Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE

DOI: 10.47750/jptcp.2023.30.13.025

Effect of Maternal Variables on some Physiological and Immunological Biomarkers in Iraqi Women Undergoing Caesarean Section

Huda Sajir Naser[1*], Makarim Qassim Al-Lami[2], Haider Faisal Ghazi[3]

[1] Ministry of science and technology, Water and Environment Directorate, Baghdad, Iraq

[2] Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

[3] Department of Microbiology, College of Medicine, AL-Nahrain University, Baghdad, Iraq

***Corresponding Author:** Huda Sajir Naser, Ministry of science and technology, Water and Environment Directorate, Baghdad, Iraq. Email: hudabio4@gmail.com

Submitted: 24 March 2023; Accepted: 13 April 2023; Published: 12 May 2023

ABSTRACT

Pregnancy and childbirth are physiological states characterized by sudden hormonal and immunologically described changes. The current study aimed to investigate the influence of maternal variables (age, previous abortion, placental position, and fetal position) on some physiological biomarkers, such as oxytocin (OT), prolactin (PRL), cortisol, and insulin growth factor 2 (IGF -2) and some immune biomarkers such as programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1) and interleukin 6 (IL-6) in Iraqi women undergoing caesarean section (CS). Blood samples were collected from 48 pregnant women in the age range (16-43 years) and serum was obtained to determine the levels of the above biomarkers.

The effect of maternal age on physiological biomarkers showed that OT levels increased significantly with maternal age ($P \leq 0.001$), whereas PRL, cortisol, and IGF-2 levels were not ($P > 0.05$), showing differences between age categories. The effect of maternal age on immune biomarkers showed that PD-1 levels decreased significantly ($P < 0.05$) and PD-L1 and PD-1/PD-L1 levels were highly significant ($P \leq 0.001$), however, IL-6 level shows a non-significant ($P > 0.05$) difference between age categories. Regarding the effect of previous abortion, statistical analysis showed that cortisol levels were significantly ($P < 0.05$) lower in women with previous abortion compared to women without previous abortion, while OT, PRL, and IGF-2 levels were not significantly different ($P > 0.05$) difference between the two groups. PD-1 and PD-L1 levels showed a significant ($P < 0.05$) decrease in women with previous abortion compared with women without abortion, while IL-6 mirror images did not differ significantly ($P > 0.05$) between the two groups. The effect of placenta position showed that when the placenta was in the anterior position, the PRL level was significantly increased ($P \leq 0.001$), IGF-2 level was significantly decreased ($P < 0.05$), while the OT and cortisol levels were significantly higher ($P \leq 0.001$) compared with the posterior position There was no significant difference between the two groups ($P > 0.05$). When the placenta was located in the anterior compared with the posterior, the level of PD-L1 was significantly ($P < 0.05$) lower, while the levels of PD-1 and PD -1/PD- were not significantly different ($P > 0.05$). L1 and IL-6 between the two groups. According to fetal position, statistical analysis showed no significant difference in all physiological and immune biomarkers when the fetus was in cephalic or breech position ($P > 0.05$).

It can be concluded that the more maternal variables which affect on the physiological and immunological biomarkers of the women undergoing caesarean section are maternal age and a previous abortion.

Keywords: *Maternal variables, Caesarean section, Abortion, Maternal age, Placenta.*

J Popul Ther Clin Pharmacol Vol 30(13):e252–e264; 12 May 2023.

INTRODUCTION

A caesarean section (CS) is a surgical technique in which a fetus is delivered through an incision in the abdomen (laparotomy) and uterine wall (hysterotomy); it is used to reduce difficulties associated with childbirth (1). In 1985, WHO (2) set an ideal CS rate range of 10-15%, noting that the risks to maternal and newborn health outweighed the benefits at 15%. There are many factors that contribute to the increase in CS rates, such as: B. Cultural factors, maternal age, especially advanced maternal age (AMA), miscarriage, abnormal placental and embryo position, and increased socioeconomic status. However, the increased rate of CS is known to be caused by AMA; this may be because the older the mother, the more likely she is to have underlying medical conditions such as hypertension, diabetes or obesity (3). The risk of cesarean delivery increases with the incidence of obstetric problems in pregnancy in older women, such as: B. gestational diabetes, preeclampsia or placenta previa, placental abruption, and preterm labor (4).

In contrast, miscarriage was defined as termination of pregnancy before the 24th week of pregnancy. 15%-20% of all clinically proven pregnancies result in miscarriage, which affects approximately one third of women and, over time, has disastrous consequences for the individual (5). Abortion history has been linked to poor pregnancy outcomes. Yet, the elevated CS rate, preterm birth, and fetal death are related to prior abortions. By scheduling the patients and providing the necessary prenatal care, these problems and fetal loss can be minimized (6). Furthermore, an abortion is a big event that can have a detrimental influence on a female's psychological health and subsequent pregnancies (7). In addition, there was a strong correlation between placental position and pregnancy outcome as well as mode of delivery (caesarean section or vaginal delivery). Anterior placenta accreta is associated with a significantly increased risk of miscarriage as well as induced hypertension, gestational diabetes, placental abruption, intrauterine growth restriction, and intrauterine fetal death. The position of the placenta is a key determinant of pregnancy outcome, although a retropositioned placenta is significantly associated with preterm birth (8). Nonetheless, childbirth can infrequently involve abnormal fetal head presentation (malpresentation) and malposition. Whenever the baby is in vertex presentation and the occiput is anterior, normal vaginal delivery occurs most frequently. Malpresentations are any circumstances in which the baby is not in vertex presentation, and malpositions are any postures other than occipito-anterior (9). In 3-4% of term pregnancies, the fetus presents with its feet, buttocks, or both in the breech position (10). Fetal malposition-occipito-transverse or occipito-posterior increases the danger of CS, prolongs labor, and increases neonatal morbidity. A round (97%) of births have a cephalic presentation of the fetus (11).

MATERIALS AND METHODS

Studied subjects:

Between December 2020 and June 2021, a total of 48 eligible women, aged 16 to 43, participated in the study and attended the Al-Immamain Al-Kadimain Hospital in Baghdad, Iraq. All women in this study gave informed consent before participating in this study. In addition, this study was approved by the Ethics Committee of the Department of Biology, Faculty of Science, University of Baghdad (Reference No. CSEC/0322/0040).

Collection of blood samples:

Obtain 5 mL of blood from each participating female by venipuncture using a disposable syringe. Distribute blood samples into gel tubes; coagulate at room temperature, centrifuge at 5000rpm for 10 minutes, collect serum, transfer to Eppendorf tubes, store at -20°C, and use for physiological and immunological examinations.

Biomarkers analysis:

The physiological biomarkers (OT, PRL, cortisol and IGF-2) and the immunological biomarkers (PD-1, PD-L1 and IL-6) were estimated by using an ELISA technique according to the manufacturer’s recommended method by using a specific kit for each biomarker.

Statistical analysis:

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 21.0 and Microsoft Excel 2013. Mean ± standard error (SE) was used to characterize numerical data. Statistical comparison between groups was performed by Student's test and analysis of variance

(ANOVA), and the significance level was set at P<0.0

RESULTS AND DISCUSSION

Effect of maternal age on levels of some hormones and IGF-2

The results shown in (Table 1) show a clear trend of increasing OT levels with increasing age. This increase was consistent across all age groups [≤20 years (247.16 ± 23.49 pg/mL), 21-30 years (356.44 ± 31.80 pg/mL) and >30 years (475.28 ± 33, 09) were highly significant (P ≤ 0.001). pg/ml)]. While other physiological parameters (PRL, cortisol and IGF-2) showed non-significant (P>0.05) differences among the three age groups.

TABLE 1: Effect of maternal age on levels of some hormones and IGF-2

Biomarkers	Age groups	Mean ± SE	P- value
OT (pg/ml)	≤ 20 years	247.16 ± 23.49	<0.001**
	21-30 years	356.44 ± 31.80	
	>30 years	475.28 ± 33.09	
PRL (ng/ml)	≤ 20 years	115.96 ± 12.57	0.547^{NS}
	21-30 years	126.82 ± 8.52	
	>30 years	114.95 ± 7.36	
Cortisol (µg/dL)	≤ 20 years	48.19 ± 1.58	0.064 ^{NS}
	21-30 years	39.25 ± 2.91	
	>30 years	36.46 ± 3.12	
IGF-2 (ng/ml)	≤ 20 years	11.09 ± 1.54	0.559 ^{NS}
	21-30 years	10.81 ± 1.90	
	>30 years	13.60 ± 2.23	

NS: Non-Significant (P>0.05), ** High Significant (P≤0.001)

It has now been found that OT increases with maternal age may be due to medical interventions, such as infusion of synthetic OT or AMA dystocia during labor. A previous study (12) reported that, in AMA, women more frequently used medical intervention for dystocia than boys. The present results are consistent with this study, which suggests that OT increases with maternal age, possibly reflecting a decreased ability of the uterus to progress adequately through labor. OT is usually administered during labor to initiate and increase uterine contractions and suppress postpartum hemorrhage. Similarly, a systematic review found that a major factor leading to cesarean delivery was inefficiency of the myometrium in older women. This in turn leads to decreased oxytocin receptor (OXTR) numbers, which may lead to the need for CS labor if adequate uterine dilatation and conduction kinetics are not achieved (13). Theoretically, medical interventions could have this effect by chronically altering the release of OT during labor and delivery (14). AMA was found to be associated with an increased risk of CS delivery after failed induction compared to younger women (15). In contrast, the present results are inconsistent with (16), which showed that labor induction and delivery in AMA had no significant effect on CS delivery rates, assisted vaginal delivery, and postpartum hemorrhage.

The non-significant effect of age on PRL levels contradicts (17), which argues that PRL levels increase in late pregnancy and explains that this increase is due to increased estrogen levels. On the other hand, a previous study observed that both emergency CS (ECS) and epidural analgesia reduced the release of PRL to breastfeeding and also reduced maternal psychological adaptation, whereas infusion of synthetic OT increased PRL and psychological adaptation. In addition, it has been suggested that medical interventions during labor and delivery, including CS, epidural analgesia, and infusion of synthetic OT, may adversely affect the initiation and continuation of breastfeeding (18). Likewise, PRL levels rise rapidly in the third trimester of pregnancy and remain elevated throughout lactation, with a marked increase in PRL levels in response to lactation (19) and then a gradual decline (20).

Furthermore, this study showed that age had no significant effect on cortisol levels. According to a previous study, cortisol levels were elevated during intrapartum CS, as indicated by cervical dilation during CS, which was significantly associated with labor intensity (21). In humans, AMA and birth pressure are risk factors for increased intrauterine growth restriction (22). On the other hand, in the relationship between PRL and cortisol hormones, PRL can assess the stress response, especially in the perinatal period (23).

Furthermore, a previous study (24) reported that chronic stress as well as increased corticosterone levels contribute to reduced PRL release. According to current OT findings, the effect of elevated OT on cortisol levels is to decrease the activity of the hypothalamic-pituitary-adrenal (HPA) axis, which in turn leads to decreased levels of adrenocorticotropic hormone (ACTH) and cortisol (25).

Insulin growth factor 2 levels did not differ significantly between age groups. This finding is consistent with a previous study (26) that showed no effect of maternal age on IGF-2 levels. However, the current findings may be due to reduced gene expression of IGF-2 and other biomarkers such as IGF-1, IL-6, and TNF in the uterus of AMA patients compared with younger women. AMA modifies the methylation of imprinted genes in essential reproductive tissues and leads to dysregulation of gene expression associated with adverse reproductive outcomes (27). Furthermore, the main role of IGF-2 as a growth stimulating factor is only in the first trimester, while its role in the third trimester is to promote the maturation of growing organs (28).

Effect of maternal age on levels of the immunological biomarkers

Regarding the immune biomarker results (Table 2), the mean value of PD-1 decreased significantly ($P < 0.05$) with increasing age [≤ 20 years old (0.88 ± 0.06 ng/mL), 21-30 years old (0.79 ± 0.06 ng/mL). 0.06 ng/mL) or >30 years (0.53 ± 0.05 ng/mL)]. Both PD-L1 levels and PD-1/PD-L1 ratios [(0.52 ± 0.02, 0.49 ± 0.02, 0.43 ± 0.02 ng/mL) and (1.66 ± 0.04, 1.82 ± 0.07, 1.45 ± 0.07)] showed highly significant ($P<0.001$) decreased with age. Although IL-6 levels showed non-significant ($P > 0.05$) differences between age groups.

TABLE 2: Effect of maternal age on immune biomarker values

Biomarkers	Age groups	Mean ± SE	P- value
PD-1 (ng/ml)	≤ 20 years	0.88 ± 0.06	<0.05*
	21-30 years	0.79 ± 0.06	
	>30 years	0.53 ± 0.05	
PD-L1 (ng/ml)	≤ 20 years	0.52 ± 0.02	<0.001**
	21-30 years	0.49 ± 0.02	
	>30 years	0.43 ± 0.02	
PD-1/PD-L1	≤ 20 years	1.66 ± 0.04	<0.001**
	21-30 years	1.82 ± 0.07	
	>30 years	1.45 ± 0.07	
IL-6 (pg/ml)	≤ 20 years	6.34 ± 0.05	0.186 NS
	21-30 years	6.42 ± 0.06	
	>30 years	7.51 ± 0.77	

* Significant ($P\leq0.05$), ** High Significant ($P\leq0.001$), NS: Non-Significant ($P>0.05$)

From a medical standpoint, women with AMA are more likely to have an increased incidence of induced labor or dystocia, including elective caesarean section and vaginal delivery, as well as an increased risk of operative vaginal delivery (29). Thus, the immune mechanism by which AMA stimulates prolonged labor or dystocia may involve changes in the pro-inflammatory environment at the fetal-maternal interface. In the current study, the decrease in PD-1 levels with increasing maternal age is consistent with (30) the observed decrease in the expression of PD-1 levels in the third trimester of pregnancy. This may indicate abnormal inflammatory signaling pathways in the decidua tissue of AMA. Pregnant women with AMA are associated with pathological delays in labor, which are associated with changes in T-cell subsets at the maternal-fetal interface (31); this figure probably explains the current results. Decreased proportion of antigen-specific effector Treg cells and decreased PD-1 expression in clonally expanded decidual (dCD8+ T) cells may stimulate fetal rejection in preeclampsia (32). Furthermore, the decrease in PD-L1 levels with maternal age is consistent with the decrease in AMA PD-L1 levels reported by (32) in late pregnancy. The present results may be due to the reduced expression of PD-L1 on the surface of CD4+ T and CD8+ T cells. However, during pregnancy, uterine and decidual macrophages (M1/M2) are differentiated or recruited from peripheral blood. Previous studies observed the polarization of embryonic trophoblast-transferred macrophages with PD-L1, which can bind to PD-1 expressed on monocytes or macrophages, and

then most macrophages were polarized into M2 subtypes. type, leading to maintenance of normal pregnancy; whereas decidua in women with pregnancy complications, such as B. recurrent miscarriage, is characterized by the presence of a high proportion of macrophages with the M1 phenotype and is associated with reduced expression of PD-1 (33).

Before parturition, the number of proinflammatory T cells and Treg cells at the maternal-fetal interface decreases with maternal age, and this decrease in T cells may lead to a decrease in T cells carrying PD-1/PD-L1 expression (31). This result is consistent with the current study showing that PD-1/PD-L1 decreases with age. Furthermore, any alterations in PD-1 and PD-L1 expression may result in increased embryo resorption due to the M1 phenotype being superior to the M2 phenotype at the maternal-fetal interface and Th1 dominance before and during lead birth (33). These immune checkpoint proteins play an important role in maintaining peripheral tolerance and controlling autoimmunity.

On the other hand, IL-6 levels did not differ significantly with age; the present results are in contrast to (34), which reported that IL-6 levels gradually increased as pregnancy progressed and reached peak. AMA Stress and depression are both associated with higher circulating levels of IL-6 and TNF-α during pregnancy (35). Interestingly, cortisol levels were not significantly affected by maternal age; perhaps this explains the current results for IL-6. However, a previous study reported that healthy AMA at delivery; placenta, amnion, chorion, decidua, and myometrium increased the production of pro-inflammatory cytokines IL-6, IL-8, and TNF-α. IL-1β, prostaglandins, and COX-2 were compared with healthy AMA at cessation of labor; their synergistic increase in all tissues confirmed their reported functional role as uterotonic agents (36).

Effect of previous abortion on levels of some hormones and IGF-2

According to the findings in (table 3), the physiological biomarkers (OT and PRL) revealed non-significant (P>0.05) differences in women with a previous abortion (339.14 ± 49.34 pg/ml) and (126.55 ± 13.37 ng/ml), respectively compared with women without previous abortion (388.79 ± 22.99 pg/ml) and (119.09 ± 5.64 ng/ml), respectively. Cortisol level revealed a significant (P<0.05) decrease in women with previous abortion (27.75 ± 5.23 µg/dl) compared with women without previous abortion (42.30 ± 1.78 µg/dl). The IGF-2 showed a non-significant (P>0.05) difference in women with and without previous abortion (8.03±0.31 ng/ml) and (12.68± 1.44 ng/ml), respectively.

TABLE 3: Effect of previous abortion on levels of some hormones and IGF-2

Biomarkers (Mean ± SE)	Previous abortion		P-value
	With	Without	
OT (pg/ml)	339.14 ± 49.34	388.79 ± 22.99	0.377 NS
PRL (ng/ml)	126.55 ± 13.37	119.09 ± 5.64	0.594 NS
Cortisol (µg/dL)	27.75 ± 5.23	42.30 ± 1.78	<0.05*
IGF-2 (ng/ml)	8.03 ± 0.31	12.68 ± 1.44	0.153 NS

NS: Non-Significant (P>0.05), * Significant (P≤0.05)

OT hormones are usually administered during labor to induce and increase uterine contractions and prevent postpartum hemorrhage. Previous studies have reported that in women with normal pregnancies but with complications, symptoms of stress and depression, miscarriages, and previous cesarean delivery, OT levels increased from 35 weeks gestation to 6 months postpartum; OT levels increased from 38 weeks gestation to two days postpartum drop (37). This result is inconsistent with the present results, indicating that there is no significant difference in OT levels. In addition, studies have shown that the effect of using labor pain medication (work analgesia) varies among women and shows a statistically significant increase in PRL and cortisol suppression (38). Homeostasis of this physiological biomarker system is maintained during pregnancy through the connection of the placenta to the maternal and fetal HPA systems. Labor may be accelerated or delayed due to elevated maternal and fetal HPA hormones due to the stress of labor (39).

The placenta helps control the activity of the enzyme 11-hydroxysteroid dehydrogenase 2 (11-HSD2) by secreting a hormone, estrogen (E2), which converts maternal cortisol to an inactive state before it reaches the fetus (possible pine),

thereby maintaining fetal homeostasis (40). Under the stress of a CS birth, the woman may release large amounts of E2, which keeps cortisol dormant. This fact is consistent with the available data showing a low rate of cortisol in the abortion group.

Furthermore, (41) reported that mothers with a history of miscarriage were more likely to undergo cesarean delivery in the third trimester and were at greater risk of adverse pregnancy outcomes, including gestational diabetes. Furthermore, a dysregulated maternal HPA axis prolongs the production of cortisol and CRH and increases the production of pro-inflammatory compounds that ultimately prevent the placenta from controlling the 11-HSD2 enzyme. This results in elevated embryonic cortisol levels, dysregulated fetal HPA activity, and abnormal organ development, including lung maturation and release of labor-inducing hormones (42). Elevated levels of stress hormones are considered key signals of the HPA axis, immune system development, lung and organ maturation, and neonatal neurogenesis. The results of the current study showed an increase in the number of mothers who had never had an abortion compared to women who had previously undergone an abortion (43).

For a pregnancy to be complete, there must be a balance between apoptosis and proliferation; IGF-2 is a cytokine that controls the pathway of this balancing process. On the surface of target tissues and cells, IGF-2 mediates a variety of metabolic and mitogenic effects. The vast majority of current findings regarding the lack of significant differences in IGF-2 levels may be due to the fact that IGF-2 function occurs at the beginning of pregnancy rather than at the end of pregnancy. Numerous studies have shown that IGF-2 is expressed in multiple tissues and regulates the proliferation and survival of multiple tissues during early pregnancy (44).

Effect of previous abortion on levels of the immunological biomarkers

The effect of prior pregnancy termination on immune biomarkers is shown in Table 4. The results showed that compared with women who had not previously had a miscarriage (0.75 ± 0.04 ng/ml and 0.49 ± 0.02 ng/ml). Although PD-1/PD-L1 and IL-6 levels were not significantly different (P>0.05) in women with a history of miscarriage compared with women without a history of miscarriage (1.55 ± 0.11 and 6.49 ± 0.15 pg/mL, respectively) previously terminated pregnancy (1.67 ± 0.05 and 6.88 ± 0.35 pg/ml).

TABLE 4: Effect of previous abortion on levels of the immunological biomarkers

Biomarkers (Mean ± SE)	**Previous abortion**		**P -value**
	With	**Without**	
PD-1 (ng/ml)	0.52 ± 0.04	0.75 ± 0.04	<0.05*
PD-L1 (ng/ml)	0.41 ± 0.02	0.49 ± 0.02	<0.05*
PD-1/PD-L1	1.55 ± 0.11	1.67 ± 0.05	0.299^{NS}
IL-6 (pg/ml)	6.49 ± 0.15	6.88 ± 0.35	0.628 ^{NS}

NS: Non-Significant (P>0.05), * Significant (P≤0.05)

Numerous studies have demonstrated the importance of the PD-1/PD-L1 association in pregnancy maintenance, and increased PD-1 expression in decidual immune cells was observed during the first trimester of healthy pregnancy (45). Indeed, there is a correlation between PD-1 and PD-L1 expression, Th1/Th2/Th17 balance, and fetal-maternal tolerance.

PD-L1 deficiency results in a reduced Treg/Teff ratio, which in turn leads to a shift in Th1 and Th17 cell proliferation, which impedes the development of tolerance and increases embryo resorption and miscarriage (46). Drugs used to relieve labor pain associated with protracted labor or dystocia can lead to blockade of the PD-1/PD-L1 signaling pathway, which may be responsible for the reduction of PD-1 and PD-L1 in the mother, and uterine contractions according to the current According to the data, they had previously miscarried their children. While dysregulation of these cells can lead to adverse pregnancy outcomes such as miscarriage, hypertensive disorders, fetal growth restriction, and maternal and infant death, pro-inflammatory immune cell stimulation can play a key role in maternal reproduction such as: B. Embryo implantation placenta accreta and parturition (47).

A previous study found that serum IL-6 levels were higher in patients with repeat abortions than in controls (48). This finding contradicts the

findings of the current study, which showed no significant changes in IL-6 levels in women who had previously had abortion. Furthermore, (49) it has been suggested that IL-6 and corticotropin-releasing hormone (CRH) are produced in a pulsatile manner during active labor and that IL-6 promotes the release of placental CRH and is directly or indirectly associated with uterine contractility.

Effect of placenta position on levels of some hormones and IGF-2

According to the present results, as reported in (Table 5), there was no significant difference (P > 0.05) in OT levels between the anteroposterior placental position (400.09 ± 35.32 pg/mL) and (399.93 ± 40.49 pg/ml), while the PRL level showed a very significant (P<0.001) increase when the placenta was located anteriorly (134.29 ± 9.05 ng/mL) compared to posteriorly (84.73 ± 8.38 ng/mL). Cortisol levels showed no significant difference (P>0.05) between the anterior and posterior placental locations (42.24 ± 3.98 µg/dl) and (40.74 ± 3.60). In addition, the results showed that IGF-2 levels were significantly (P<0.05) decreased in anterior placenta (10.23 ± 1.12 ng/mL) compared with retroplacenta (19.21 ± 3.66 ng/mL).

TABLE 5: Effect of placenta position on levels of some hormones and IGF-2

Biomarkers (Mean ± SE)	Placental position		P -value
	Anterior	Posterior	
OT (pg/ml)	400.09 ± 35.32	399.93 ± 40.49	0.998^{NS}
PRL (ng/ml)	134.29 ± 9.05	84.73 ± 8.38	<0.001**
Cortisol (µg/dL)	42.24 ± 3.98	40.74 ± 3.60	0.781^{NS}
IGF-2 (ng/ml)	10.23 ± 1.12	19.21 ± 3.66	<0.05*

NS: Non-Significant (P>0.05), * Significant (P≤0.05), ** High Significant (P≤0.001)

In the past, multiple studies have shown a strong association between anterior placenta and the incidence of certain birth disorders and postpartum problems. Labor onset is delayed, labor induction rates are higher, and cesarean delivery due to labor failure is more common, especially when the placenta is located in the anterior wall of the uterus (50). The placenta located in the fundus has a greater association with preterm, preterm birth; moreover (51) suggested that placental placement may be associated with poor fetal growth.

The position of the placenta at the end of pregnancy has a major impact on the duration of the third stage of labor. A fundal position of the placenta may be closely related to a shorter third stage of labor, whereas a posterior position may be directly related to a longer third stage of labor. The bipolar separation of the fundoplacenta, as opposed to the more typical unipolar downward-to-upward separation of an anterior or posterior placenta, could explain the shorter length. Treatment of the third stage with OT infusion may also be an important factor (52).

Pregnant women have a longer posterior wall of the uterus. The uterus expands to accommodate the pregnancy, and the source of the mother needs to be more spread over this larger area, therefore, these pregnant women may experience severe pain due to the reduction. Mother's source. This is true, although a placenta on the posterior wall of the uterus may not be suitable due to the anatomy of the uterine wall (52). Anterior placenta may also be associated with labor dysfunction, including prolonged third stage of labor, higher levels of labor induction, higher risk of cesarean delivery, and higher frequency of manual placental removal (50).

As a neuro-immune cytokine that actively participates in the interaction among the immunological and endocrine systems throughout pregnancy, PRL performs a crucial role in the development of an immune-privileged environment (53). It is not regulated by conventional hormonal feedback. PRL instead increases the release of its own inhibitor, dopamine, through short loop feedback. The hypothalamus contains dopamine neurons. In order to prepare for the postpartum breastfeeding process, estrogen promotes the synthesis of maternal PRL hormone near the end of pregnancy. Present finding is in line with findings by (54) who indicated an increase in the PRL when placenta position is anterior. Earlier studies indicated a high level of placental lactogen and PRL at various stages of pregnancy indicates that the PRL-receptor (PRL-R) is likely highly activated throughout the whole pregnancy (55). In the current study, cortisol levels were not affected by placental position; although it plays

an important role in delivery. Negative perceptions and psychological effects that affect the childbirth experience include women's stress and feelings of powerlessness during childbirth, according to researchers (56). IGF is critical in signaling that promotes placental and fetal development. As the endometrium develops in the first trimester, IGF-2 and IGF-2 receptors may be less effective later in pregnancy; this fact is consistent with existing studies showing decreased IGF-2 levels (57).

Effect of placenta position on levels of the immunological biomarkers

Table (6) shows no significant difference ($P > 0.05$) in PD-1 levels between anterior placental locations (0.58 ± 0.04 ng/ml) and posterior placental locations (0.67 ± 0.07 ng/ml). PD-L1 levels were significantly ($P < 0.05$) decreased when the placenta was positioned anteriorly (0.39 ± 0.01 ng/mL) compared to posterior (0.47 ± 0.03 ng/mL). PD-1/PD-L1 and IL6 levels showed no significant difference between the anterior (1.47 ± 0.07, 6.42 ± 0.09 pg/mL) and posterior (1, 71 ± 0.10, 7.83 ± 0.98 pg/ml) ($P > 0.05$), respectively.

TABLE 6: Effect of placental position on levels of the immunological biomarkers

Biomarkers (Mean ± SE)	Placental position		P -value
	Anterior	Posterior	
PD-1 (ng/ml)	0.58 ± 0.04	0.67 ± 0.07	0.324 ^{NS}
PD-L1 (ng/ml)	0.39 ± 0.01	0.47 ± 0.03	<0.05*
PD-1/PD-L1	1.47 ± 0.07	1.71 ± 0.10	0.063 ^{NS}
IL-6 (pg/ml)	6.42 ± 0.09	7.83 ± 0.98	0.194 ^{NS}

NS: Non-Significant ($P>0.05$), * Significant ($P\leq0.05$)

The present results indicate that the immune pathway, occurrence of dystocia, and risk of CS are not significantly affected by placental position. At present, it is believed that immune checkpoint molecules such as PD-1 and PD-L1 are mainly produced by the placenta during pregnancy. Elevated PD-L1 levels control the mother's immune response, which helps induce immune tolerance of the mother to her child (30). While PD-1 increases in the second trimester of pregnancy but not in the first and third trimesters; (58) found that PD-L1 increases in the third trimester, which increases the suppression of maternal immunity during pregnancy. This result contradicts the current study.

Unlike PD-L1, PD-1 has been reported to competitively inhibit the interaction of PD-L1 and PD-1 on T cells; therefore, it was hypothesized that both elevated PD-L1 and decreased PD-1 are present in maternal serum. Thus, in maternal serum, the PD-1/PD-L1 ratio is very low and can inhibit lymphocyte proliferation in response to autoantigens and alloantigens (59). Another study confirmed that pregnant women had higher serum PD-L1 levels in late pregnancy, 8.3 times higher than their offspring and 6.9 times higher than non-pregnant women (60). Furthermore, the levels of PD-L1 molecules on the surface of CD8+ T and CD4+ T cells were lower in the third trimester compared with non-pregnant women, and the levels of PD-L1 molecules were lower in CD4+ T cells compared with the second trimester. In contrast, pregnant women in late pregnancy have higher serum levels of PD-L1 than non-pregnant women, which may also reduce maternal immunity (61).

Furthermore, a study by (62) found that women who underwent elective cesarean delivery had significantly higher serum levels of PD-1 during labor than women who did not undergo cesarean delivery; therefore, identifying immune changes during normal pregnancy, particularly is about the immune mechanisms that lead to labor and may help reduce the rate of preterm birth and the risk of emergency caesarean section. Anti-PD1 therapy can also affect the mother's ability to tolerate the fetus, increasing the likelihood of miscarriage. Anti-PD-1 and anti-CTLA-4 antibodies have been used in a safety study of checkpoint inhibitors during pregnancy, which showed an increased risk of stillbirth, miscarriage, low birth weight, infant death, and preterm birth during the third trimester of pregnancy (63).

According to previous studies, high levels of IL-6 in uterine smooth muscle are beneficial to regulate the expression and binding ability of OXTR mRNA. At birth, IL-6 and CRH are also released in a pulsatile manner, and the secretion of IL-6 directly or indirectly causes CRH. This suggests that IL-6 may function as an action that stimulates and activates uterine contractions (49). These facts contradict current evidence showing

no differences in IL-6, OT, and cortisol hormone levels. Prolonged dosing duration and subsequent exposure to CS may be mainly due to the lack of physiological changes in the concentrations of these parameters. However, decreased decidual PRL and PRL-R were associated with higher levels of the pro-inflammatory cytokine IL-6, which may adversely affect labor and pregnancy (64). Finally, maintaining the balance between pro-inflammatory and anti-inflammatory cytokines is critical for protecting the fetus. All prenatal tissues had higher levels of common pro-inflammatory biomarkers, demonstrating their coordinated functional role in facilitating labor (36).

Effect of fetal position on levels of some hormones and IGF-2

Table 7 shows the effect of fetal position on some hormones and IGF-2 levels. Results showed non-significant (P > 0.05) differences in all hormones between the two groups (head and breech): OT (368.70 ± 52.13 pg/mL, 388.26 ± 35, 87 pg/ml), PRL (135.37 ± 12.24 ng/ml, 110.48 ± 8.87 ng/ml) and cortisol (40.55 ± 3.43 µg/dL, 39.44 ± 3, 61 µg/dL). IGF-2 levels also showed non-significant (P > 0.05) differences between the two groups (8.71 ± 0.93 ng/mL, 8.71 ± 0.93 ng/mL).

TABLE 7. Effect of fetal position on levels of some hormones and IGF-2 hormones and IGF-2

Biomarkers (Mean ± SE)	Fetal position		P -value
	Cephalic	Breech	
OT (pg/ml)	368.70 ± 52.13	388.26 ± 35.87	0.751 NS
PRL (ng/ml)	135.37 ± 12.24	110.48 ± 8.87	0.099 NS
Cortisol (µg/dL)	40.55 ± 3.43	39.44 ± 3.61	0.833 NS
IGF-2 (ng/ml)	8.71 ± 0.93	8.71 ± 0.93	0.134 NS

NS: Non-Significant (P>0.05)

The present results show that the position of the embryo at the onset of labor is critical in determining the mode of delivery (normal or cesarean section), thus protecting the mother and fetus from possible intrapartum and postpartum complications. However, it turns out that fetal position does not directly affect biomarkers at physiological levels. A common cause of CS delivery risk during dystocia and labor is abnormal fetal position. The most common type of deformity is breech presentation, which increases maternal morbidity and the risk of neonatal hypoxia and death (65). However, following a successful study of external skull conversion, birth rates and infant outcomes with CS were comparable to those with natural skull placement, and CS should be considered if delivery did not proceed as expected (66). Early conversion of the external cranium is associated with an increased risk of preterm birth, premature birth, and low birth weight (67).

According to (68), a total of 6.35% of newborns presented with breech presentation (total sample size = 3240 cases, of which 206 were breech and 126 delivered, accounting for 3.8% of all newborns). Of the 126 women with breech babies, 42 delivered vaginally and 84 delivered vaginally. Most patients are between 20 and 30 years of age, and infants delivered vaginally have higher perinatal morbidity than infants delivered by cesarean section.

Effect of fetal position on levels of the immunological biomarkers

Effect of fetal position on levels of immunological biomarkers is shown in (table 8). The findings revealed that non-significant (P>0.05) differences were found between the two groups (cephalic and breech fetal positions) in all the immunological biomarkers: PD-1(0.73 ± 0.09 ng/ml, 0.70 ± 0.07 ng/ml), PD-L1 (0.46 ± 0.03 ng/ml, 0.48 ± 0.03 ng/ml), PD-1/PD-L1 (1.56 ± 0.10, 1.71 ± 0.08), and IL-6 (6.31 ± 0.04 pg/ml, 7.14 ± 0.69 pg/ml).

TABLE 8: Effect of fetal position on levels of the immunological biomarkers

Biomarkers (Mean ± SE)	Fetal position		P -value
	Cephalic	Breech	
PD-1 (ng/ml)	0.73 ± 0.09	0.70 ± 0.07	0.759 NS
PD-L1 (ng/ml)	0.46 ± 0.03	0.48 ± 0.03	0.638 NS
PD-1/PD-L1	1.56 ± 0.10	1.71 ± 0.08	0.247 NS
IL-6 (pg/ml)	6.31 ± 0.04	7.14 ± 0.69	0.331 NS

NS: Non-Significant (P>0.05)

Based on the present results, it turns out that fetal position has no direct effect on immune biomarkers; however, knowledge of fetal position at the onset of labor signs is important for determining delivery method, thus protecting mother and fetus from possible intrapartum and postpartum complications disease. Because a diagnosis of a non-cephalic presentation after the onset of labor is associated with increased infant and maternal morbidity. The third trimester is critical for timely management and clinical decision-making related to achieving external cranial conversion. (69) Furthermore, previous studies have shown that administration of neuraxial analgesia to women in labor or late preterm labor significantly increases the success rate of the external cranial version, which in turn significantly increases the likelihood of vaginal delivery (70).

External cranial conversion is an obstetric procedure performed to prevent breech presentation, CS and its potential dangers. In addition, previous studies have shown that approximately 3% and 4% of term singleton pregnancies result in breech presentation at delivery (71). Another embryonic deformity is the posterior occipital deformity, which accounts for 33.3% of birth deformities, and the dystocia rate is as high as 93.5%. Therefore, although the embryo must enter the pelvis in an anterior occiput position, a posterior occiput entry is still acceptable since most fetuses will automatically rotate to an anterior occiput position (72). However, in rare cases, spontaneous rotation cannot be accomplished, and approximately 5% of infants have a permanent occipital position (73).

CONCLUSION

It can be concluded that the more maternal variables which affect on the physiological and immunological biomarkers of the women undergoing caesarean section are maternal age and a previous abortion.

ACKNOWLEDGMENTS

The authors are grateful to all the participants in the current study, and also to the staff of Al-Immamain Al-Kadimain Hospital/ Baghdad/ Iraq.

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