|| Print ISSN: 2589-7837 || Online ISSN: 2581-3935 ||
International Journal of Medical Science and Diagnosis Research (IJMSDR)
Available Online at www.ijmsdr.com
PubMed (National Library of Medicine ID: 101738825)
Volume 3, Issue 10; October: 2019; Page No. 40-46
Original Research Article
crossref DOI: https://dx.doi.org/10.32553/IJMSDR/v3i10.7

ROLE OF SALIVARY ESTRIOL TO PROGESTERONE RATIO IN PREDICTION OF PRETERM LABOUR

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Conflicts of Interest: Nil
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Abstract:

Objectives: To evaluate the ratio between Salivary Estriol and progesterone in preterm labour prediction.

Study design: Department of Obstetrics and Gynecology in collaboration with Department of Biochemistry, King George Medical University, Lucknow. Study was done over one year.

Material & Methods: Antenatal women were enrolled between 28 and <37 weeks of gestation with labour pains or without labour pains attending the antenatal clinic and those admitted in Queen Mary's Hospital, Lucknow. From each patient 5 ml of saliva was collected in cryo-vials to estimate Estriol and Progesterone by Immunoenzymatic colorimetric method of the supernatant of salivary sample.

Results: In study group mean value (0.84±0.26) of Estriol to Progesterone ratio in gestation age of <32 weeks was significantly higher that mean value (0.65±0.18) of control group. Mean value of estriol to progesterone ratio in gestational age group of 32-35 weeks in study group was 0.93±0.13 also significantly higher than the mean value of control group (0.65± 0.18). The p value 0.027 is statistically significant.

Conclusion: Thus, salivary estriol/progesterone ratio can be used as a predictor for detecting symptomatic and asymptomatic women at risk for preterm birth.

Keywords: Preterm labour, Estriol, Progesterone, Saliva.

Introduction

Preterm delivery is the leading cause of perinatal morbidity and mortality throughout the world. Preterm birth is a heterogeneous condition; nearly 30-40% of all cases of preterm birth are the result of elective delivery for a maternal or fetal complications [1]. The remaining 60-70% of preterm birth is probably the result of covert or sub-clinical infective/ Inflammatory processes, cervical dysfunction, idiopathic (unknown causes), multiple gestations and possible social, nutritional and environmental interaction [2]. This report focuses on this latter group of so-called, spontaneous preterm births.

Spontaneous onset of labour at term is produced by a definite increase in the saliva estriol to progesterone ratio; this rise in free estrogen should precede the onset of labour according with the known effect of estrogen & progesterone on myometrial activity.[3]

Concentration of steroid in saliva reflects unbound, unconjugated & biologically active fration. As saliva is easy to collect & store, measurement can be readly introduced. Saliva estriol levels showed a very high correlation (r=0.98) with serum levels of free estriol in pregnant women and salivary estriol levels were suggested as a means for the assessment of fetoplacental function.[4-5] This study is undertaken to evaluate the ratio between Salivary Estriol and progesterone in preterm labour prediction and normal pregnancy.

Material and Method:

The study was conducted in Department of Obstetrics and Gynecology, King George Medical University, Lucknow in collaboration with Department of Biochemistry, King George's Medical University, Lucknow.

Study Design:

This is a case control study, conducted for period of One year (July 2013 to July 2014). Selection of cases: Total number of 115 patients were enrolled and followed till delivery and they were divided into two Groups:

Control Group (Group I):

Patient with normal pregnancy with no preterm labour pain. Total 60 patients were enrolled they were followed till delivery. Out of 60, 8 were lost, 50 delivered at full term and two patients had preterm delivery, they were excluded from study. So in control group total 50 patients were included (n=50).

Study Group (Group II)

Patients who came with preterm labour pain. Total 55 patients were enrolled, patient followed till delivery, 5 patients were lost to follow (n =50). The study group was further subdivided into two groups.

Study Group, (IIA):

Patient who came with preterm labour pain and tocolysis given but had preterm delivery (n=41).

Study Group (IIB):

Patient who came with preterm labour pain and tocolysis given and patient delivered at full term (n=09).

Inclusion criteria:

Gestational age between 28 to <37 weeks having threatened preterm labour (uterine contractions with cervix <80% effaced and <1cm dilated) or established preterm labour pains with according to guidelines of ACOG (1997).

Exclusion criteria:

1. Not willing to participate in the study, 2. Patients who were taking steroid, 3. Who were having connective tissue disorder, gout, or metabolic disorder like viral hepatitis?, 4. Fetal congenital anomalies, 5. Intrauterine death, 6. Fetal distress, PPROM, 7. Patients with oral infection, poor oral hygiene, recent oral injuries.

Sample collection:

After per abdominal and per vaginal examinations verbal consent was taken from patients. Participants were instructed to rinse mouth with water for 10 minutes to remove food residue before sample collection. In women who came with preterm labour sample was collected before any intervention.

From each patient 5ml of salivary sample was collected in cryovials and transport to Department of Biochemistry. All samples were centrifuged at 3000 rpm for 15 minutes. The supernatant of salivary sample was quantitatively tested for estriol and progesterone.

The samples were maintained at 4°C for no longer than 2 hrs. If the specimens cannot assayed with in this time, the samples were stored at temperature of -20°C.

Estimation of Progesterone and Estriol:

The estimation of saliva progesterone was done by Immunoenzymatic Colorimetric method manufactured by Diametra S.r.l. headquater: Garibaldi, 18-20090 SEGRATE (MI) Italy. The lowest detectable concentration of Progesterone and estriol is 3.27 pg/ml and 1 pg/ml respectively at the 95% confidence interval.

Results:

In this study we observed that saliva progesterone levels of pregnant women with preterm delivery (study group IIA and II B) was lower than the control group (group I) but the difference was statistically not significant (3814.46$\pm$751.14 pg/ml vs 3945.16$\pm$ 577.11 pg/ml, p=0.351) ; 3588.67$\pm$ 1022.60 vs 3945.16$\pm$ 577.11 pg/ml ,p>0.140).

In the present study the mean estriol level in control group was 2691.72$\pm$681.08 pg/ml and in study group II was 3333.02$\pm$759.87 pg/ml , which was higher than control group but the difference was not statistically significant (p=0.222)

Mean value of study group IIA (3512.85$\pm$586.16 pg/ml) was higher as compared to the mean value of control group (2691.72$\pm$681.08 pg/ml) and difference was statistically significant (p value <0.001)

In our study mean value of Estriol/ Progesterone ratio in gestation age <32 wks group (0.84$\pm$0.26) was significantly higher than mean value of group I (0.65$\pm$0.18)

Mean value of estriol to progesterone ratio in gestational age group 32-35 wks in group II was 0.93$\pm$0.13 was also significantly higher than the mean value of control group (0.65$\pm$ 0.18) , p = 0.027.

But the mean value of estriol/Progesterone ratio in gestational age >35 wks-<37 wks in control group and study group were not significantly different

Table 1: Age Profile of Study Population

Age (years)	Group I (n=50)		Group IIA (n=41)		Group IIB (n=9)		Group II (n=50)	
	No.	%	No.	%	No.	%	No.	%
Up to 25	26	52.00	20	48.78	6	66.67	26	52.00
26-30	20	40.00	12	29.27	2	22.22	14	28.00
30-35	4	8.00	9	21.95	1	11.11	10	20.00
Statistical Significance (χ^2 test)	Group I, Group IIA & Group IIB				χ^2=4.811; p=0.307			
	Between Group I & Group II				χ^2=3.630; p=0.163			
	Between Group I & Group IIA				χ^2=3.853; 'p'=0.146			
	Between Group I & Group IIB				χ^2=1.036; 'p'=0.596			
	Between Group IIA & Group IIB				χ^2=1.018; p=0.601			

Table No.1 shows that majority of the study population (52%) were aged below 25 years and the differences between groups are not significant.

Obstetric Profile

Table 2: Gravida Status of Study Population

Gravida	Group I (n=50)		Group IIA (n=41)		Group IIB (n=9)		Group II (n=50)	
	No.	%	No.	%	No.	%	No.	%
1	24	48.00	20	48.78	4	44.44	24	48.00
2	15	30.00	7	17.07	1	11.11	8	16.00
3	9	18.00	8	19.51	2	22.22	10	20.00
4+	2	4.00	6	14.63	2	22.22	8	16.00
Statistical Significance (χ^2 test)	Group I, Group IIA & Group IIB				χ^2=6.379; p=0.382			
	Between Group I & Group II				χ^2=5.783; p=0.123			
	Between Group I & Group IIA				χ^2=4.485; p=0.214			
	Between Group I & Group IIB				χ^2=4.832; 'p'=0.184			
	Between Group IIA & Group IIB				χ^2=0.486; p=0.922			

Table No.2 shows Nearly half (48%) of the study population was primi-gravida. Intergroup comparison of Group I, Group IIA and Group IIB showed that proportion of Gravida 1 women was almost equal in above groups.

Table 3 : Gestation period status of Study Population

Period of Gestation	Group I (n=50)		Group IIA (n=41)		Group IIB (n=9)		Group II (n=50)	
	No.	%	No.	%	No.	%	No.	%
<32 wks.	16	32.00	5	12.20	7	77.78	12	24.00
32-35 wks.	30	60.00	23	56.10	1	11.11	24	48.00
>35 wks.	4	8.00	13	31.71	1	11.11	14	28.00
Statistical Significance (χ^2 test)	Group I, Group IIA & Group IIB				χ^2=22.635; p<0.001			
	Between Group I & Group II				χ^2=6.794; p=0.033			
	Between Group I & Group IIA				χ^2=10.665; p=0.005			
	Between Group I & Group IIB				χ^2=7.657; p=0.022			
	Between Group IIA & Group IIB				χ^2=17.455; p<0.001			

Table No.3 shows that Between Group comparison of Group I and Group II, period of gestation <32 weeks and 32-35 weeks was found in higher proportion of women of Group I as compared to Group II and period of gestation >35 weeks was found in higher proportion of Group II as compared to Group I and this difference was found to be statistically significant (p=0.033).

Table 4: Estriol levels (pg/ml) of Study group and control group

	Number of Subjects	Minimum ESTRIOL level	Maximum ESTRIOL level	Mean ESTRIOL level	Standard Deviation
Group I	50	1048.00	4022.00	2691.72	681.08
Group IIA	41	2227.00	4230.00	3512.85	586.16
Group IIB	9	1259.00	3902.00	2513.78	947.55
Statistical Significance	Group I, Group IIA & Group IIB (ANOVA)				F=19.606; p<0.001
	Between Group I & Group IIA (Student 't' test)				't'=6.088; p<0.001
	Between Group I & Group IIB (Student 't' test)				't'=0.678; p=0.500
	Between Group IIA & Group IIB (Student 't' test)				't'=4.111; p<0.001

Table No.4 shows that Estriol levels of Group IIA (3512.85±586.16 units) were found to be higher than that of Group I (2691.72±681.08 units) and Group IIB (2513.78±947.55 units) and this difference was found to be statistically significant (p<0.001).

Table 5: Estriol/Progesterone Ratio in Study group and control group

	Number of Subjects	Minimum level	Maximum level	Mean level	Standard Deviation
Group I	50	0.30	1.17	0.69	0.18
Group IIA	41	0.60	1.18	0.94	0.14
Group IIB	9	0.28	1.03	0.74	0.25
Statistical Significance	Group I, Group IIA & Group IIB (ANOVA)			F=23.886; p<0.001	
	Between Group I & Group IIA (Student 't' test)			't'=7.219; p<0.001	
	Between Group I & Group IIB (Student 't' test)			't'=0.660; p=0.512	
	Between Group IIA & Group IIB (Student 't' test)			't'=3.361; p=0.002	

Table No.5 shows that Estriol/Progesterone ratio of Group IIA was found to be higher (0.94±0.14) as compared to Group IIB (0.74±0.25) and Group I (0.69±0.18) and this difference was found to be statistically significant (F=23.886; p<0.001).

Table 6: Comparison of Estriol/Progesterone Ratio

	Number of Subjects	Minimum ESTRIOL/Progesterone ratio	Maximum ESTRIOL/Progesterone ratio	Mean ESTRIOL/Progesterone ratio	Standard Deviation
A)At Gestational age< 32 weeks					
Group I	16	0.30	0.92	0.65	0.18
Group II	12	0.28	1.16	0.84	0.26
Group IIA	5	0.87	1.16	0.98	0.11
Group IIB	7	0.28	1.03	0.74	0.29
Statistical Significance	Group I, Group IIA & Group IIB (ANOVA)			F=4.925; p=0.016	
	Between Group I & Group II (Student 't' test)			't'=2.346; p=0.027	
	Between Group I & Group IIA (Student 't' test)			't'=3.849; p=0.001	
	Between Group I & Group IIB (Student 't' test)			't'=0.973; p=0.342	
	Between Group IIA & Group IIB (Student 't' test)			't'=1.660; p=0.128	
	Number of Subjects	**Minimum ESTRIOL/Progesterone ratio**	**Maximum ESTRIOL/Progesterone ratio**	**Mean ESTRIOL/Progesterone ratio**	**Standard Deviation**
B)At Gestational age 32-35 weeks					
Group I	30	0.38	1.17	0.70	0.19
Group II	24	0.64	1.14	0.93	0.13
Group IIA	23	0.64	1.14	0.95	0.13
Group IIB	1	0.68	0.68	0.68	.

Statistical Significance	Group I, Group IIA & Group IIB (ANOVA)				F=14.734; p<0.001
	Between Group I & Group II (Student 't' test)				't'=5.130; p<0.001
	Between Group I & Group IIA (Student 't' test)				't'=0.5378; p<0.001
	Between Group I & Group IIB (Student 't' test)				't'=0.088; p=0.931
	Between Group IIA & Group IIB (Student 't' test)				't'=2.045; p=0.053
C) Comparison of Estriol/Progesterone Ratio					
	Number of Subjects	**Minimum ESTRIOL/Progesterone ratio**	**Maximum ESTRIOL/Progesterone ratio**	**Mean ESTRIOL/Progesterone ratio**	**Standard Deviation**
At Gestational age >35 weeks -<37 wks					
Group I	4	0.71	0.91	0.79	0.09
Group II	14	0.60	1.18	0.90	0.17
Group IIA	13	0.60	1.18	0.91	0.17
Group IIB	1	0.74	0.74	0.74	.
Statistical Significance	Group I, Group IIA & Group IIB (ANOVA)				F=1.357; p=0.287
	Between Group I & Group II (Student 't' test)				't'=1.240; p=0.233
	Between Group I & Group IIA (Student 't' test)				't'=1.375; p=0.189
	Between Group I & Group IIB (Student 't' test)				't'=0.517; p=0.641
	Between Group IIA & Group IIB (Student 't' test)				't'=0.998; p=0.338

Table 7: Comparison of various parameter for Estriol and Estriol to Progesterone

Parameters	Sensitivity	Specificity	PPV	NPV	ROC
Diagnostic Estimation of Estriol	75.6%	72.9%	68.9%	81.8%	81.5%
Estriol to Progesterone ratio	85.4%	66.1%	63.6%	86.7%	84.9%

Discussion:

Preterm delivery continues to be leading cause of perinatal morbidity and mortality throughout the world. Recent advances in perinatal health care have markers that facilitated more accurate prediction of preterm birth. The use of biological markers to enhance clinical approach in predicting preterm birth has been recently proposed. In the present study our aim was to determine whether saliva estriol and progesterone can be used as a predictor of preterm birth. Near term 90% estriol was produced in the placenta from fetal 16-α (OH) DHEAS. Spontaneous onset of labour at term & preterm is preceded by definite increase in free estriol in serum. Recently estriol level have been also studied in the saliva of pregnant women. Steroid in saliva correlate well with the unbound and free form of steroid in plasma.

Similarly progesterone which is secreted by placenta and its value in saliva also correlate well with free steroid in serum, fall in saliva progesterone with high E3 progesterone ratio may be associated with preterm labour.

Luisi *et al.* (1981) were first to measure progesterone level in saliva which showed good correlation with free serum progesterone [6]. Kundu *et at* (1983) measured estriol in saliva showed a very high correlation with free estriol in pregnant female and suggested estriol as a measure for assessment of fetoplacental function [5].

In this study we observed that saliva progesterone levels of pregnant women with preterm delivery (study group IIA) was lower than the control group (group l) but the difference was statistically not significant (3814.46± 751.14 pg/ml vs 3945.16± 577.11 pg/ml, p=0.351)

In study group IIB (patients who came with preterm labour pain and delivered at terms) the mean level of progesterone was 3588.67± 1022.60 pg/ml. This value is lower than the mean value of control group (3945.16± 577.11 pg/ml) but difference was not statistically significant (p>0.140)

A study by Lachelin *et al.* (2009) reported that saliva progesterone concentrations in women who delivered before 34 week following the spontaneous onset of preterm labour were significantly lower than those of the terms group (p=0.009) or those delivering after spontaneous onset between 34 and 37 wks of gestation (p=0.007) [7].

Another study by Klebanoff *et al* (2 008) conducted on 386 women made similar observation and reported low progesterone concentration associated with increased risk of preterm labour.[8]

In the present study the mean estriol level in control group was 2691.72±681.08 pg/ml and in study group II was 3333.02±759.87 pg/ml, which was higher than control group but the difference was not statistically significant (p=0.222)

Mean value of saliva estriol of study group IIA (3512.85±586.16 pg/ml) was higher as compared to the mean value of control group (2691.72±681.08 pg/ml) and difference was statistically significant (p value <0.001)

Similar observation was made by McGregor *et al.* (1995) they reported that detection of an early estriol surge or increased level (>2.3 ng/ml) may be clinically helpful in identifying women at elevated risk for preterm labour [9].

Another study of Heine *et al* (2000) in their study on 267 women reported elevated saliva estriol is associated with increased risk of preterm birth in asymptomatic women and symptomatic women who presented for evaluation of preterm labour (p value <0.005, RR=4)[10]. They observed a single positive (>2.1 ng/ml) salivary E3 test presented an increased risk of spontaneous preterm labour and delivery in the total, low risk and high risk population.[10]

In our study the mean of saliva estriol in study group II in gestational age <32 wks. was 2880.25± 1056.24 pg/ml which was significantly higher than mean value of estriol in control group I (2476.75± 577.11 pg/ml, p<0.001).The mean value of saliva estriol in group llA (3512.85± 586.16pg/ml) is significantly raised than control group (p <0.001). On comparing the mean value of group I (control group) and group llB (2343.57± 1000.01 pg/ml) no statistically significant difference was found.

The mean value of saliva estriol in group II in gestational age group 32-35 wks (3658.63±512.24 pg/ml) was significantly higher than mean value of control group (2727.3±412.66 pg/ml & p value <0.001) in the same gestational age.

The mean value of estriol in gestational age between >35wks and <37 wks were not significantly different in both study group and control group.

A study conducted by Lachelin *et al* (2009) shows saliva estriol in these gestational age group <34,b/w 34-37 wks> 37 wks but they found that there was no significant difference[7] in between different gestational age group.

Another study by Mcgregors *et al* (1995) conducted on 267 women shows estriol concentration were significantly higher from 24 to 34 wks in women with singleton pregnancies delivering preterm (p<0.05) [9]. They also reported a surge in estriol concentration approximately 3 weeks before in women delivering at term and preterm. This increase occurs approximately 4wks earlier in women delivering at preterm.

In present study mean value of estriol to progesterone ratio (E3/Prg ratio) in study group (0.91± 0.18) was significantly higher than the mean value of E3/ Prg ratio in control group (0.69± 0.18 p<0.001)

Lachelin *et al* (2009) reported that the E3/Prg ratio was significantly higher in the women who went into spontaneous labour and delivered before 34 wks than those delivered at term (p=0.047) [7]

In our study mean value of E3/Prg ratio in gestation age <32 wks group (0.84± 0.26) was significantly higher than mean value of group l (0.65± 0.18)

Mean value of estriol to progesterone ratio in gestational age group 32-35 wks in group II was 0.93± 0.13 was also significantly higher than the mean value of control group(0.65± 0.18) , p = 0.027. But the mean value in gestational age >35 wks-<37 wks in control group and study group were not significantly different.

A study by Lachelin (2009) reported E3/ Prg ratio was higher in the women who went into spontaneous labour and delivered before 34 wks of gestation than those delivering between 34 to 37 wks of gestational (p=0.041) and those at term (p-0.047) [7].

Another study by Darne *et al* (1987) shows, out of 23 women who went into preterm labour with intact membrane had a saliva estriol to progesterone ratio greater than 1 in every case and greater than 95th percentile for their length of gestation in 12 cases [11].

In present study the cut off value for E3 was ≥3107.50pg/ ml (3.1 ng/ml) with sensitivity and specificity of 75.6% and 72.9% respectively. In study of Heine *et al* (2000) cut off value of 2.1ng/ml was associated significantly with preterm birth in each

group (p≤0.05) with sensitivity of 57% and specificity of 78% in combined population , and sensitivity of 64%(lower than our study) and specificity of 68% (higher than our study)in high risk group[10] while a study of McGregor *et al* (1995) shows threshold of 2.3ng/ml for estriol with sensitivity 71% (lower than our study), specificity 77% (higher than our study), false-positive rate 23% .[9]

Conclusion

Salivary estriol and estriol/progesterone ratio can be used as a predictor for detecting symptomatic and asymptomatic women at risk for preterm birth. Whole saliva collected in non – invasive manner by individual with modest training including patient. Estriol/progesterone ratio is better screening method compared to estriol. Estriol/ Progesterone ratio has slightly high burden of false positive value but sensitivity is higher than E3. So further studies with larger sample are required to evaluate the accuracy of the saliva estriol and progesterone as a biochemical marker for identifying those symptomatic and who are at risk for preterm birth. However before salivary estriol or salivary estriol/progesterone ratio as diagnostic test, further investigation are necessary to evaluate its accuracy as a biochemical marker for predicting preterm delivery.

References:

1. Lockwood CL, Senyei AE, Dische MR, Casal D, Shah KD, Thung SN *et al.* Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery. N Engl J Med 1991; 325:669-674.
2. Villar J, Abalos, Lanoli G, Giordano D. Heterogeneity of perinatal outcomes in the preterm delivery syndrome. Obsetet Gynecol 2004; 104:78-87
3. Lumley J. Defining the problem the epidemiology of preterm birth. BJOG 2003; 110 Suppl 20:3-7.
4. Mc Garrigle HHG, Lachelin GCL. Increasing saliva (free) estriol to progesterone ratio in late pregnancy: a role for estriol in intiating spontaneous onset of labour at term. BMJ 1984;287:457-9
5. Kundu N, Novak N, Petersen LP (1983). Salivary unconjugated estriol levels in normal third trimester pregnancy–direct correlation with serum levels. Steroids 41:145-153.
6. Luisi M, Franchi F, Kicovic PM, Silvestri D, Cossu G, Catarsi AL, *et al.* (1981). Radioimmunoassay for progesterone in human saliva during the menstrual cycle. J Steroid Biochem 14:1069-1073.
7. Lachelin GCL, Mc Garrigle, Seed PL el al: Low saliva progesterone concentrations are associated with spontaneous every preterm labour (before 34 weeks of gestation) in women at increased risk of preterm delivery. British Journal of Obstetrics and Gynaecology2009;116:1515-1519
8. Klebanoff MA, Meis PJ, Dombrowski MP, Zhao Y, Moawad AH, Northen A, *et al.* Salivary progesterone and estriol among pregnant women treated with 17-a- hydroxyprogesterone caproate or placebo. Am J Obstet Gynecol 2008;199:506 e1–7. Saliva progesterone and preterm labour 2009.
9. McGregor JA, J, Jackson GM, Lacheln GCL, Goodwin TM, Artal R, Hastings C, *et al.* Salivary estriol as risk assessment for preterm labour: A prospective trial. Am J Obstet Gynecol 1995; 173:1337-42
10. Heine RP,McGeregor JA, Goodwin TM, el al. Serial Salivari Estriol to detect an increased risk of preterm birth .Am J Obstet Gynecol, 2000; vol. 96, no.1, pp. 490-497
11. Darne J, Mc Garrigle HHG, Lachelin GCL. Saliva estriol, estadiol, estrone and progesterone levels in pregnancy: spontaneous labour at term is preceded by arise in the saliva estrol progesterone ratio. Br J Obstet Gynaecol.