sciendo

ENDOCRINE REGULATIONS, Vol. 58, No. 4, 225–232, 2024

doi:10.2478/enr-2024-0027

Hormonal biomarkers and preterm birth: insights from a study of pregnant women in Lahore, Pakistan

Sundas Akram [1], Shaaf Ahmad [2], Kaleem Maqsood [1], Javeria Malik [1], Muhammad Amir Iqbal [1], Husna Ahmad [1], Nabila Roohi [1]

[1]Institute of Zoology, University of the Punjab, Lahore, Punjab, Pakistan; [2]King Edward Medical University, Mayo Hospital, Lahore, Punjab, Pakistan
E-mail: nabila.zool@pu.edu.pk

Objective. Reduced calciferol (vitamin D) levels in pregnant women have been associated with an increased risk to infant health. Progesterone sustains pregnancy and reduces the risk of premature birth through its metabolites affecting myometrial contractility. Sex hormone-binding globulin protein (SHBG) is a biomarker of premature birth. The present study aimed to find out if early pregnancy levels of vitamin D, SHBG, and progesterone metabolites may predict preterm birth risk.

Methods. Five hundred pregnant women aged 18–43 years during their 2nd and 3rd trimesters from multiple civilian regional medical centers in Lahore participated in the study. Blood samples taken from participants were used to determine vitamin D, SHBG, 11-deoxycorticosterone (DOC), and 16α-hydroxyprogesterone (16α-OHP) levels using specific ELISA kits. Statistical analysis was performed by one-way ANOVA using the latest GraphPad Prism software.

Results. A significant decrease in vitamin D, DOC, and SHBG levels ($p<0.001$, $p<0.001$, and $p<0.05$, respectively) in the preterm birth cohorts in the 2nd and 3rd trimester was found compared to the corresponding control groups. Furthermore, 16α-OHP levels in the preterm birth cohorts in the 2nd and 3rd trimesters were significantly increased ($p<0.001$ and $p=0.0062$, respectively) compared to their control cohorts.

Conclusion. The results of our study confirm that calciferol deficiency in pregnant women is associated with an increased risk of premature birth and indicate that SHBG and progesterone metabolites may be useful biomarkers for the early identification and prediction of preterm birth.

Keywords: 11-deoxycorticosterone, vitamin D, preterm birth, SHBG, 16α-hydroxyprogesterone

Neonatal Intensive Care Unit (NICU) births are linked to a higher incidence of neonatal syndromes and significantly increased healthcare costs due to the need for specialized, intensive care. Decreased calciferol levels have been associated with adverse pregnancy disorders (Dror and Allen 2010). Calciferol controls mineral regulation and bone homeostasis. It is a lipid-soluble molecule functioning as a precursor hormone. Humans can produce calciferol in the presence of appropriate sunlight, and its deficiency is responsible for many diseases, such as immune system dysfunction, insulin signaling dysfunction, and cancer (Heaney 2008; Perez-Lopez et al. 2011).

In 2016, as reported by UNICEF, 34.8% of early childhood deaths occurred internationally as a result of preterm birth (PTB) (Dror and Allen 2010). The genesis of PTB is polyfactorial and complex. Myometrium infection, over-distension of uterine tissues, asymmetrical stimulation of evolving young infants, endocrine signaling cascade, and inflammatory reactions are predisposing issues (Romero et al. 1994). This analysis was planned to find the

Corresponding author: Prof. Dr. Nabila Roohi, Institute of Zoology, University of the Punjab, Lahore - 54590, Pakistan; phone: 03334053953; e-mail: nabila.zool@pu.edu.pk, director.zool@pu.edu.pk.

correlation of PTB with calciferol levels in pregnant women to clarify the purpose of calciferol supplementation as an economical and modest practice to reduce the risk of PTB in the Pakistani community.

Recently, sex hormone-binding globulin protein (SHBG) has been acknowledged as a prognostic biomarker of PTB. In the course of pregnancy, the level of SHBG progressively increases in maternal blood, but in the case of low gestational age birth, the concentration of this protein falls sharply. SHBG provides insight into the status of the immune response and structural integrity of the supporting tissues of the fetal membrane in diagnostically asymptomatic pregnant women (Savitz et al. 2005; Saade et al. 2016).

During the gestational period, the baby's pituitary gland provides the primary stimulus for birth. The adrenal gland of the fetus produces corticosteroids under the control of adrenocorticotropic hormone (ACTH) from the fetus's master gland, which comes into the maternal circulation and reduces the progesterone level. A decrease in progesterone concentration stimulates oxytocin release from the maternal posterior lobe of the pituitary gland and produces a contraction of the endometrium wall (labor pain) (Mastorakos and Ilias 2003). Progesterone has been used to reduce the threat of PTB by maintaining pregnancy. Progesterone is catabolized into numerous metabolites as well as steroid hormones, like mineralocorticoids (aldosterone) and glucocorticoids. Progesterone has beneficial effects on gestation; however, the effects of its metabolites can be diverse. Progesterone metabolic chemical variations (6β-hydroxyprogesterone, 6β-OHP, and 16α-hydroxyprogesterone, 16α-OHP) affect the oxytocin-stimulated endometrium contraction (Patil et al. 2015). In the same way, higher glucocorticoid (cortisol) concentration has been associated with PTB. Steroid hormone pathway imbalance is associated with an increased risk of extremely/very preterm birth (e/vPTB) and may specify maternal stress hormone profile (Sandman et al. 2006).

A chronic aspect associated with spontaneous PTB comprises the placental-adrenal axis (fetal/maternal HPA axis) (Sandman et al. 2006). Gestational sac production of corticotropin-releasing hormone (CRH) exists as a systematic means of allowing prenatal stressors to affect the pregnancy (Sandman 2018). Gestational stress is considered to be the main cause of almost 59% of PTBs (Manuck et al. 2015). Fetal/maternal neuroendocrine axis stimulation has been interrelated with the gestational stress responses and results in a molecular cascade, triggering premature delivery. Through the fetal/maternal cortisol axis, hormonal stimuli control the regulation of PTB. It has previously been established that mainly 16α-OHP and 11-deoxycorticosterone (DOC), while measured at the beginning of the 2nd trimester, allow the rapid identification of pregnant women at risk of vPTB (Patil et al. 2020).

A baby born before the 32nd week of the prenatal period shows a deficiency of corticosteroids, which are crucial for the control of ionized substances and H_2O levels in the renal organs. Corticosteroid deficiency has been attributed to a higher risk of neonatal death and neonatal lung disease. Decreased steroid chemical messengers have been associated with adrenocortical insufficiency, which is adversely associated with the fetal stage of development. Endogenous corticosteroids have been documented as essential promoters of the genetic manifestation of CRH in pregnancy tissues (Whittle et al. 2001; Wang et al. 2013), while progesterone reduces this effect.

Glucocorticoids and 16α-OHP have remained associated with antagonistic effects on myometrium muscle fiber contraction. Progesterone biomolecules have been discovered to have inconsistent efficiency in reducing uterine contraction, while some bioactive molecules, contrasting with progesterone, increase contractility (Patil et al. 2015). Increased blood cortisol level at the beginning of the 2nd stage of gestation was associated with an increased CRH concentration in the 3rd term and PTB (Sandman et al. 2006). However, another study failed to determine that CRH predicted PTB while it was quantified at the mid-stage of gestation (Sibai et al. 2005).

The purpose of the present study was to reveal whether variations in serum vitamin D levels of pregnant women are associated with an increased risk of PTB. In addition, the study aimed to estimate progesterone derivatives and SHBG as potential predictive biomarkers of PTB.

Material and Methods

Sample collection. This study was endorsed by the Research Ethics Board (REB) of Punjab University, Lahore. Five hundred pregnant females were recruited from various primary care hospitals in Lahore, Pakistan (Sheikh Zaid, Jinnah, and Lady Wellington Medical Center) during their prenatal consultation in the 2nd and 3rd term of pregnancy. Participants or their relatives signed a voluntary agreement form clearly describing the aims of the research. All participants were asked about age, pregnancy, previous medical

history, parity, miscarriage history, miscarriages, PTB genetic background, and last menstrual period (LMP) date.

Exclusion criteria. The participants aged <18 and >43 years were not included in the study. Females with complications like miscarriages, hepatitis, polycystic ovarian syndrome, carcinoma, uterine fibroids, and further ambiguous abnormalities were also omitted.

Study cohorts. As mentioned above, 500 pregnant women were recruited at the beginning of the study. Blood samples were collected during their first visit. All participants were monitored until they delivered and their gestational length was documented after delivery.

The initial cohort of 500 participants was divided into the PTB and reference (control) groups based on the delivery outcomes. One hundred and eighty-six participants included in the PTB cohorts were those who experienced PTBs, defined as delivery before 37 weeks of gestation. The remaining 314 participants had full-term deliveries and were placed in the reference cohort. There were no significant differences between the included and excluded participants in terms of baseline characteristics such as age, body mass index (BMI), or socio-economic status, ensuring that the groups were comparable.

Out of 500 subjects, 186 pregnant women who gave birth before the estimated due date (EDD) were chosen and distributed into the 2nd (n=111) and 3rd trimester (n=75) cohorts. Furthermore, PTB females were divided into mPTB (≤32 prenatal weeks) (n=106) and vPTB (≤28 prenatal weeks) (n=80) cohorts. Of the remaining 314 women who gave birth at full term, 140 were nominated as the reference cohort. Out of 140 subjects, 70 pregnant females were 13–26 weeks pregnant at the time of blood collecting and classified as control I, and 70 were 27–32 weeks pregnant and treated as control II.

Serum collection. About 5 ml of venous blood was taken from the participants. Centrifugation of all specimens was completed for 10 min at 3500 rpm. Eppendorf tubes were utilized and tagged for serum storage with date and sample ID. Sera were stored at –80 °C.

Sample analysis. Maternal serum mid-gestational biomarkers (16α-OHP, SHBG, vitamin D, and DOC) were assessed with commercially accessible ELISA kits (Glory BioScience, China).

Statistical analysis. The results found were presented as mean±SEM. One-way ANOVA performed the quantitative examination to compare the outcomes in different cohorts. A p-value <0.05 was considered statistically significant.

Results

A comparison of the endocrine profile (vitamin D, SHBG, DOC, and 16α-OHP levels) of individual groups, moderate preterm birth (mPTB) cohorts, vPTB cohorts, and reference cohorts in the 2nd and 3rd gestational period is shown in Table 1.

Calciferol (vitamin D). Calciferol levels in the PTB cohorts in the 2nd and 3rd gestational periods showed a significant decrease (p<0.0001 and p=0.0008, respectively) compared to their reference groups (Table 1). In the 2nd trimester, the concentration of calciferol in the mPTB and vPTB cohorts was significantly reduced (p<0.05 and p=0.001, respectively) compared to the control-I group. In addition, a significantly lower calciferol level (p<0.05) was also observed in the vPTB cohort compared to the mPTB cohort (Figure 1A). In the 3rd trimester, there was a

Table 1
Cumulative endocrine profile association across study cohorts

Parameter	Trimester	Mean ± SEM			p-value
		Reference (Control)	m-PTB	v-PTB	
Vitamin D (ng/ml)	2nd	32.490±0.756	27.66±1.80	22.670±1.432	<0.0001
	3rd	29.35±0.98	25.61±0.978	23.47±1.09	0.0008
SHBG (pg/ml)	2nd	51.62±2.528	48.57±2.599	42.08±2.634	0.0346
	3rd	52.89±2.207	45.74±2.028	43.95±1.972	0.0129
11-DOC (pg/ml)	2nd	51.65±1.11	47.47±1.258	40.49±1.616	<0.0001
	3rd	54.3±1.161	45.89±1.541	39.46±2.127	<0.0001
16α-OHP (pg/ml)	2nd	8.123±0.189	9.044±0.252	9.812±0.323	<0.0001
	3rd	8.077±0.254	8.708±0.308	9.79±0.601	0.0062

Abbreviations: 11-DOC – 11-deoxycorticosterone; 16α-OHP – 16α-hydroxyprogesterone; m-PTB – moderate preterm birth; SHBG – sex hormone-binding globulin; v-PTB – very preterm birth.

significant decline in calciferol concentration in the mPTB and vPTB cohorts ($p<0.05$ and $p<0.01$, respectively) compared to the control-II (Figure 1B).

Sex hormone binding globulin (SHBG). SHBG levels in the PTB cohorts in the 2nd and 3rd trimesters were lowered ($p=0.034$ and $p=0.013$, respectively) compared to their reference cohorts (Table 1). In the 2nd trimester, SHBG concentrations were significantly decreased ($p<0.05$) only in the vPTB cohort compared to the control-I group (Figure 2A). In contrast, a significant reduction of SHBG levels in both mPTB and vPTB cohorts in the 3rd trimester was observed compared with the control-II group (Figure 2B).

11-deoxycorticosterone (DOC). Concentrations of DOC in the PTB cohorts in the 2nd and 3rd gestational periods showed a significant decrease ($p<0.001$) compared to their reference cohorts (Table 1). In the 2nd trimester, significantly lower levels of DOC were measured in the vPTB cohorts compared to the control-I group and the mTPB cohorts ($p<0.001$ and $p<0.01$, respectively) (Figure 3A). In the 3rd trimester, the DOC concentration was significantly decreased in both mPTB and vPTB cohorts ($p<0.001$) compared to the control-II group and in the vPTB cohorts ($p<0.05$) compared to the mPTB cohorts (Figure 3B).

16α-hydroxyprogesterone (16α-OHP). Levels of 16α-OHP in both PTB cohorts in the 2nd and 3rd trimesters were significantly increased ($p<0.0001$ and $p=0.0062$, respectively) compared to their reference cohorts (Table 1). A significant increase in 16α-OHP concentration was found in the mPTB and vPTB cohorts ($p<0.05$ and $p<0.001$, respectively) in the 2nd trimester compared to the control-I group (Figure 4A) and in the vPTB cohorts ($p<0.01$) in the 3rd trimester compared to the control-II group (Figure 4B).

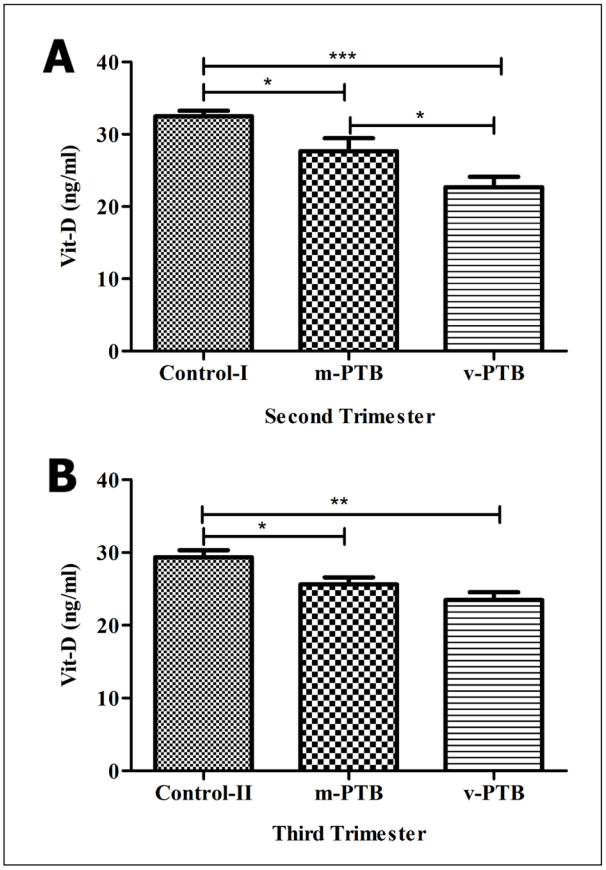

Figure 1. Vitamin D (ng/ml) concentrations in the reference (control-I and control-II), moderate preterm birth (mPTB) and very preterm birth (vPTB) cohorts in the second **(A)** and the third **(B)** trimester of pregnancy. Data are presented as mean±SEM. *p<0.05; **p<0.01; ***p<0.001.

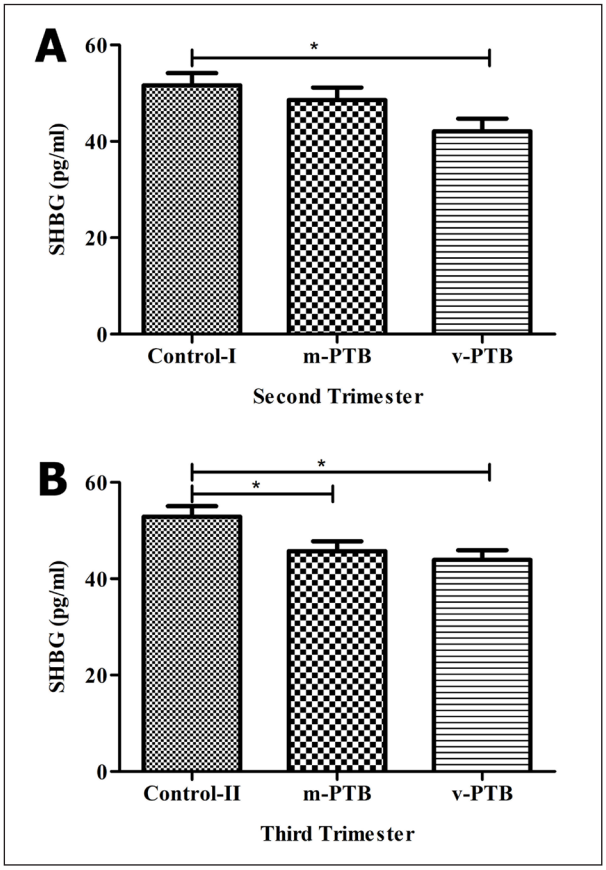

Figure 2. Sex hormone binding globulin protein (SHBG) concentrations (pg/ml) in the reference (control-I and control-II) groups, moderate preterm birth (mPTB) and very preterm birth (vPTB) cohorts in the second **(A)** and the third **(B)** trimester of pregnancy. Data are presented as mean±SEM. *p<0.05; **p<0.01; ***p<0.001.

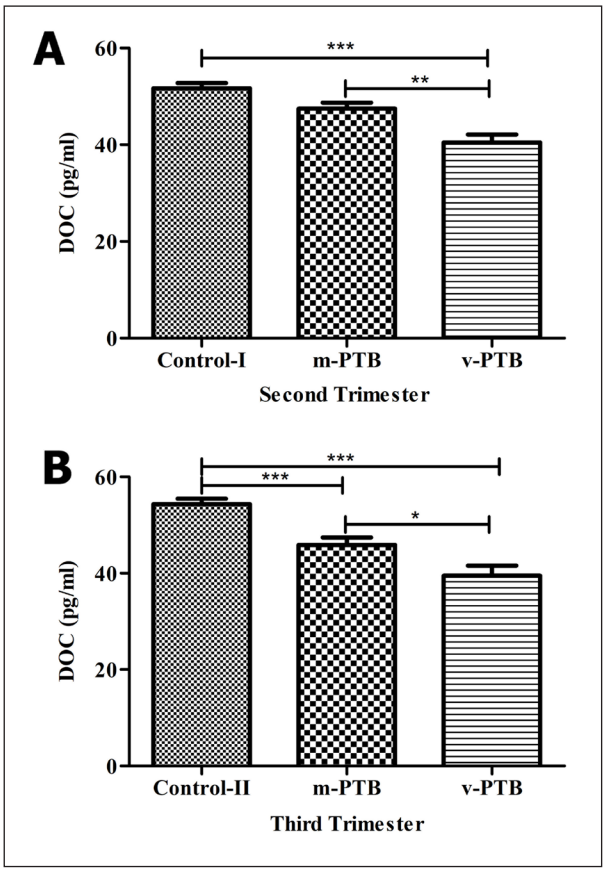

Figure 3. 11-deoxycorticosterone (DOC) concentrations (pg/ml) in the reference (control-I and control-II) groups, moderate preterm birth (mPTB) and very preterm birth (vPTB) cohorts in the second **(A)** and the third **(B)** trimester of pregnancy. Data are presented as mean±SEM. *p<0.05; **p<0.01; ***p<0.001.

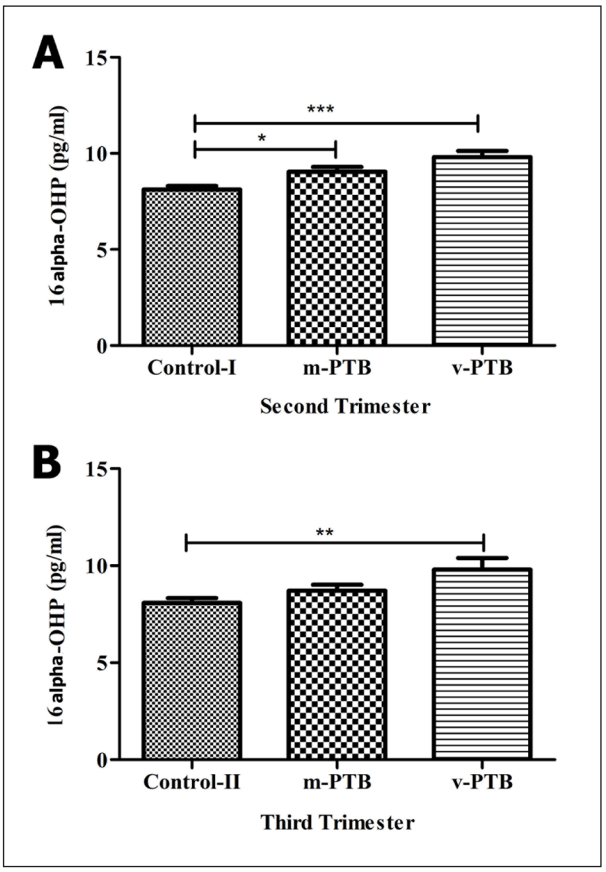

Figure 4. 16α-hydroxyprogesterone (16α-OHP) concentrations (pg/ml) in the reference (control-I and control-II) groups, moderate preterm birth (mPTB) and very preterm birth (vPTB) cohorts in the second **(A)** and the third **(B)** trimester of pregnancy. Data are presented as mean±SEM. *p<0.05; **p<0.01; ***p<0.001.

Discussion

Mothers in premature labor face a high risk of both neonatal and maternal complications during pregnancy. Decreased calciferol concentration during the gestation period has been associated with severe prenatal complications. Vitamin D level is influenced by components that control its production, such as latitude, epithelial layer pigmentation, and climatic phase (De-Regil et al. 2016).

In this study, calciferol levels were statistically lower in the subjects in the 2nd and 3rd trimesters who delivered before EDD compared to women who delivered at term. In addition, in the 2nd trimester, a significantly decreased calciferol level ($p<0.05$) was also observed in the vPTB cohort compared to the mPTB cohort. The present study demonstrates that vitamin D deficiency was significantly associated with PTB. Gestational age and vitamin D are directly proportional to each other. The findings of Shibata et al. (2011) have suggested that there is a high prevalence of hypovitaminosis D in perinatal pregnant Japanese women throughout the year, which seems to affect bone metabolism and is associated with the threat of PTB. In addition, they found that early labor pains were related to vitamin D deficiency. Results of a study from Thailand have shown that vitamin D deficiency in patients with PTB was 48%, equivalent to the current cohort. A meta-analysis of 10 studies has revealed that vitamin D deficiency during pregnancy is associated with an increasing risk of PTB (Qin et al. 2016). On the contrary, Bhupornvivat and Phupong (2017) observed no differences in serum vitamin D concentrations and the prevalence of vitamin D deficiency and insufficiency in Thai pregnant women with and without PTB.

In case of sudden PTB, the muscles of the uterine myometrium shift from relaxation to contraction before the newborn matures. Myometrium contractions are based on calciferol-controlled Ca^{+2} release in uterine involuntary muscle cells. Therefore, vitamin D is important in reducing the risk of sudden PTB by promoting uterine smooth muscle relaxation (Tribe 2001).

In the present study, the concentration of SHBG was significantly reduced in the vPTB and mPTB cohorts. During the 16th–27th week of gestation, SHBG concentration increased due to the elevated level of estrogen. It has been shown that SHBG is expressed in umbilical cord and placental blood (Morisset et al. 2011). SHBG has recently been demonstrated to function as a predictor of PTB. The concentration of SHBG in the gestational blood increases significantly during the stage of fetal growth. However, it decreases in cases of spontaneous PTBs (Saade et al. 2016).

SHBG is an androgen-binding globulin that may also be involved in receptor-regulated mechanisms. One steroid biomolecule binds to one binary molecule (dimer). SHBG controls the availability of an unattached physiologically active steroid biomarker. During the period of fetal growth, the serum SHBG level increases 5–10 times (Hammond 2011). Biologically, the amount of SHBG is inversely correlated with body mass ratio, insulin concentration, and neutral fats (Simo et al. 2015). Inflammatory reactions and intrauterine infections are associated with PTB (Mendelson 2009; Gomez-Lopez et al. 2014). SHBG transcription is inhibited by TNF-α signaling, a pathway involved in the induction of regular or abnormal labor (Lindstrom and Bennett 2005). A decrease in the amount of SHBG in pregnant women predisposed to PTB may be caused by an inflammatory reaction or pathogenic invasion. Therefore, SHBG is likely critical for estrogen and gonadal steroid regulation of the immune response triggers in the placental-fetal complex.

Our findings demonstrate that the concentration of 16α-OHP was significantly increased in the PTB cohorts as compared to the controls. On the other hand, DOC levels remained significantly decreased in the PTB cohorts compared to their reference cohorts. These results suggest that when we detect an increase in the concentration of 16α-OHP and a decrease in the level of DOC, we can predict the occurrence of PTB. Our results confirm that the amount of DOC and 16α-OHP in serum, measured in the middle and third stages of the gestational period, can predict the risk of PTB in women. Progesterone is a biomarker responsible for maintaining pregnancy. It is hydrolyzed by 17α-hydroxylase and 21-hydroxylase into many metabolic regulators. Patil et al. (2015) have found that 6β-OHP and 16α-OHP have opposing effects on oxytocin-inducing uterine smooth muscle contraction. Pregnant women with increased contractility are exposed to PTB due to shock, desiccation and pathogen invasion. In the same way, elevated glucocorticoid levels are associated with PTB (Sandman et al. 2006).

CRH in the fetal blood flow stimulates estrogens and prostaglandin secretion, enhancing myometrial contractility. Endogenously produced steroids such as glucocorticoids are altered by processes that control CRH production in placental tissues (Whittle et al. 2001; Wang et al. 2013). Increased CRH in the placental barrier stimulates the production of glucocorticoids in the fetal adrenal glands, which reach their peak during labor. As the labor process progresses, serum cortisol levels rise. This improvement appears to play a vital role in maintaining the health of newborns and mothers and aids in the normal delivery process. DOC is a progenitor of aldosterone with electrolyte regulator activity. Analysis of pregnant women showed that elevated cortisol levels during the middle trimester of pregnancy were associated with increased CRH concentration in the 3rd trimester and PTB (Sandman et al. 2006).

In the present study, a reduced DOC level may direct a dysfunctional physiological process and change of CRH formation in the chorionic villi and uterine smooth muscle contractility as a component of the maternal-fetal stress axis that leads to PTB. 16α-OHP is one of two metabolites that resulted from the metabolism of progesterone by cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17A1) (Storbeck et al. 2011; Quinney et al. 2017). 16α-OHP is not metabolized in steroidogenic tissue, although many progesterone metabolites are continuously metabolized to androgens, estrogens, and corticosteroids. Progesterone hydroxylation CYP proteins produce molecules that enhance the myometrium smooth muscle contraction rate and result in PTB (Mizrachi et al. 2011; Patil et al. 2015).

The study has some limitations. First, during gestation, maternal blood specimens were collected and chemically treated either in the 2nd or 3rd trimester of pregnancy. Second, the sera were collected over a wide range of the prenatal period (gestational age from 13 to 32 weeks).

Another promising research goal may be to investigate the role of other biomarkers, such as inflammatory cytokines, which could provide

further insight into the mechanisms underlying PTB. Furthermore, investigation of genetic predispositions related to calciferol metabolism in pregnant women may reveal new aspects of PTB risk.

Conclusion

At formative stages of fetal development in symptom-free pregnant women, the proportion of these prognostic clinical markers permits well-timed and cautious identification of women at risk of PTB. This appropriate outcome may recover pregnancy difficulties via upgraded medical research and the emergence of therapeutic intervention for PTB prevention.

Acknowledgment

We gratefully acknowledge the Institute of Zoology, University of the Punjab, Lahore for providing resources for this study.

Conflict of interest: *The authors declared no conflict of interest.*

References

Bhupornvivat N, Phupong V. Serum 25-hydroxyvitamin D in pregnant women during preterm labor. Asia Pac J Clin Nutr 26, 287–290, 2017.

De-Regil LM, Palacios C, Lombardo LK, Pena-Rosas JP. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev 1, CD008873, 2016.

Dror DK, Allen LH. Vitamin D inadequacy in pregnancy: Biology, outcomes, and interventions. Nutr Rev 68, 465–477, 2010.

Gomez-Lopez N, StLouis D, Lehr MA, Sanchez-Rodriguez EN, Arenas-Hernandez M. Immune cells in term and preterm labor. Cell Mol Immunol 11, 571–581, 2014.

Hammond GL. Diverse roles for sex hormone-binding globulin in reproduction. Biol Reprod 85, 431–441, 2011.

Heaney RP. Vitamin D in health and disease. Clin J Am Soc Nephrol 3, 1535–1541, 2008.

Lindstrom TM, Bennett PR. The role of nuclear factor kappa b in human labour. Reproduction 130, 569–581, 2005.

Manuck TA, Esplin MS, Biggio J, Bukowski R, Parry S, Zhang H, Huang H, Varner MW, Andrews W, Saade G. The phenotype of spontaneous preterm birth: Application of a clinical phenotyping tool. Am J Obstet Gynecol 212, 487.e1–487.e411, 2015.

Mastorakos G, Ilias I. Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. Ann N Y Acad Sci 997, 136–149, 2003.

Mendelson CR. Minireview: Fetal-maternal hormonal signaling in pregnancy and labor. Mol Endocrinol 23, 947–954, 2009.

Mizrachi D, Wang Z, Sharma KK, Gupta MK, Xu K, Dwyer CR, Auchus RJ. Why human cytochrome P450C21 is a progesterone 21-hydroxylase. Biochemistry 50, 3968–3974, 2011.

Morisset AS, Dube MC, Drolet R, Robitaille J, Weisnagel SJ, Tchernof A. Sex hormone-binding globulin levels and obesity in women with gestational diabetes: Relationship with infant birthweight. Gynecol Endocrinol 27, 905–909, 2011.

Patil AS, Swamy GK, Murtha AP, Heine RP, Zheng X, Grotegut CA. Progesterone metabolites produced by cytochrome P450 3A modulate uterine contractility in a murine model. Reprod Sci 22, 1577–1586, 2015.

Patil AS, Gaikwad NW, Grotegut CA, Dowden SD, Haas DM. Alterations in endogenous progesterone metabolism associated with spontaneous very preterm delivery. Hum Reprod Open 2020, hoaa007, 2020.

Perez-Lopez FR, Chedraui P, Fernandez-Alonso AM. Vitamin D and aging: Beyond calcium and bone metabolism. Maturitas 69, 27–36, 2011.

Qin LL, Lu FG, Yang SH, Xu HL, Luo BA. Does maternal vitamin D deficiency increase the risk of preterm birth: A meta-analysis of observational studies. Nutrients 8, 301, 2016.

Quinney SK, Benjamin T, Zheng X, Patil AS. Characterization of maternal and fetal CYP3A-mediated progesterone metabolism. Fetal Pediatr Pathol 36, 400–411, 2017.

Romero R, Mazor M, Munoz H, Gomez R, Galasso M, Sherer DM. The preterm labor syndrome. Ann N Y Acad Sci 734, 414–429, 1994.

Saade GR, Boggess KA, Sullivan SA, Markenson GR, Iams JD, Coonrod DV, Pereira LM, Esplin MS, Cousins LM, Lam GK, Hoffman MK, Severinsen RD, Pugmire T, Flick JS, Fox AC, Lueth AJ, Rust SR, Mazzola E, Hsu C, Dufford MT, Bradford CL, Ichetovkin IE, Fleischer TC, Polpitiya AD, Critchfield GC, Kearney PE, Boniface JJ, Hickok DE. Development and validation of a spontaneous preterm delivery predictor in asymptomatic women. Am J Obstet Gynecol 214, 633.e1–633.e24, 2016.

Sandman CA, Glynn L, Schetter CD, Wadhwa P, Garite T, Chicz-DeMet A, Hobel C. Elevated maternal cortisol early in pregnancy predicts third trimester levels of placental corticotropin releasing hormone (CRH): Priming the placental clock. Peptides 27, 1457–1463, 2006.

Sandman CA. Prenatal CRH: An integrating signal of fetal distress. Dev Psychopathol 30, 941–952, 2018.

Savitz DA, Dole N, Herring AH, Kaczor D, Murphy J, Siega-Riz AM, Thorp Jr JM, MacDonald TL. Should spontaneous and medically indicated preterm births be separated for studying aetiology? Paediatr Perinat Epidemiol 19, 97–105, 2005.

Shibata M, Suzuki A, Sekiya T, Sekiguchi S, Asano S, Udagawa Y, Itoh M. High prevalence of hypovitaminosis D in pregnant Japanese women with threatened premature delivery. J Bone Miner Metab 29, 615–620, 2011.

Sibai B, Meis PJ, Klebanoff M, Dombrowski MP, Weiner SJ, Moawad AH, Northen A, Iams JD, Varner MW, Caritis SN, O'Sullivan MJ, Miodovnik M, Leveno KJ, Conway D, Wapner RJ, Carpenter M, Mercer B, Ramin SM, Thorp JM Jr, Peaceman AM, Gabbe S; Maternal Fetal Medicine Units Network of the National Institute of Child Health and Human Development. Plasma CRH measurement at 16 to 20 weeks' gestation does not predict preterm delivery in women at high-risk for preterm delivery. Am J Obstet Gynecol 193, 1181–1186, 2005.

Simo R, Saez-Lopez C, Barbosa-Desongles A, Hernandez C, Selva DM. Novel insights in SHBG regulation and clinical implications. Trends Endocrinol Metab 26, 376–383, 2015.

Storbeck KH, Swart P, Africander D, Conradie R, Louw R, Swart AC. 16α-hydroxyprogesterone: origin, biosynthesis and receptor interaction. Mol Cell Endocrinol 336, 92–101, 2011.

Tribe RM. Regulation of human myometrial contractility during pregnancy and labour: Are calcium homeostatic pathways important? Exp Physiol 86, 247–254, 2001.

Wang B, Palomares K, Parobchak N, Cece J, Rosen M, Nguyen A, Rosen T. Glucocorticoid receptor signaling contributes to constitutive activation of the noncanonical NF-κB pathway in term human placenta. Mol Endocrinol 27, 203–211, 2013.

Whittle W, Patel F, Alfaidy N, Holloway A, Fraser M, Gyomorey S, Lye S, Gibb W, Challis J. Glucocorticoid regulation of human and ovine parturition: The relationship between fetal hypothalamic-pituitary-adrenal axis activation and intrauterine prostaglandin production. Biol Reprod 64, 1019–1032, 2001.