4 weeks apart) in pregnant women who delivered at term.

Medical decision limit (optimum cut off value) of salivary E3 levels at mean gestational age of 28 weeks, 30 weeks and increase in salivary estriol between these serial measurements, for the prediction of PTL was found out by receiver -operating characteristic (ROC) curve analysis.

RESULTS

A total of 257 pregnant women were recruited in this study. All were asymptomatic of PTL at the time of enrolment for the study. All were followed up till the delivery to know the type of delivery as preterm or term but 51 lost follow up and hence the final number became 206. Out of these 12 (5.8 %) were preterm and 194 (94.2 %) term deliveries. The general characteristics of pregnant women who participated in the study are given in Table 1.

Reference interval

The reference interval of increase in salivary E3 over a period of 2-4 weeks in pregnant women who delivered at term (nonparametric method) was 16 to 1468 pg/ml (Table 2).

Reference intervals of salivary estriol during the period 26- 28 weeks, 29-30 weeks and 31 to 32 weeks found by this study were 74 -3227,119 -3422 and 203-4346 pg/ml respectively. Increase in salivary estriol as per the time interval was compared. Median values were 98,162,460 and 954 for 1 week, 2 weeks, 3 weeks and ≥4 weeks respectively. Also, inter-quartile ranges were 54, 343, 490 and 1125 for 1 week, 2 weeks, 3 weeks and ≥4 weeks respectively (Table 3, Fig. 1). According to the Kruskal Wallis H test, there was a statistically significant difference in the increase in salivary estriol among different time intervals (p=0.001).

Diagnostic accuracy

ROC curve analysis (Fig. 1, table 4) revealed diagnostic cut off values (clinical decision limit) for predicting preterm labor:

i) increase in salivary estriol during two serial measurements, as 159 pg/ml with sensitivity of 75% and specificity of 45%. Area under the curve (AUC) was 0.537 with a p value of 0.683, NPV 94% and PPV 13%.
ii) salivary estriol at 1st sitting (mean gestational age 28 weeks) as 492 pg/ml (sensitivity 83% and specificity 35%; AUC – 0.502 with a p value 0.986), NPV 94% and PPV 13%.

iii) salivary estriol at 2nd sitting (mean gestational age 30 weeks) as 561 pg/ml (sensitivity 67% and specificity 50%; AUC – 0.541 with a p value 0.6695), NPV 93% and PPV 13%.

Table 1: Demographic and patient characteristics

Characteristic	Term (n=194)	Preterm labor (n=12)	Total (n=206)
Age			
18-20	17	1	18
21-25	94	4	98
26-30	51	2	51
31-35	25	3	28
36-40	6	2	8
41-45	1	0	1
Socio economic status			
Above poverty line	91	5	96
Below poverty line	103	7	110
Level of education			
8th to 9th std	4	1	5
10th	40	0	40
Plus 2	74	4	78
Diploma	15	0	15
UG	54	7	61
PG	5	0	5
Professional Degree	2	0	2
Job status			
Not working	178	12	190
Working	16	0	16
Parity			
Primi	98	6	104
Multi	96	6	102
Gravida			
G1	100	6	106
G2	65	3	68
G3	24	1	25
G4	3	2	5
G5	2	0	2
Interval between salivary estriol measurements			
1 w	4	0	4
2 w	162	11	173
3 w	13	0	13
≥4 w	15	1	16

Table 2: Reference intervals of increase in salivary E_3 (pg/ml) over 2 to 4 weeks in 3rd trimester and week-wise reference interval

Variables	Lower limit	90 % Confidence interval	Upper limit	90 % Confidence interval
Increase in salivary estriol over 2 – 3 weeks (between 26 to 36 weeks)	16	4 - 20	1468	1364 - 2016
Salivary E_3(single measurement)				
26 -28 weeks (n= 131)	74	60 - 87	3227	2688 - 3918
29 -30 weeks (n= 135)	119	75 - 235	3422	2568 - 3979
31-32 weeks (n= 45)	203	145 to 283	4346	3110 - 6072

Table 3: Comparison of values of salivary estriol increase as per period

Third trimester	Median	Interquartile range	P Value*
26 -28 weeks	98.0	54	0.001
29 -30 weeks	162	343	
31 -32 weeks	460	490	
≥ 32 weeks	954	1125	

*Kruskal Wallis H test statistically significant difference between different periods at $p<0.05$

Table 4: Comparison of diagnostic cut off values of salivary estriol

Salivary estriol	Gestational age	Diagnostic cut off value of SE (pg/ml)	AUC	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Accuracy (%)
1st sitting	28 weeks (mean)	492	0.502	83	35	94	13	48
2nd sitting	30 weeks (mean)	561	0.541	67	50	93	13	51
Increase in E3	28 – 30 weeks	159	0.537	75	45	94	13	48

AUC-Area Under Curve; NPV-Negative predictive value; PPV-Positive predictive value

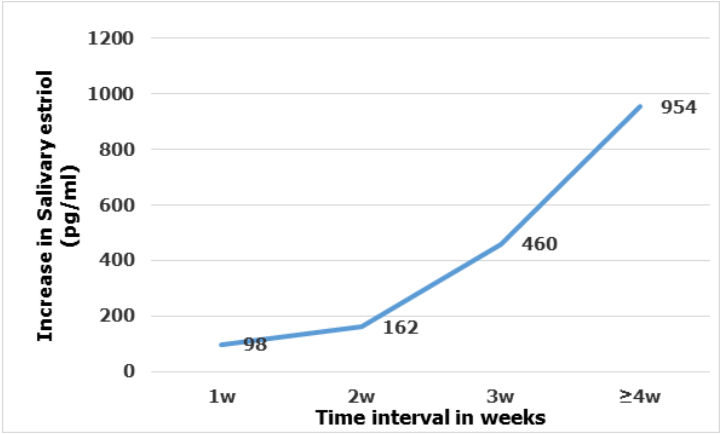

Fig. 1: Increase of salivary estriol according to time interval of salivary estriol collection

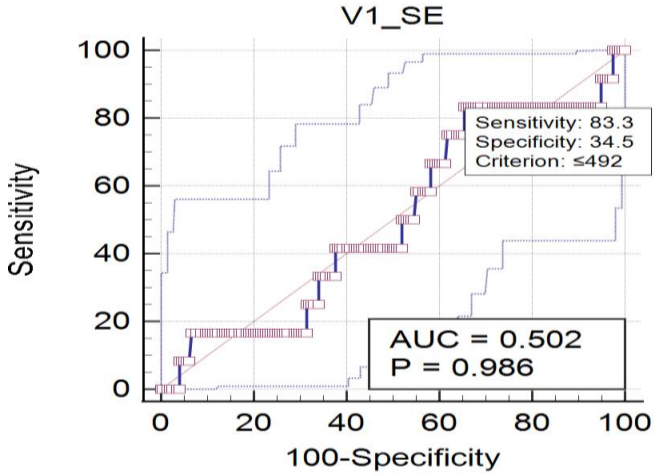

Fig. 2: ROC curve analysis to show cut off value of salivary estriol for predicting PTL at first sitting (Mean gestational age of 28 weeks)

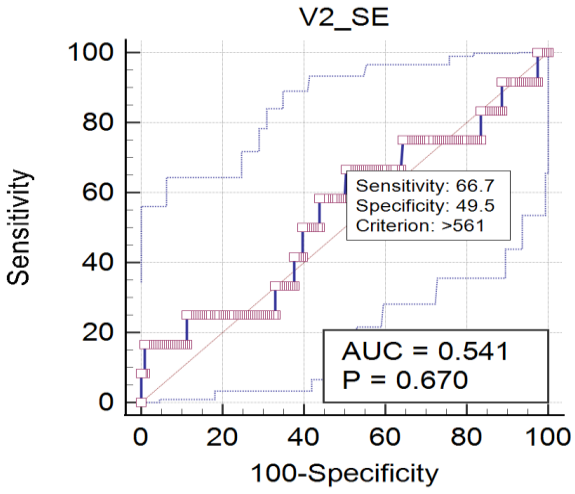

Fig. 3: ROC analysis to show diagnostic cut off value of salivary estriol for predicting PTL at second sitting (Mean gestational age 30 weeks)

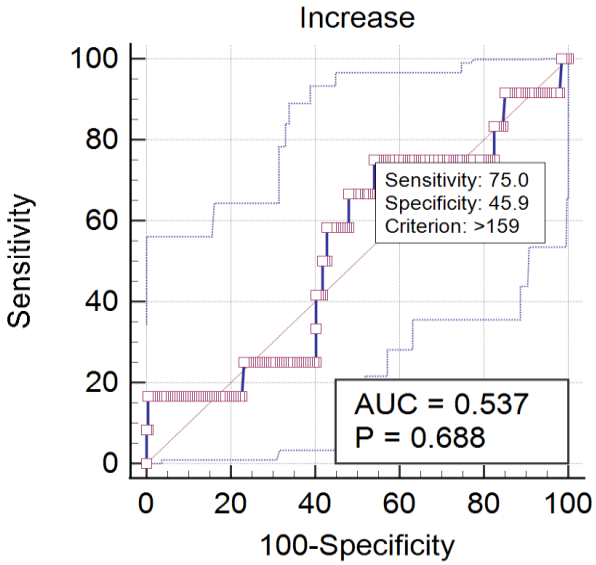

Fig. 4: ROC Analysis to show diagnostic cut off value of increase in salivary estriol between two serial prenatal checkup visits (28 and 30 weeks)

DISCUSSION

The estriol (E3) is the main estrogen of pregnancy circulating in the blood and extracellular fluid like saliva (14)**.** Advantages of salivary samples over plasma/serum or urine specimens for doing a monitoring test is that it is non-invasive and therefore stress free for pregnant women and does not require privacy as in the case of urine collection. For the person collecting the salivary sample, it causes least risk for contracting infections such as HPV, HCV, and HIV. It has been found that there is a correlation between maternal salivary and serum estriol levels (15). The rate of preterm birth is around 10 % even in developed countries indicating the need of further medical care for a considerable number of neonates worldwide (16). An awareness of preterm labor will be useful to act for preterm birth such as transfer to tertiary care centers, prophylactic administration of corticosteroids, magnesium sulfate treatment for neuroprotective effect, and antibiotic treatment in case of infection.

The high negative predictive value of salivary estriol cut off values (Table 4), at mean gestation weeks of 28 (94%), 30 (93%) and increase in salivary E3 during this interval is useful to reduce unwanted admission of mothers with symptoms of PTL to tertiary or regional centers and related expenses. But the diagnostic cut off values derived for the salivary E3 levels at 28 and 30 weeks of gestation (mean) and increase in salivary estriol between two serial measurements by ROC analysis have low statistical significance. High levels of E3 or a sudden increase in maternal E3 levels between two serial measurements are potential markers of impending labor. In accordance with our study, Smith et al also revealed E3 cannot be considered as a reliable marker for prediction of PTL (16,17).

Our study showed that there is a statistically significant difference in the increase in salivary estriol among different time intervals (26-28, 29-30, 31-32 and >32 weeks) during the 3rd trimester of pregnancy which is useful to assess fetoplacental function (16). During pregnancy E3 has a role of regulating uteroplacental blood flow and placental vascularization (18,19).

According to McGregor *et al.*, two repeated salivary estriol values ≥ 2.1 ng/ml were associated with a mean time to delivery of 2.3 weeks with NPV of 97 % (20). Our study showed cut off values of single measurements as 492 pg/ml (0.5 ng/ml) and 561 pg/ml (0.6 `ng/ml) at mean gestational age of 28 weeks, and 30 weeks with NPV of 94 % and 93% respectively to predict PTL.

A study (n= 115) conducted by Yadav and Singh on asymptomatic pregnant women revealed a significant increase in salivary estriol in women who delivered preterm-Salivary E3: 3513 ±586 pg/ml in preterm and 2692± 681 pg/ml with a p value <0.001 (21).

In this study, the increase in salivary estriol was 159 pg/ml at an interval between mean gestation weeks of 28 and 30 was found to have low diagnostic accuracy (AUC =537, P= 0.688). Lim *et al.*, also showed serial E_3 measurements in saliva are not strong biomarkers for preterm birth (22). Hence the prediction of preterm labor cannot solely depend upon increase in salivary E3 between two serial measurements during the third trimester.

Limitations of the study

Serum unbound estriol could have been tested which correlates well with salivary E3 (23) simultaneously to know whether any inconsistencies occurred during the salivary sample collection which needs mouth

rinsing prior to collection of saliva. But due to funding constraints we could not include serum testing for E3.

CONCLUSION

Weekly rise in salivary estriol during the third trimester of pregnancy can be utilized to monitor fetoplacental well-being. The high negative predictive value of salivary estriol is useful to exclude chances for preterm labor. But cannot rely upon it solely as the other metrics of diagnostic accuracy of salivary estriol are not statistically significant.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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