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COMPARATIVE EVALUATION OF SALIVARY ESTRIOL AND PROGESTERONE LEVELS IN WOMEN WITH PRETERM LABOR AND NORMAL PREGNANCY

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Abstract

Background: Preterm labour is a major obstetric complication and an important cause of neonatal morbidity and mortality. Estriol and progesterone play significant roles in maintaining pregnancy and regulating the onset of labour. Salivary estimation of these hormones offers a convenient, non-invasive approach for evaluating endocrine changes associated with preterm labour.

Aim: To determine the levels of salivary estriol and progesterone in women with preterm labour and normal pregnancy.

Material and Methods: A comparative observational study was designed involving 152 pregnant women divided into four groups, with 38 participants in each group: Group I, Group IIA, Group IIB and Group II. Demographic and obstetric characteristics, including maternal age, gravida status and gestational age, were evaluated. Salivary samples were collected under standardized conditions, and estriol and progesterone concentrations were estimated using enzyme-linked immunosorbent assay (ELISA). The results were compared using appropriate statistical methods, including one-way analysis of variance (ANOVA).

Results: The majority of participants belonged to the age group up to 25 years, and primigravida women constituted the largest proportion across all four groups. Regarding salivary progesterone, Group I demonstrated the highest mean concentration (3944.40 ± 635.56 pg/ml), followed by Group IIA (3812.98 ± 656.62 pg/ml), Group II (3772.30 ± 660.70 pg/ml) and Group IIB (3576.91 ± 773.86 pg/ml). The difference was not statistically significant ($F = 1.879$, $p = 0.136$). Salivary estriol concentrations were highest in Group IIA (3548.54 ± 633.69 pg/ml), followed by Group II (2680.53 ± 647.38 pg/ml), Group I (2647.74 ± 648.36 pg/ml) and Group IIB (2611.85 ± 969.76 pg/ml). The difference among groups was statistically significant ($F = 14.222$, $p < 0.001$).

Conclusion: Salivary estriol demonstrated significant differences among the four study groups, whereas salivary progesterone did not show statistically significant variation. These findings indicate the potential relevance of salivary estriol in investigating hormonal changes associated with preterm labour. Further studies are required to establish the clinical utility of these hormones as predictive biomarkers.

Keywords: Preterm labour, Salivary estriol, Salivary progesterone, Pregnancy, ELISA, Hormonal changes.

Introduction

Preterm labour is defined as the onset of regular uterine contractions accompanied by progressive cervical changes before 37 completed weeks of gestation. It remains one of the leading causes of neonatal morbidity and mortality worldwide. Infants born prematurely are at increased risk of respiratory distress syndrome, intraventricular haemorrhage, necrotizing enterocolitis, neurological impairment and long-term developmental complications. Despite advances in obstetric and neonatal care, prediction of spontaneous preterm labour remains a significant clinical challenge. Early identification of women at increased risk is essential for timely obstetric intervention and improvement of maternal and neonatal outcomes [1].

The initiation of normal labour involves complex hormonal and biochemical changes affecting the maternal, fetal and placental systems. Among the various hormones involved, estriol and progesterone play important roles in maintaining pregnancy and regulating the onset of labour. Progesterone helps maintain uterine quiescence by suppressing myometrial contractions, reducing inflammatory activity and maintaining cervical stability. Estriol, a major estrogen produced during pregnancy, reflects fetoplacental endocrine activity and increases progressively with advancing gestation. Changes in the balance between these hormones may contribute to the physiological processes responsible for the initiation of labour [2,3].

Salivary hormone estimation has emerged as a convenient, non-invasive method for assessing hormonal changes during pregnancy. Unlike serum sampling, saliva collection is simple, painless and suitable for repeated measurements. Salivary estriol represents the biologically available fraction of circulating estrogen and may reflect changes in fetoplacental function. Previous studies have demonstrated that increasing salivary estriol concentrations and alterations in the estriol-to-progesterone ratio may precede the onset of spontaneous labour [4-6].

Progesterone concentrations also undergo physiological changes during pregnancy and are considered important in maintaining uterine relaxation. Reduced salivary progesterone levels have been associated with an increased risk of spontaneous preterm delivery, particularly among women with additional risk factors. Studies evaluating salivary progesterone and estriol have suggested that these hormones may provide useful information regarding endocrine changes associated with premature activation of the parturition process [7,8]. Furthermore, an increasing salivary estriol-to-progesterone ratio during late pregnancy supports the potential role of hormonal balance in the initiation of labour [9,10].

The comparative assessment of salivary estriol and progesterone in women with preterm labour and normal pregnancy may provide valuable information regarding hormonal alterations associated with premature onset of labour. Salivary hormone estimation may also offer a convenient approach for investigating endocrine changes during pregnancy without requiring invasive blood collection. Therefore, the present study was undertaken to determine and compare salivary estriol and progesterone levels in women with preterm labour and normal pregnancy across different study groups.

Material and Methods

The present comparative observational study was designed to evaluate salivary estriol and progesterone levels in pregnant women with preterm labour and normal pregnancy. A total of 152 pregnant women were included in the study and divided into four groups, with 38 participants in each group. Group I consisted of 38 women with preterm labour, Group IIA included 38 women belonging to the first subgroup of the study population, Group IIB comprised 38 women belonging to the second subgroup,

and Group II consisted of 38 women with normal pregnancy serving as the comparison group. Participants were categorized according to predefined obstetric and clinical criteria.

Relevant demographic and obstetric details, including maternal age, gestational age, parity, obstetric history and presenting complaints, were recorded for all participants. Women with multiple pregnancies, known endocrine disorders, pregnancy-related medical complications or current hormonal therapy that could influence steroid hormone concentrations were excluded according to the predefined eligibility criteria. Written informed consent was obtained from all participants before enrolment and sample collection.

Unstimulated whole saliva samples were collected from all participants under standardized conditions, preferably during morning hours to minimize variations associated with sampling time. Participants were instructed to avoid eating, drinking, smoking and oral hygiene procedures for at least 30 minutes before sample collection. Saliva samples were collected in sterile, properly labelled containers, ensuring that samples were free from visible blood contamination. The collected samples were appropriately stored and transported under controlled conditions until biochemical analysis.

Salivary estriol and progesterone concentrations were estimated using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Hormone concentrations were recorded in appropriate units, and the estriol-to-progesterone ratio was calculated wherever applicable. The results obtained from the four groups were compared to identify variations in salivary hormone concentrations associated with preterm labour and normal pregnancy.

The collected data were systematically tabulated and analysed using appropriate statistical methods. Continuous variables were expressed as mean and standard deviation, whereas categorical variables were presented as frequencies and percentages. Comparisons among the four groups were performed using one-way analysis of variance (ANOVA) or an appropriate non-parametric test, depending on the distribution of data. Post-hoc analysis was performed wherever required to identify differences between individual groups. A p-value of less than 0.05 was considered statistically significant.

Results

Table 1 demonstrates the age-wise distribution of the study population across the four groups. In Group I, the majority of participants (52.6%) belonged to the age group up to 25 years, followed by 39.5% in the 26–30 years age group and 7.9% in the 30–35 years age group. Similarly, in Group IIA, 50% of participants were aged up to 25 years, 28.9% belonged to the 26–30 years age group and 21.1% were in the 30–35 years age group. In Group IIB, the maximum proportion (65.8%) belonged to the age group up to 25 years, followed by 21.1% in the 26–30 years group and 13.2% in the 30–35 years group. In Group II, 52.6% of participants were aged up to 25 years, 28.9% belonged to the 26–30 years group and 18.4% were aged 30–35 years. Overall, the younger age group constituted the largest proportion of participants in all four groups.

Table 2 illustrates the distribution of participants according to gravida status. In Group I, primigravida women constituted the largest proportion (47.4%), followed by gravida 2 (28.9%), gravida 3 (18.4%) and gravida 4 or above (5.3%). In Group IIA, 50% of participants were primigravida, while 15.8% belonged to gravida 2, 18.4% to gravida 3 and 15.8% to gravida 4 or above. In Group IIB, primigravida women accounted for 44.7% of cases, followed by 10.5% with gravida 2, 21.1% with gravida 3 and 23.7% with gravida 4 or above. In Group II, 47.4% were primigravida, 15.8% belonged to gravida 2, 21.1% to gravida

3 and 15.8% to gravida 4 or above. Thus, primigravida women represented the predominant category across all four groups.

Table 3 shows the distribution of participants according to period of gestation. In Group I, the majority of women (60.5%) were between 32 and 35 weeks of gestation, while 31.6% were below 32 weeks and 7.9% were above 35 weeks. In Group IIA, 55.3% of participants belonged to the 32–35 weeks category, 13.2% were below 32 weeks and 31.6% were above 35 weeks. In Group IIB, the majority (78.9%) were below 32 weeks, whereas 10.5% belonged to each of the remaining gestational-age categories. In Group II, 47.4% of women were between 32 and 35 weeks, followed by 28.9% above 35 weeks and 23.7% below 32 weeks. The distribution indicates variation in gestational age among the four study groups.

Table 4 demonstrates the comparison of salivary progesterone levels among the four study groups. The mean salivary progesterone concentration was highest in Group I, measuring 3944.40 ± 635.56 pg/ml. Group IIA showed a mean concentration of 3812.98 ± 656.62 pg/ml, while Group II demonstrated a mean value of 3772.30 ± 660.70 pg/ml. The lowest mean progesterone concentration was observed in Group IIB, measuring 3576.91 ± 773.86 pg/ml. One-way analysis of variance revealed an F-value of 1.879 with a p-value of 0.136, indicating that the difference in mean salivary progesterone levels among the four groups was not statistically significant.

Table 5 illustrates the comparison of salivary estriol levels among the four study groups. The highest mean salivary estriol concentration was observed in Group IIA (3548.54 ± 633.69 pg/ml). Group II showed a mean concentration of 2680.53 ± 647.38 pg/ml, followed by Group I with 2647.74 ± 648.36 pg/ml. The lowest mean concentration was recorded in Group IIB (2611.85 ± 969.76 pg/ml). Statistical analysis using one-way ANOVA demonstrated an F-value of 14.222 with a p-value of less than 0.001, indicating a statistically significant difference in salivary estriol concentrations among the four groups.

Table 6 demonstrates the comparison of salivary progesterone and estriol concentrations according to gestational age across the four study groups. Among participants below 32 weeks of gestation, Group I showed mean progesterone and estriol concentrations of 3900.00 pg/ml and 2476.75 pg/ml, respectively. In Group IIA, the corresponding values were 3750.00 pg/ml and 3631.60 pg/ml, whereas Group IIB showed values of 3365.29 pg/ml and 2343.57 pg/ml. Group II demonstrated mean concentrations of 3525.33 pg/ml for progesterone and 2880.25 pg/ml for estriol.

Among women between 32 and 35 weeks of gestation, Group I demonstrated mean progesterone and estriol concentrations of 3940.00 pg/ml and 2691.00 pg/ml, respectively. Group IIA showed values of 3963.17 pg/ml and 3512.85 pg/ml, while Group IIB demonstrated concentrations of 4019.00 pg/ml and 2513.78 pg/ml. In Group II, the corresponding values were 3965.50 pg/ml and 2691.00 pg/ml.

In women above 35 weeks of gestation, Group I showed mean progesterone and estriol concentrations of 4155.73 pg/ml and 3000.04 pg/ml, respectively. Group IIA demonstrated corresponding values of 3576.39 pg/ml and 3576.39 pg/ml. Group IIB showed concentrations of 4721.97 pg/ml and 4722.02 pg/ml, while Group II demonstrated mean values of 3658.22 pg/ml and 2500.00 pg/ml. Overall, the findings demonstrate variations in salivary progesterone and estriol concentrations across different gestational-age categories and study groups.

Table 1: Age-wise distribution of study population

Age (years)	Group I (n=38)	Group IIA (n=38)	Group IIB (n=38)	Group II (n=38)
Up to 25	20 (52.6%)	19 (50.0%)	25 (65.8%)	20 (52.6%)

26–30	15 (39.5%)	11 (28.9%)	8 (21.1%)	11 (28.9%)
30–35	3 (7.9%)	8 (21.1%)	5 (13.1%)	7 (18.5%)
Total	38 (100%)	38 (100%)	38 (100%)	38 (100%)

Table 2: Distribution of study population according to gravida status

Gravida	Group I (n=38)	Group IIA (n=38)	Group IIB (n=38)	Group II (n=38)
1	18 (47.4%)	19 (50.0%)	17 (44.7%)	18 (47.4%)
2	11 (28.9%)	6 (15.8%)	4 (10.5%)	6 (15.8%)
3	7 (18.4%)	7 (18.4%)	8 (21.1%)	8 (21.1%)
4+	2 (5.3%)	6 (15.8%)	9 (23.7%)	6 (15.8%)
Total	38 (100%)	38 (100%)	38 (100%)	38 (100%)

Table 3: Distribution according to period of gestation

Period of gestation	Group I (n=38)	Group IIA (n=38)	Group IIB (n=38)	Group II (n=38)
Less than 32 weeks	12 (31.6%)	5 (13.2%)	30 (78.9%)	9 (23.7%)
32–35 weeks	23 (60.5%)	21 (55.3%)	4 (10.5%)	18 (47.4%)
More than 35 weeks	3 (7.9%)	12 (31.6%)	4 (10.5%)	11 (28.9%)
Total	38 (100%)	38 (100%)	38 (100%)	38 (100%)

Table 4: Comparison of salivary progesterone levels among study groups

Group	No. of subjects	Mean progesterone (pg/ml)	SD
Group I	38	3944.40	635.56
Group IIA	38	3812.98	656.62
Group IIB	38	3576.91	773.86
Group II	38	3772.30	660.70
ANOVA	152	F = 1.879	p = 0.136

Table 5: Comparison of salivary estriol levels among study groups

Group	No. of subjects	Mean estriol (pg/ml)	SD
Group I	38	2647.74	648.36
Group IIA	38	3548.54	633.69
Group IIB	38	2611.85	969.76
Group II	38	2680.53	647.38
ANOVA	152	F = 14.222	p < 0.001

Table 6: Comparison of salivary progesterone and estriol levels according to gestational age

Gestational age	Group	No. of subjects	Mean progesterone (pg/ml)	Mean estriol (pg/ml)
Less than 32 weeks	Group I	12	3900.00	2476.75
	Group IIA	5	3750.00	3631.60

	Group IIB	30	3365.29	2343.57
	Group II	9	3525.33	2880.25
32–35 weeks	Group I	23	3940.00	2691.00
	Group IIA	21	3963.17	3512.85
	Group IIB	4	4019.00	2513.78
	Group II	18	3965.50	2691.00
More than 35 weeks	Group I	3	4155.73	3000.04
	Group IIA	12	3576.39	3576.39
	Group IIB	4	4721.97	4722.02
	Group II	11	3658.22	2500.00

Discussion

Preterm labour is a major obstetric concern and remains an important contributor to perinatal morbidity and mortality. The hormonal changes associated with the initiation of labour involve a complex interaction between maternal, fetal and placental endocrine mechanisms. Among the various hormonal markers investigated, estriol and progesterone have received considerable attention because of their potential roles in maintaining pregnancy and regulating the onset of parturition. Salivary estimation of these hormones offers a non-invasive approach for evaluating endocrine changes during pregnancy.

In the present analysis, the age-wise distribution demonstrated that women aged up to 25 years constituted the largest proportion of participants across all four groups. Group IIB showed the highest proportion of women in this age category (65.8%), followed by Group I (52.6%) and Group II (52.6%). The distribution of maternal age is an important consideration while evaluating pregnancy outcomes, as maternal characteristics may influence obstetric risk. Similarly, the gravida-wise distribution showed that primigravida women constituted the largest category in all four groups, accounting for 47.4% in Group I, 50% in Group IIA, 44.7% in Group IIB and 47.4% in Group II. These observations indicate that both primigravida and multigravida women were represented in the study population.

The distribution according to gestational age demonstrated differences among the four groups. In Group I, the majority of participants (60.5%) belonged to the 32–35 weeks gestational-age category. In Group IIB, however, 78.9% of participants were below 32 weeks of gestation. The variation in gestational age is particularly relevant when interpreting hormonal concentrations because both estriol and progesterone undergo physiological changes as pregnancy advances. Vining et al. [11] investigated salivary estriol measurements as an alternative to serum unconjugated estriol assessment and highlighted the potential usefulness of salivary hormone estimation in evaluating fetoplacental function. These observations support the importance of considering gestational age while interpreting salivary estriol concentrations.

The comparison of salivary progesterone levels revealed that Group I had the highest mean concentration (3944.40 ± 635.56 pg/ml), followed by Group IIA (3812.98 ± 656.62 pg/ml) and Group II (3772.30 ± 660.70 pg/ml). The lowest mean concentration was observed in Group IIB (3576.91 ± 773.86 pg/ml).

Although variations were observed among the groups, the difference was not statistically significant (F = 1.879, p = 0.136). This suggests that the observed differences in salivary progesterone concentrations were insufficient to establish a statistically significant distinction among the four groups in this analysis. The role of progesterone in maintaining pregnancy is well established. It contributes to uterine quiescence by reducing myometrial contractility and suppressing inflammatory pathways associated with labour. A decline in progesterone activity, particularly at the maternal-fetal interface, has been proposed as one of the mechanisms involved in the initiation of parturition. However, circulating progesterone concentrations do not necessarily reflect the local functional withdrawal of progesterone activity. Choe et al. [12] examined progesterone and estradiol concentrations in saliva and plasma during the menstrual cycle, demonstrating the relevance of salivary steroid hormone assessment. Their work provides a physiological basis for investigating salivary reproductive hormones, although its findings cannot be directly extrapolated to preterm labour.

The comparison of salivary estriol concentrations demonstrated a more pronounced variation among the study groups. Group IIA exhibited the highest mean estriol concentration (3548.54 ± 633.69 pg/ml), followed by Group II (2680.53 ± 647.38 pg/ml) and Group I (2647.74 ± 648.36 pg/ml). Group IIB demonstrated the lowest mean concentration (2611.85 ± 969.76 pg/ml). The overall difference among the groups was statistically significant (F = 14.222, p less than 0.001). This finding indicates that salivary estriol concentrations differed significantly across the four study groups and may reflect variations in fetoplacental endocrine activity.

The biological significance of estriol during pregnancy is related to its production through the fetoplacental unit. Maternal estriol concentrations generally increase with advancing gestation and reflect fetal adrenal activity, placental metabolism and maternal-fetal endocrine interactions. Kundu et al. [10] investigated salivary unconjugated estriol concentrations during normal third-trimester pregnancy and reported a relationship between salivary and serum concentrations. Similarly, Purba [13] examined salivary estriol levels in relation to preterm delivery and highlighted the potential relevance of estriol estimation in evaluating premature birth. These studies provide a basis for further investigating salivary estriol as a possible marker of pregnancy-related endocrine changes.

Vining et al. [11] also demonstrated the potential application of salivary estriol measurements in assessing fetoplacental function. The findings of the present analysis, particularly the statistically significant variation in estriol concentrations, support the importance of further evaluating this hormone in relation to preterm labour. However, the higher estriol concentration observed in Group IIA should be interpreted in the context of its clinical classification and gestational-age distribution rather than being considered an independent predictor of preterm delivery.

The gestational-age-wise comparison further demonstrated variations in progesterone and estriol concentrations across the study groups. Among women below 32 weeks, Group IIA showed a relatively higher mean estriol concentration, whereas Group IIB demonstrated comparatively lower progesterone and estriol concentrations. In the 32–35 weeks category, Group IIA continued to show higher estriol concentrations than the other groups. Among women above 35 weeks, Group IIB demonstrated comparatively higher mean progesterone and estriol concentrations. These variations suggest that gestational age and the underlying obstetric characteristics of participants should be considered when evaluating hormonal differences. McGarrigle and Lachelin [9] discussed the increasing salivary estriol-to-progesterone ratio during late pregnancy and its possible relationship with the initiation of spontaneous

labour. Their observations provide a physiological explanation for investigating the relative changes in these hormones rather than considering their concentrations independently.

The relationship between progesterone and estriol is particularly important because labour initiation involves alterations in the balance between estrogenic and progestational activity. An increase in estrogenic influence may contribute to increased myometrial responsiveness, cervical remodelling and the development of uterine contractions. A relative reduction in progesterone activity may further facilitate these processes. The combined assessment of salivary estriol and progesterone may therefore provide additional information regarding the endocrine environment associated with pregnancy maintenance and labour initiation. Reference 14, concerning the role of the salivary estriol-to-progesterone ratio in predicting preterm labour, is relevant to this aspect of the present investigation.

Overall, the present analysis demonstrates that salivary estriol exhibited statistically significant differences among the four groups, whereas salivary progesterone did not show a statistically significant overall difference. These findings suggest that estriol may be a useful hormone for further investigation in relation to preterm labour. Nevertheless, hormonal concentrations are influenced by gestational age, individual physiological variation and obstetric characteristics. Further studies involving clearly defined clinical subgroups, gestational-age matching, serial saliva sampling and larger patient populations are necessary to establish the diagnostic and predictive value of these hormonal markers.

Conclusion

The present study examined salivary estriol and progesterone concentrations among pregnant women with different obstetric characteristics and normal pregnancy. Salivary estriol demonstrated statistically significant differences among the four study groups, whereas progesterone concentrations did not show a statistically significant overall variation. Gestational-age-wise comparisons also demonstrated differences in hormonal concentrations, highlighting the importance of considering the period of gestation during hormonal assessment. Salivary estriol may have potential as a non-invasive biochemical marker for investigating endocrine changes associated with preterm labour. However, its clinical usefulness, either independently or in combination with progesterone, requires further evaluation through appropriately designed prospective studies.

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