Maternal serum corticotrophin releasing hormone and ACTH levels as a predictive marker of uncomplicated preterm labor

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ABSTRACT

Back ground:
Numerous factors were looked into as preterm birth continues to be a key treatment issue.

Objectives: - To ascertain whether the plasma levels of corticotrophin releasing hormone (CRH) and (ACTH) in women who have been identified as having preterm labor may have clinical importance in identifying the risk of premature birth.

Methods: - Al-Kadhmiya Teaching Hospital hosted this investigation from October 2022 to March 2023. Plasma samples from 80 women with preterm labor diagnoses were used in this experiment. Samples were divided into three groups (weeks 24-28, 29-32, and 33-37) based on the week of gestation. A few weeks before the end of the second trimester, twenty low-risk women were enlisted. Values for CRH and ACTH were evaluated using ELISA. All of the women in the control group gave birth to healthy babies after more than 37 weeks of pregnancy.

Results: - In a research population of 100 women, 64 of the (80) women who reported preterm labor and 20 women who served as the control group gave delivery preterm. Our research demonstrates that CRH and ACTH levels are higher in preterm birthing women; CRH varied from 18.30 to 95.03 pg/ml and ACTH levels were between 21.20 and 26.63 pg/ml. The serum levels of CRH and ACTH were considerably lower in the (16) women who had term births while having the same diagnosis, with CRH levels between (13.5-14.9) pg/ml and levels of ACTH between (21.20-26.63) pg/ml, respectively (p 0.001). CRH and ACTH have 80% sensitivity and 100% specificity, respectively.

Conclusion: - In comparison to women who delivered at term, preterm mothers' maternal blood levels of CRH and ACTH were considerably greater.

Keywords: CRH, ACTH, Trichomonasiasis, and preterm labor.

1.Introduction

Preterm labor is characterized as uterine contractions occurring often and intensely enough to induce progressive cervicovaginal effacement and dilation between (24–37) weeks of pregnancy [1]. Preterm labor should be separated into three gestational groups for reasons related to etiology, prognosis, and recurrence risk: Extreme preterm birth occurred between 24+0 and 27+6 weeks, with an incidence of 0.4%, severe preterm birth occurred between 28+0 and 32+6 weeks, and mild preterm birth occurred between 32+0 and 36+6 weeks (1,2,3). In prosperous nations, preterm birth rates range from 7 to 12% [1]. Preterm birth rates have slowly increased in correlation with

assisted reproduction and a rising propensity for obstetric intervention.

Preterm birth rates before 32 weeks have stayed around 1-2% for a while now. Pre-eclampsia, intrauterine growth restriction, or maternal illness are the three most common causes of elective deliveries, which account for around a quarter of preterm labor. The remaining amount results from premature labor and delivery. Women in their 20s experience the lowest incidence. Teenagers and women in their 30s are at higher risk [1, 3]. Women in their 20s have the lowest incidence rates. Teenagers and women in their 30s are more at risk than other age groups [4]. Although there has been a decline in the death rate over the previous three decades, this is more likely due to advancements in neonatal care than to a decline in premature labor.

Preterm labor has higher mortality, morbidity, and financial expenses at earlier gestational ages; for instance, preterm labor fatality rates are 90% at 23 weeks. By 34 weeks, a decline to 2%. Even in neonates who survive, there is a significant risk of both short- and long-term morbidity [4, 5].

1-Short term morbidity: -

breathing difficulty, periventricular leukomalacia, necrotizing enterocolitis, patent ductus arteriosus, intraventricular bleeding, and retinopathy of prematurity [4].

2-Long term morbidity: -

A) Cerebral palsy.

Specifically, vulnerable to damage between 20 and 32 weeks after conception is the fetal and newborn brain. Infants born before 28 weeks or with birth weights under 1 kg have the highest risk of long-term morbidity from issues with brain development [4]. Because fetal and neonatal mortality rates are higher in boys after preterm birth, they are more likely to have long-term neurological and motor impairments [6,7].

B) Vision loss or Refractory errors.
C) Hearing loss.
D) Intellectual impairment including difficulty in learning and academic achievement.
E) Visual –motor integration.
F) Impairment growth in later child hood.
G) Abnormal airway function in preterm labour in infants who suffered respiratory distress syndrome [7, 8].

Etiology of Preterm Labor: -

1- There are three identified possible causal chains that could result in preterm delivery:
2. Cervical pathway infection.
3. Placental vascular route number two.
4. Placental vascular route number two.

These components provide direction for identifying at-risk patients and using viable remedies [9,10].

Pathogenesis: -

1. Preterm labor with an idiopathic cause is 60–70% of all preterm labors [11].

2. Premature Hypothalamic-Pituitary-Adrenal Axis Activation

Infection: -

If there is a high concentration of virulent diseases in the vagina, even a healthy cervix may allow bacteria to penetrate the lower pole of the uterus. The start of spontaneous labor and uterine infection are tightly connected. All biochemical pathologies within the uterus have the potential to be activated by infection, which could then result in uterine contraction and cervical ripening by activating inflammatory mediators. The strongest direct connection between infection and preterm labor is bacterial vaginosis. [15,16]. After a spontaneous preterm birth, the Histological chorioamionitis is very prevalent. Most occurrences of chorioamionitis are asymptomatic, with overt clinical signs of infection present in only 10% of histopathological preterm cases [17,18]. The majority of these infections, which are not limited to the uterus and affect 5 to 10% of those who experience premature labor, is a urinary tract infection [17]. Romero and Mazor's research indicates that an extra uterine infection may cause preterm labor by way of a mechanism involving the production of IL and TNF by maternal macrophages, which then causes the amnion to produce PG [17].

Hemorrhage: -

Preterm placental abruption is assumed to be the cause of preterm labor because the

release of thrombin causes myometrial contraction to increase without prostaglandin production. Another potential contributing factor to premature labor brought on by chorioamnionitis is the generation and subsequent release of thrombin as a result of deciduas bleeding [3].

Uterine over distension: -

Multiple pregnancies most likely trigger two processes that cause premature labor. When the uterus is too distended, the contraction-related protein and factor—all of which have been demonstrated to be sensitive to mechanical stretch—that mediates cervical ripening are prematurely upregulated. Multiple pregnancy is linked to multiple placenta, which causes the circulation's level of placental CRH to increase early [2, 3].

Prediction of preterm labour

Preterm birth risk in pregnant women is anticipated for both screening and diagnosis using a combination of patient features, symptoms, physical signs, and investigation [19, 20].

For the risk of preterm delivery, there are two target populations of pregnant women that need to be assessed and treated:

Asymptomatic pregnant women, such as: Risk assessment [21, 22], maternal anthropometry [20], ultrasound measurement of cervical length [25, 26], cervico- vaginal fetal fibronectin [23,24], and testing for bacterial vaginosis [11].

1. Symptomatic pregnant women; which include:

Cervico-vaginal fetal fibronectin, corticotrophin releasing hormone [12,13,14,28,29], and salivary oestriol [27].

Serum CRH and ACTH level: -

The hypothalamic peptide corticotrophin-releasing hormone, which is abundantly produced in the human placenta and is present in high amounts in both maternal and fetal plasma throughout late pregnancy, regulates the pituitary-adrenal axis [13,14]. Multiple lines of evidence suggest that this hormone may control human parturition and fetal maturation. Positive feedback loops that start labor and parturition may be created by the interaction of CRH with oestrogen, adrenal hormones, prostaglandins, and oxytocin. [28]. Increased plasma concentrations of CRH and ACTH may be related to the pathophysiology of premature labor [29,30]. Numerous variables, including as ethnicity, stress, and hormonal placental changes, seem to be connected to CRH concentrations [12,13].

Prevention of preterm labour [2, 11]:

- Potentially effective intervention.
- Progesteronesupplementation.
- Smoking cessation.
- Reduce rate of multiple pregnancy.
- Cervical cerclage.
- Nutrition.
- Reduce occupational stress.
- Early diagnosis and treatment of infection.

Management's aims [2,11]:

- • Early recognition of the risk factors for preterm birth.Timely diagnosis of preterm labour.
- determining the cause of preterm labor.
- Evaluating fetal well-being.
- pharmacological prophylaxis is used to lengthen pregnancy and lower the risk of respiratory distress syndrome and intra-amniotic infection.
- Initiating tocolytic therapy when indicated.
- creating a plan for maternal and fetal surveillance and educating patients and healthcare professionals to improve newborn outcomes

Steps of Management:

Initial:

1. Preterm labor evaluation and laboratory research as specified.Treat genitourinary infections if present.
2. Bed rest.
3. Consider intravenous fluid for dehydration:
4. Treat underlying causes:
    1. Urinary tract infection.
    2. Consider ampicillin for group B-streptococcus.
5. Inform your primary care physician about a potential delivery.
6. Labour precautions:

1. Limit maternal Narcotics for pain control.
2. Anticipate malpresentations.
3. Complete cervical dilation may be less than 10 cm.
4. Elective cesarean <36 weeks offered in some settings.

7. Consider transport to tertiary center with NICU:
    1. Strongly consider if <34 weeks gestation.
    2. Contraindications [24,26]:
        A- Imminent delivery.
        B- Fetal Distress or maternal status unstable.
        C- No safe transport to referral center

Corticosteroids: -

1. Indications:
    1. Intact membranes at 24-34 weeks.
    2. PPROM without Chorioamnionitis at 24 to 32 weeks.
2. Mechanism:
    1. Promotes fetal lung maturity.
3. Preparations:
    1. Two doses of betamethasone 12 mg IM every 24 hours.
    2. Dexamethasone 12 mg IM twice every 12 hours.
8. Course and safety:
    1. Delay delivery at least 24-48 hours after steroids.
    2. No longer recommended to repeat weekly but administer only at first week [6].

According to studies conducted in France, betamethasone appeared to have a protective effect against periventricular leukomalacia while dexamethasone did not [1].

Tocolytic agents:-

In contrast to dexamethasone, betamethasone appears to have a protective effect against periventricular leukomalacia, according to investigations carried out in France [1 ,3 , 4].

Corticotrophin-releasing hormone (CRH):

Numerous lines of evidence point to this hormone's potential role in the regulation of human parturition and fetal maturation. Positive feedback loops that start labor and parturition may be created by the interaction of CRH with oestrogen, adrenal hormones, prostaglandins, and oxytocin [12]. The hypothalamus can release CRH in response to a variety of psychological or physical dangers, and CRH is crucial for coordinating the neuroendocrine response to stress. The central nervous system has regions where CRH serves as a neurotransmitter. Additionally, it is produced in numerous organs outside of the central nervous system, such as the stomach, ovary, and testis [13]. Large amounts of CRH are produced by the placenta and fetal membranes during human pregnancy. By term, there are significant quantities of CRH in the blood of both the mother and the fetus as well as in the amniotic fluid due to the exponential growth of CRH synthesis in both tissues with increasing gestation [26].

Production: - The major capillary plexus of the hypothalamo-hypophyseal portal system receives CRH at the median eminence from the neurosecretory terminals of parvocellular neuroendocrine cells that are connected with the paraventricular nucleus in the hypothalamus. The anterior lobe of the pituitary gland receives the CRH through the portal system, where it stimulates corticotropes to release adrenocorticotrophic hormone [12,13].

ACTION: -

Through the hypophyseal portal arteries, CRH is rapidly and directly delivered from the hypothalamus to the anterior pituitary [13]. The hypophyseal portal arteries allow the hypothalamus to quickly and directly discharge CRH into the anterior pituitary [32]. High levels of cortisol signal the hypothalamus to reduce the secretion of CRH, which is another way that the CRH is controlled. The central nervous system's extensive connectivity triggers the activation of the HPA axis [13,14,30,33].

Adrenocorticotrophic hormone ACTH:

High levels of cortisol signal the hypothalamus to reduce the secretion of CRH,

which is another way that the CRH is controlled. The central nervous system's extensive connectivity triggers the activation of the HPA axis [32, 33].

Structure: - The molecular weight of ACTH, which includes 39 amino acids and a total of 4500 atomic mass units, is 4500.

Action: - Glucocorticosteroids, mineralocorticoids, and androgenic steroids are produced and secreted when cell surface ACTH receptors on the adrenocortical cells of the adrenal cortex are stimulated by ACTH [12]. Glucocorticosteroids, mineralocorticoids, and androgenic steroids are produced and secreted when cell surface ACTH receptors on the adrenocortical cells of the adrenal cortex are stimulated by ACTH [13].

- ACTH must act at a number of key locations in the adrenal cortex in order to influence the steroidogenic pathway: In turn, this increases the bioavailability of cholesterol in the cells of the adrenal cortex. ACTH enhances the uptake of lipoproteins into cortical cells.
- The transfer of cholesterol into mitochondria that initiates hydrolysis is increased by ACTH.
- The rate-limiting step in steroidogenesis, cholesterol side-chain cleavage enzyme, is stimulated by ACTH. Pregnenolone is produced as a result [32, 33, 34].

Placental Corticotrophin-Releasing Hormone production:

CRH was discovered in the placenta soon after being found in the hypothalamus. Since the CRH gene is expressed in the placenta as well, the concentration of the peptide and its mRNA rises during pregnancy there as well, corresponding to an exponential rise in maternal plasma CRH levels [31]. The hypothalamus expresses the 41-amino acid CRH peptide, which is found in placental extracts [33].

Controlling the release of placental corticotrophin-releasing hormone:

Due to a particular increase in placental CRH gene expression, placental CRH synthesis increases dramatically throughout pregnancy [35]. Despite the fact that the placenta lacks nerve tissue and that circulating and locally produced humeral hormones are most likely the main factors influencing placental CRH release, The neurological system controls the hypothalamus's release of CRH [33]. When exposed to the cytokine polypeptide, prostaglandins E2 and F2, noradrenaline, interleukin (IL-1), and oxytocin, human syncytiotrophoblasts grown in culture can produce CRH. [36]. The cytokine polypeptide, prostaglandins E2 and F2, noradrenaline, interleukin (IL-1), and oxytocin can all induce the production of CRH in human syncytiotrophoblasts grown in culture. The positive feedback effect of glucocorticoids on placental CRH may be essential for parturition [29, 32]. Figure (1).

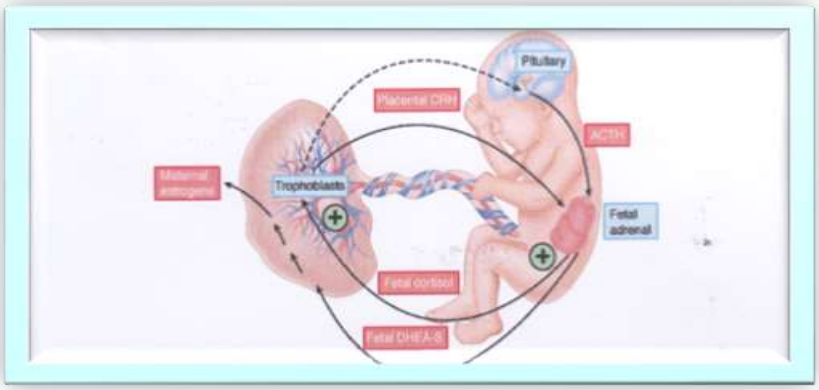

Figure (1): depicts the embryonic adrenal endocrine cascade from the placenta.

A critical link in the chain of events that starts parturition in humans is the production of DHEA and DHEA-S in the fetal zone. These androgenic hormones pass into the placenta and account for 80–90% of the substrate used to produce placental estrogen. These androgenic hormones start labor in the maternal compartment by affecting the uterus, cervix, and fetal membranes [4].

Fetal pituitary drenal axis

By the mid-trimester, the pituitary-adrenal axis is operating in the human fetus (12). Although placental CRH concentrations in the fetal circulation are roughly 10 times lower than those in the mother's blood, they are nevertheless sufficient to stimulate fetal pituitary ACTH secretion [34]. Under the impact of CRH's activation, the placenta produces ACTH. The fetal adrenal gland is stimulated to produce steroids by the hormone ACTH, which is produced by the pituitary and the placenta [12]. Dehydroepiandrosterone in addition to cortisol, the primary androgenic steroids generated by the embryonic adrenal gland are (DHEA) and its sulphate (13). Due to the fact that ACTH powerfully induces cortisol release, an increase in fetal cortisol synthesis in the last stages of pregnancy is essential for the development of fetal organ maturation before to delivery. The specialized fetal zone of the adrenal, which makes up more than 50% of the adrenal mass in late fetal life but totally involutes shortly after birth (12, 13), is where DHEA and DHEA-S are created. Fetal adrenals are able to release When CRH directly activates the fetal zone, DHEA and DHEA-S are produced in a dose-dependent manner without the assistance of ACTH. The rapid withdrawal of CRH during delivery may be what causes the fetal zone's post-natal disappearance because placental CRH may stimulate the fetal zone's hypertrophy throughout pregnancy [28]. One of the key steps in the procedures that cause human parturition is the creation of DHEA and DHEA-S in the fetal zone. These androgenic hormones, which make up 80–90% of the substrate for placental oestrogen production, are absorbed by the placenta. Oestrogen. The production of DHEA and DHEA-S in the fetal zone is one of the crucial steps in the processes that lead to human parturition. The placenta absorbs these androgenic hormones, which account for 80–90% of the substrate for placental oestrogen synthesis. Oestrogen [27].

Fetal Membranes and Amniotic Fluid:

CRH is expressed by every layer of the fetal membranes, and it is also present in the amniotic fluid. The amniotic fluid and all layers of the fetal membranes both express CRH [37]. Early in pregnancy, the amniotic fluid concentration is about comparable to that of maternal plasma; but, by late pregnancy and at term, it is comparable to that of fetal blood and substantially lower than in the mother's circulation [33,37]. CRH and prostaglandin production in the amniotic compartment may be significantly related. PGE2 and PGF2 are potent inducers of cervical maturation and uterine contractility that are produced in preparations by CRH in physiological levels [38]. These prostaglandins have the ability to encourage the placenta and fetal membranes to secrete even more CRH, creating a positive feedback loop. Additionally, CRH stimulates the production of interleukin 6 by peripheral blood mononuclear cells, which invade the fetal membranes, placenta, and cervix in increasing numbers after delivery and intrauterine infection. It has been demonstrated that IL-6 and IL-1, in turn, increase CRH secretion from cultured human placental trophoblasts [26].

Positive-Feedback Circuits in Human Parturition

Fetal cortisol and DHEA-S synthesis is stimulated by placental CRH, and these hormones then return to the placenta via the umbilical blood supply, where cortisol stimulates the release of more CRH. The fetal-placental unit would unavoidably be driven toward the end result of fetal maturation and delivery as this positive feedback loop was intensified over time [33, 26]. Activation of the fetal pituitary-adrenal axis in response to stress may hasten fetal organ maturation and feedback to the placenta to increase CRH secretion and promote the beginning of parturition in cases of fetal distress or compromise (intrauterine infection or hypoxia), providing a way to stop a threat to

fetal survival in utero [30,34,35,39]. In the amniotic compartment, a second feed-forward circuit is created where prostaglandins and CRH encourage one another's creation. When labor first begins, a positive feedback loop, however, clearly has advantages. The placenta and fetus must be born in order to break these amplification loops. The hormonal signals that urge parturition steadily intensify up until labor and delivery [26]. The hormonal mechanism (a negative feedback loop) that promotes uterine quiescence may switch to a positive feedback circuit at the beginning of parturition [39, 40].

2. Materials and Methods

From October 2022 to March 2023, 100 pregnant patients at Al-Kadhmiya Teaching Hospital participated in this case-control study. The Iraqi Scientific Council for Obstetrics and Gynecology's ethical committee gave its approval to the study. Every patient's consent was obtained.

- Of the 100 pregnant women, 80 were brought to the hospital's obstetrics and gynecology department with a diagnosis of impending preterm labor between (24-36+6) weeks of gestation. All patients had their demographic information collected, and details about the result of the birth were entered into a database. Out of 80 women, 64 had preterm births, while 16 had full-term pregnancies. All cases were monitored, and any complications that developed during the women's current pregnancies were noted.
- Twenty low-risk pregnant women were included in the study between weeks 24 and 28 of their second trimester (control group), and their serum was utilized as a measure of control. The control group consisted of healthy women free of any medical conditions or uterine contractions. Every woman in the control group gave birth to a healthy child at a gestational age greater than 37 weeks and with a pgar score greater than 7 at one minute.

Inclusion criteria: -

1. pregnancy with one healthy fetus.

2- The presence of regular uterine contractions lasting 30 seconds, induration occurring at a rate of four contractions every 30 minutes, and dilatation or effacement of the cervix are required for the diagnosis of impending preterm labor.

3 - Before any interventions, such as tocolysis and/or steroid injections, serum samples were taken.

Exclusion criteria: -

1- Preterm premature rupture of membrane.
2-Antepartum hemorrhage (Abruption+ previa).
3- Fetal and uterine anomalies.
4- Intra uterine growth restriction and fetal death.
5- Cervical dilation more than 4cm.
6- Women with HELLP syndrome.
7- Pre-eclampsia.
8- Diabetes mellitus.
9- Patient with cervical surgery.
10- Drugs and clinical sign of infection.

- Based on gestational age, the study's patient population was divided into three groups: 24-28 weeks, 29-32 weeks, and 33-36+6 weeks. the females' ages ranging from 19 to 35.
- Each patient's complete medical history was obtained, and the gestational age was calculated using the menstrual cycle and a first or early second trimester ultrasound. Patients who participated in this study got a general obstetrical checkup that included a pelvic and abdominal check, as well as an abdominal ultrasound and a general urine examination.

• The systemic digital cervix examination and uterine contractions were used to make the decision to admit the patient to the hospital. Additionally, corticosteroids and tocolytic treatment were administered based on the attending obstetrician's assessment. Calcium channel blockers were used as the tocolytic therapy for our patients.

Measurement of the CRH and ACTH level:

Peripheral blood samples were drawn into heparinized tubes, promptly centrifuged at 4 c for 20 min, and then incubated in a

microplate well with streptavidin coating. Samples were frozen at -20°C, and then 25 Reagent 1 (Biotinylated Antibody) and 25 Reagent 2 (Peroxides Enzyme Labeled Antibody) were added to each sample. The microplate was then covered to prevent light exposure, and it was placed on an orbital rotator set at (170 10) rpm for 4 hours. Aspirate the fluid and wash it five times with working wash solutions made from ELISA REAGENT A (ELISA Wash Concentrate). After allowing the samples to dry, we added 150 l of ELISA REAGENT B (tetra methylbenzidine) into each well and covered the microplate to prevent light exposure. We then added 100 l of ELISA REAGENT C (stop solution 1 N sulfuric acid) and gently mixed.In less than 10 minutes, we used a microplate reader set to 450 nm to measure the solution's absorbance against 250 ml of pure water.

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Statistical analysis: -

SPSS 21 and Microsoft Office Excel 2010 were used to analyze the data. The data were shown as mean + SEM. When comparing more than two groups, the ANOVA test was used to evaluate continuous (numeric) data; however, when comparing just two groups, the student test was utilized. The Pearson correlation coefficient was used to look into how ACTH and CRH are related. P-values below 0.05 were regarded as significant. To provide sensitivity and specificity values, the CRH and ACTH cut-offs were selected. For each test individually, predictive values were computed. The proportion of all disease-present patients with positive test results is known as a test's sensitivity. The proportion of healthy patients that get negative test results is known as a test's specificity. A test's predictive value is a proportion that shows the likelihood that the outcome, whether positive or negative, will actually occur. For instance, the positive predictive value is the percentage of all positive results that will actually occur as genuine positives.

3. The Results:

A total of 100 women participated in the trial, of which 20 low risk women served as the control group and 80 patients were admitted to the hospital with complaints of preterm labor. Table 1 displays the clinical characteristics of the study groups, including age, gravidity, parity, gestational age, and neonatal birth weight. In the three gestational age groups (24–28, 29–32, and 33–36+6 weeks), there was no difference in the mean gestational ages between women who delivered preterm babies and those who had a successful delivery at term. There was a substantial difference in birth weight between term and preterm births, although age, gravidity, and parity did not differ significantly across the groups.

Table -1- Maternal demographic characteristics in the study groups.

Groups	**24-28 weeks NO.=20**		**29-32 weeks NO.=30**		**33-36^{+6} weeks NO.=30**	
Cases	**Term 5**	**Preterm 15**	**Term 6**	**Preterm 24**	**Term 5**	**Preterm 25**
Age	**27.25±3.75**	**27.75±4.08**	**28.10±4.45**	**27.93±4.31**	**27.80±3.75**	**28.20±4.50**
Gravidity	**2.75±1.82**	**2.94±1.95**	**3.30±2.40**	**2.94± 1.95**	**3.30±2.40**	**2.94±1.95**
Parity	**1.77±1.65**	**1.73±1.84**	**1.77±1.65**	**2.23±2.30**	**1.73±1.84**	**2.23±2.30**

Neonatal birth wt.	**3450.20±105.6**	**898.00±89.2**	**3370.30±115.6**	**1246.00±105.6**	**3505.35±85.8**	**2164.33±89.8**

Table 2 shows that maternal blood levels of CRH and ACTH were significantly higher in women who delivered preterm babies than in patients who delivered healthy babies in each of the three gestational age groups (p 0.001).

Table -2- Levels of CRH and ACTH in the research groups.

Groups	**24-28 weeks n=20**		**29-32 weeks n=30**		**33-36^{+6}weeks n=30**	
Cases	**Term n=5**	**Preterm n=15**	**Term n=6**	**Preterm n=24**	**Term n=5**	**Preterm n=25**
CRH (pg/ml)	**12.6±3.2**	**18.30±2.81**	**13.9±7.2**	**31.63±6.56**	**23.7±25.5**	**95.03±2.76**
ACTH (pg/ml	**13.5±3.5**	**21.20±2.63**	**14.2±6.1**	**23.27±2.03**	**14.9±6.5**	**26.63±3.65**

Table 3 (p 0.001) displays the range of CRH and ACTH levels throughout three gestational age groups and the P value for patients who had preterm deliveries.

Table 3 shows the P value and the CRH and ACTH range.

	Groups	**Minimum**	**Maximum**	**P-Value**
CRH	**24 - 28 w**	**14.00**	**22.00**	**<0.001**
	29 - 32 w	**23.00**	**44.00**	
	33 - 36^{+6}w	**91.00**	**100.00**	
ACTH	**24 - 28 w**	**18.00**	**26.00**	**<0.001**
	29 - 32 w	**20.00**	**28.00**	
	33 -36^{+6}w	**22.00**	**32.00**	

The data from the gestational age groups of preterm babies born at (24-28w), (29-32w), and (33-36+6 w) show that the amount of CRH in maternal plasma increases significantly close to the end of gestation (Fig. 1; p0.001).

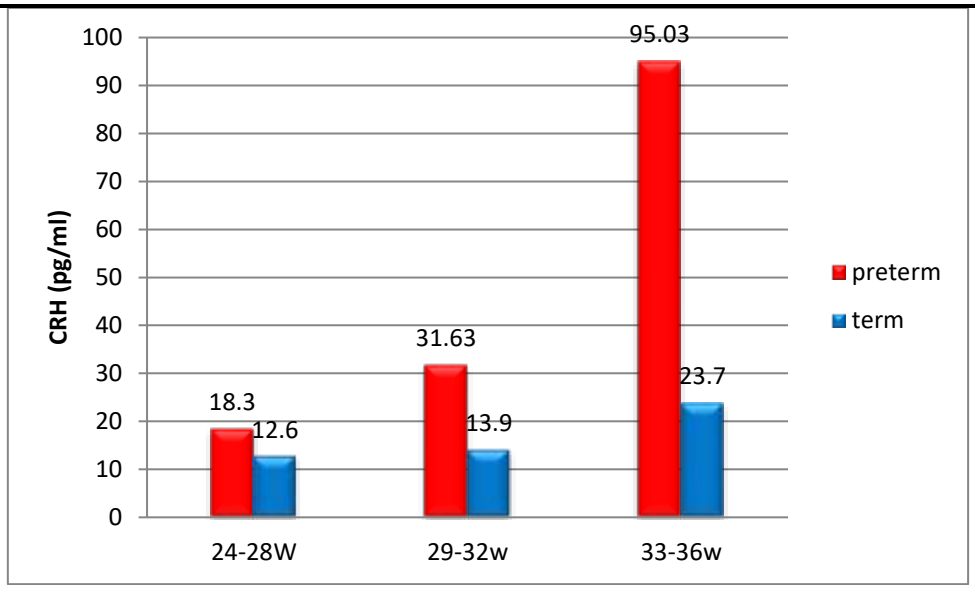

Fig-1-Mean of CRH levels in three gestational age groups.

According to the findings obtained from the (24-28w), (29-32w), and (33-36+6 w) gestational age groups who gave birth preterm, the maternal plasma ACTH level rises considerably near the end of gestation (Fig. 2, p0.001).

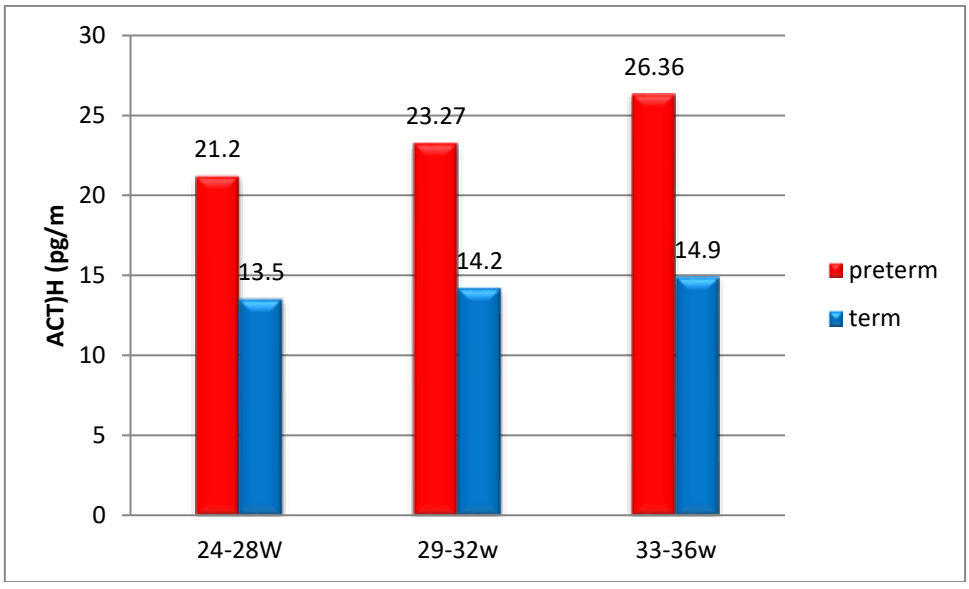

Fig-2-Mean of ACTH levels in three gestational age groups.

Fig. 3 compares the CRH levels between the control group and patients who were about to go into preterm labor from the 24th to the 28th week of pregnancy. Preterm birthing women have significantly higher mean serum CRH concentrations than term birthing women (18.3pg/ml versus 10.1pg/ml, respectively). Furthermore, the CRH values of the control women were considerably (p 0.001) lower than the corresponding values of the women in the 24- to 28-week group who had their kids early.

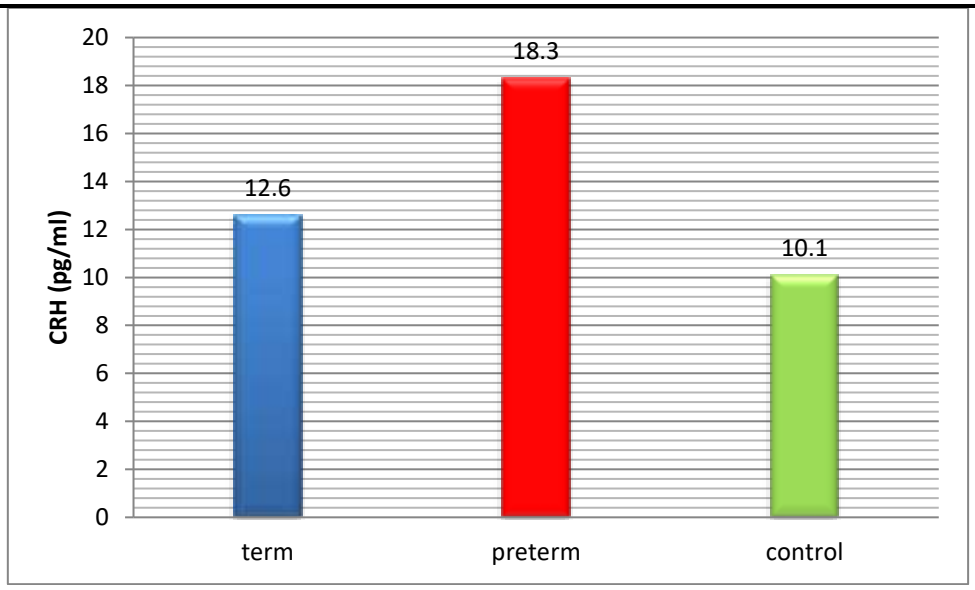

Fig.-3-CRH levels in (24-28 weeks) patients and control group.

In Fig. 4, Between the 24th and 28th week of gestation, the ACTH levels of patients who are about to go into preterm labor are compared to those of the control group. Women who give birth preterm have mean serum ACTH concentrations that are substantially greater than those who give birth at term (21.2pg/ml versus 12.79pg/ml, respectively). Additionally, the control women's ACTH levels were significantly lower than those of the women in the 24- to 28-week group who delivered babies too soon (p 0.001).

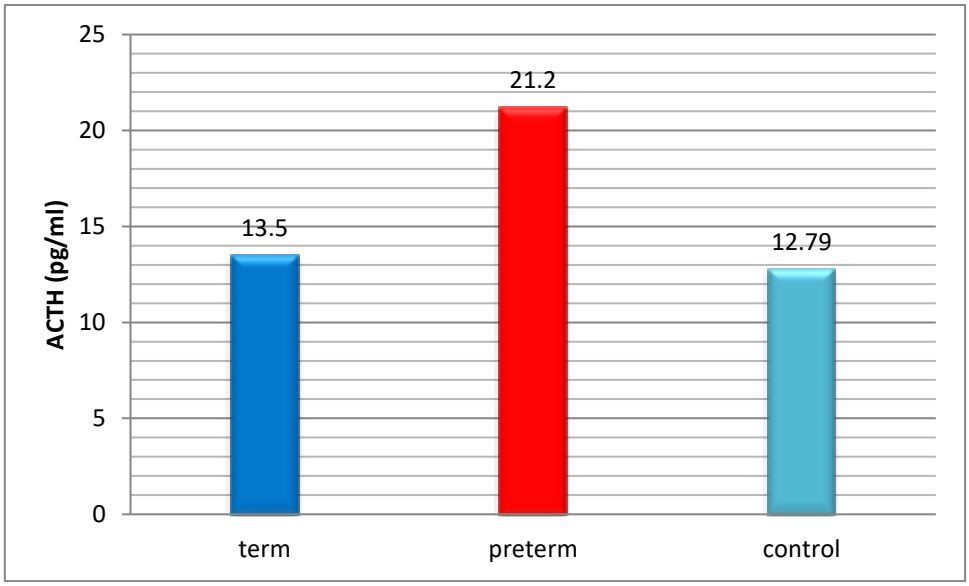

Fig. 4- ACTH level in (24-28 weeks) patients and control group.

The sensitivity and specificity of CRH and ACTH are shown in Table 4. Using a cutoff of 10.45 pg/ml for CRH and a cutoff of 14.65 pg/ml for ACTH. The sensitivity and specificity of CRH and ACTH are 80% and 100%, respectively (positive predictive value is 100%, negative predictive value is 55.56%).

Table-4- demonstrates CRH and ACTH's sensitivity and specificity.Using a cutoff of 10.45 pg/ml for CRH and a cutoff of 14.65 pg/ml for ACTH. The sensitivity and specificity of CRH and ACTH are 80% and 100%, respectively (positive predictive value is 100%, negative predictive value is 55.56%).

Table -4- The sensitivity and specificity of CRH and ACTH.

Predictor	Sensitivity. (%(95%CI)	Specificity. (%(95%CI)	PPV. (%(95%CI)	NPV. (%(95%CI)	Accuracy.
CRH pg/ml	80%	100 %	100%	55.56%	84%
ACTH pg/ml	80%	100 %	100%	55.56%	84%

4.The Discussion: -

The primary cause of perinatal deaths, preterm labor, is a serious global health issue. Additionally, premature labor can be seen as a major social issue because it may put an end to many families' hopes of having healthy children. Determining the preterm labor predicting factor is crucial. New preterm birth risk markers have been proposed as main predictors in low risk populations in recent years. They might be more pertinent than traditional markers, which would increase the effectiveness of our management (34, 36). The effectiveness of CRH and ACTH in predicting preterm labor has been the subject of numerous studies published worldwide.

According to the results of our study, women who have been given the prognosis of approaching preterm labor had increased levels of CRH and ACTH in their maternal serum. While unwell mothers who had term babies had significantly lower serum levels of CRH and ACTH. Our study found that women whose preterm deliveries were predicted to be life-threatening had higher levels of CRH and ACTH in their maternal serum. Women with the same condition who gave birth to term babies had significantly lower CRH and ACTH serum levels than those who did not.

. The study that showed how placental CRH can increase fetal cortisol production and act as a positive feedback mechanism to increase placental CRH production. High amounts of CRH may influence myomaterial contractility by interacting with the CRH receptor isoform, which is known to improve myomaterial contractile response. Cortisol may also have indirect effects on myomaterium by promoting the production of prostaglandins in the fetal membranes. [41].

Kalantaridou S.N. and others (2010). which concentrate on the possible effects of CRH on the pathophysiology and physiology of implantation, fetal immunological tolerance, parturition, and fetal programming of the hypothalamic-pituitary-adrenal axis. According to certain theories, the "CRH placental clock" regulates the duration of pregnancy as well as the time of parturition and delivery [42].

Jeanne Ruiz and colleagues (2011). The correlations between felt stress, specific clinical risk factors, and the amount of corticotropin-releasing hormone in maternal plasma and preterm labor and gestational age at birth were examined, as well as the prognosticative abilities of these variables. Combining the assessment of stress with the detection of CRH from maternal plasma may improve the identification of pregnant women at risk for preterm delivery [43].

BD Pearce and colleagues (2010). Studies on biological mediators (cytokines, stress hormones), psychological variables, obstetric history, and demographic variables have all been suggested in the early prediction of preterm birth [44].

In a study undertaken in 2009 by Roger Smith and others, 500 pregnant women were followed from the time of their first prenatal consultation until birth. Maternal blood samples were examined for the ratios of estradiol to estriol, progesterone to estriol, and progesterone to estradiol as well as the association between corticotrophin-releasing hormone (CRH) and estriol concentrations in the placenta during the last month before to the onset of labor. There was a substantial positive association between CRH and estriol levels in late pregnancy, and preterm singletons had much higher percentage daily

variations in CRH levels at 26 weeks compared to their term counterparts [45].

According to Thomas Vrekoussis and associates (2010). which emphasizes how the hypothalamic-pituitary-ovarian (HPO) axis is inhibited at the levels of the hypothalamus, pituitary, ovary, and uterus, among other suppressive effects on female reproduction caused by the stress system. Studies on humans have demonstrated that harmful prenatal stimuli, whether of maternal or fetal origin, that impact the growing embryo in utero can lead to both short- and long-term health issues. Some of these include low birth weight, premature delivery, the beginning of adult diseases including the metabolic syndrome, and other defects in neurodevelopment [46].

T. Field et al. (2008). study done recently on potential psychological, physical, and biological indicators. Depression, anxiety, challenging relationships, and a lack of supportive social networks are a few examples of psychological stressors. C-reactive protein, cortisol, and fetal fibronectin are examples of biochemical predictors. An investigational strategy that connects a preterm intervention to a prematurity predictor [47]. According to our study, Manscuo RA et al., Wadhwa PD et al., Mendelson CR et al., Rich Edwards et al., and Zhang LM et al.'s study focuses on the connection between maternal prenatal anxiety and CRH related to timing of deliveries [48,30, ,49,50,31].

Emily.Harville et al. (2009) studied the association between preterm birth and stress indicators and hormones like cortisol and corticotropin-releasing hormone, which was different from our study. It is uncertain if psychological parameters are empirically linked to stress biomarkers in pregnant women, despite the fact that these hormones have been interpreted as biomarkers of stress. In contrast to our research, Emily.Harville et al. (2009) employed stress markers and hormones such cortisol and corticotrophin-releasing hormone to evaluate the relationship between premature birth. Although these hormones have been considered to be indicators of stress, it is unknown whether psychosocial interventions are experimentally linked to stress biomarkers in expectant mothers [51].

Additionally, in contrast to our study, Aurelija Klimaviciute et al. (2006) split 67 women into five groups: those in preterm labor, those in term labor, those in term labor but not in labor, and those who were not pregnant. Biopsies of the cervical, isthmic, and fundal tissues were all taken (from only non-pregnant patients). The relevant proteins were identified and the levels of mRNA were measured using immunohistochemical analysis. Pregnant participants' tissues contained less CRH-R1 and CRH-R2 mRNA than non-pregnant subjects did. As a result, it was unable to tell the difference between the preterm and term groups. [52]. In addition to Sibai 2005. analyzed a study in which women at risk for preterm delivery were not predicted to have it by the level of maternal plasma CRH at 16 to 20 weeks of pregnancy [53].

Amar Chatterjee, et al. (2006) published a study that is similar to ours on the involvement of ACTH in the prediction of preterm labor. Studies have shown that stress brought on by persistent worry, depression, or physical effort consistently activates the hypothalamic-pituitary-adrenal axis. The hypothalamic-pituitary-gonadal axis is adversely impacted by each HPA axis component, including CRH, ACTH, B-endorphin, and glucocorticoids, which reduces fertility[54].

J.D. Iams and others, 2007. They found that parturition appeared to involve the fetus as well. It is thought that the mature fetal hypothalamus secretes more corticotrophin-releasing hormone, which in turn prompts the adrenals to produce cortisol and ACTH. These pathological modifications lead to preterm birth [55].

Sandman and others (2006). Recognize the connection between stress hormones (ACTH, cortisl) and their effects by an increase in preterm births or an increase in the maternal blood level of CRH during pregnancy [56].

Sandman and others (2006). Recognize the connection between stress hormones (ACTH, cortisl) and their effects by an increase

in preterm births or an increase in the maternal blood level of CRH during pregnancy [12].

4. The Conclusion: -

Symptomatic women with impending preterm births exhibited significantly greater maternal blood levels of CRH and ACTH compared to women who delivered their babies at term. They use assays that are incredibly accurate and sensitive.

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