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Original article

The Measurement of *Cortisol* Levels in the Blood Pregnant Trimester III With ELISA (*Enzyme Linked Immunosorbent Assay) Method*

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Abstract:

Background: During pregnancy, maternal cortisol increases 2-4 times. Every type of body response in the form of stress, both physical and psychological stress can increase ACTH secretion, which in turn can increase cortisol levels.

Methods: This study uses quasi experimental / quasi experimental one group design pre and post test design with group control. The sample in this study was 24 respondents. . Cortisol level examination tool uses a microplate reader with the ELISA examination method.

Results: There was a significant difference ($p = 0.000 < \propto$) of the average cortisol level in the control group before observation (54.43 ± 25.86) and after observation (459.15 ± 28.08Whereas in the treatment group showed a significant difference ($p = 0.004 < \propto$) the average cortisol level before being treated (65.26 ± 28.74) and after being treated (53.65 ± 23.68) Yoga exercises.

Conclusion: There was a significant difference ($p = 0{,}000 < \propto$), mean cortisol levels in the control group before observation (54.43 ± 25.86) and observations after (459.15 ± 28.08), whereas in the treatment group there were significant differences ($p = 0.004 < \propto$) it can be concluded that prenatal yoga reduces cortisol levels before and after treatment.

Keywords: Cortisol, blood level, ELISA, pregnant, Trimester III

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Introduction

Cortisol is the main product of the hypothalamus-pituitary-adrenal (HPA) axis, secreted both in response to threats and in a strong and consistent pattern throughout the day (diurnal cortisol rhythm). Cortisol has many effects on the body, including building up energy and shutting down systems that are not essential for dealing with threats, and also affect memory and mood[1,2].

Under normal circumstances, stress hormones are released in small amounts throughout the day, but when faced with stress these hormone levels increase dramatically (in the range of 5-20 times). Each type of body response in the form of stress, both physical stress and psychological stress can increase secretion ACTH, which can ultimately increase cortisol levels. In conditions of anxiety, anxiety and depression, cortisol secretion increased[3,4,5] Increased cortisol affects the function of almost every cell and tissue in the body by activating glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) that modify gene expression programs through direct binding to specific DNA sequences and recruitment of transcription complexes[6,7].

Several clinical studies have examined stressors that occur naturally and most report insignificant or low relationships. This is especially true for studies that use a single measure of cortisol from amniotic fluid, serum or saliva. Ecological momentary assessments are used to simultaneously assess mood and cortisol 5 times a day for 3 days in 83 women (6-37 weeks gestational age). possible cortisol as a biological relationship between maternal psychological stress during pregnancy and fetal development[8,9].

The results from multilevel analysis showed a strong relationship between negative mood and cortisol. For every 1.0% increase in negative moods there is an appropriate 1.9% increase in cortisol. This association was not affected by advanced gestational age cross-sectional studies at the end of pregnancy in 79 women to investigate the relationship between the factors involved in anxiety and depression[10,11,12].

Some regression analysis shows that depressed subjective feelings explain more than 50% of the variation of BDI and STAI scores. Plasma cortisol is not a significant predictor of psychometric scores and did not show a significant correlation with them in correlation analysis, and subjects with low and high cortisol levels showed the same psychometric scores. The mechanism that mediates the effects of subjective pressure remains unclear: candidates who might include monoamine neurotransmission and / or changes caused by stress in the expression or distribution of glucocorticoid receptors[13,14]..

Research to test one potential mechanism by examining the relationship between pregnancy anxiety and maternal salivary cortisol on 4 occasions during pregnancy in a sample of 448 women. Strong evidence from animal models shows that maternal pressure predicts increased fetal glucocorticoid exposure through synthesis and release of maternal glucocorticoids but similar evidence in humans that links pregnancy anxiety (or general maternal pressure) with increased levels of maternal cortisol during pregnancy is still lacking[5,9]

During pregnancy, maternal cortisol increases 2-4-fold, and crosses the placenta but is limited because the pathway is regulated by the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD2: Maternal cortisol accounts for about 30-40% of the variability in fetal cortisol concentration. Physiological increase in glucocorticoid levels during the pregnancy, facilitating fetal organ maturation by promoting cell differentiation Synthetic glucocorticoids (including dexamethasone or

betamethasone) are given during the third trimester of pregnancy at risk of preterm delivery to increase the survival rate and maturation of the lungs[15,16]. Based on these references, researchers are interested in examining cortisol levels in pregnant women with prenatal yoga treatment.

MATERIALS AND METHODS

This study used quasi experimental one group design pre and post test design with group control. In this study the researchers' treatment or intervention was prenatal Yoga exercises in third trimester pregnant women (UK 28-40 weeks) ,The sample in this study was 24 respondents.

Cortisol examination procedure;

a) Prepare samples, reagents, main solutions and equipment
b) Let the blood sample freeze for 2 hours at 4 ° C. Do it
c) Centrifugation for 15 minutes, then immediately assay checks or can be divided and stored at temperatures <-20 ° C (≤ 1 month), or -80 ° C (≤ 6 months). Blood tubes must be disposible, sterile.
d) Reagents are prepared at room temperature
e) Dilute 30 ml of WBC to 750 ml of wash buffer with distilled water
f) If crystalline deposits are found in the Wash Buffer Consentrate, warm at 40 ° C and shake gently until the crystals dissolve.
g) The solution must be cooled to room temperature before use
h) Standard Solution
    1. Prepare a standard solution 15 minutes before use
    2. Standard with 1 ml Sample Diluent, allow 10 minutes to dissolve.
    3. This reconstruction produces 40 ng / ml stock solution. Then make serial dilutions (40, 20, 10, 5, 2.5, 1.25, 0.63, 0 ng / mL).
i) Procedure:

1. Add 50 µl of standard or sample to each microplate hole.
2. Add 50 µl Biotinylated Detection AB. Incubation 45 minutes at 37 ° C
3. Aspiration and wash 3 times
4. Add 100 µl HRP Conjugate. Incubation 30 minutes at 37 ° C
5. Aspiration and wash 5 times
6. Add 90 µl Substrate Reagent. Incubation for 15 minutes at 37 ° C
7. Add 50 µl Stop Solution. Read at 450 nm right away.
8. Calculation of results in the form of OD values. Real values or concentrations are generated by entering OD values in standard curves

Table 1 Characteristics of education level and type of work

Variable	Group		*p-value*
	Control (%)	Treantment (%)	
Level education			**0.590**
– SMA	1 (8.3%)	3 (25%)	
– D3	11 (91.7%)	9 (75%)	
Type work			
– House wife	10 (83.3%)	11 (91.7%)	**1.000**
– Wiraswasta	2 (16.7%)	1 (8.3%)	

The results level education test analysis on the distribution of data on the education level of pregnant women in Table 3 showed no significant difference (p = 0.590> ∝) which was divided into 2 levels of education. Namely: high school and D3. Whereas there was no significant difference in the type of work (p = 1.00> ∝)

Table 2: The Comparison Of Cortisol Levels Before And After Treatment

Group observation	Before Rerata ± SD	After Rerata ± SD	*p-value*
Control	54.43±25.86	459.15±28.08	0.000
Treatment Yoga	65.26±28.74	53.65±23.68	0.004

Table 2 shows that there is a significant difference (p = 0,000 <∝) of the average cortisol level in the control group before observation (54.43 ± 25.86) and after observation (459.15 ± 28.08). it means Whereas in the treatment group showed a significant difference (p = 0.004 <∝) the average cortisol level before being treated (65.26 ± 28.74) and after being treated (53.65 ± 23.68) Yoga exercises

Table 3 The Correlation Test Results Between Variables In The Group Treatment After Yoga Exercises

Correlation of variable	n	Koefisien korelasi (r)	*p-value*
Level anxiety and kortisol	12	0.853	0.000
Cortisol With FKBP5	12	0.937	0.000

DISCUSSION

In the results of this study the treatment of Yoga exercises in third trimester pregnant women can significantly reduce cortisol levels. So the third minor hypothesis has been proven, that is, there is a difference in effect between non-action and prenatal Yoga exercises on cortisol levels in primigravida trimester III.

In line with previous studies where anxiety disorders were associated with increased levels of basal cortisol in saliva as well as in plasma. Stress conditions during pregnancy will affect hormonal changes and fetal growth, especially in the hormone cortisol and will result in impaired fetal growth such as low birth weight and abnormal body length. Several studies have shown that depression is associated with hyperactivity from the hypothalamic-pituitary-adrenal (HPA) axis which results in high cortisol levels[17,18,19].

During pregnancy, maternal cortisol increases 2-4 times. The physiological increase in glucocorticoid levels during pregnancy, facilitates maturation of the fetal organs by promoting cell differentiation. Synthetic glucocorticoids (including dexamethasone or betamethasone) given during the third trimester of pregnancy are at risk of preterm delivery to increase the survival rate and maturation of the lungs[8,110,114].

Research conducted on white rats (Rattus Norvegicus) that formation of cortisol and cortisol secretion increased six times in a mouse within 4 to 20 minutes after fracture of the two bones in his legs which caused the rat to be stressed. This is in line with the literature that stress can cause an increase in ACTH secretion by anterior pituitary gland followed by an increase in cortisol hormone secretion within a few minutes, prenatal yoga reduces anxiety and decreases cortisol levels. [20]

Stressors activate the hypothalamic-pituitary-adrenal (HPA) axis, which triggers the secretion of glucocorticoid hormones (cortisol for humans and corticosterone for mice) to drive systemic changes in physiological patterns, which aim to return the body to homeostasis. Exposure to high levels of glucocorticoids can cause changes in fetal programming, as well as affect the development of glucocorticoid receptors in the fetal brain. For mothers, glucocorticoids induce physiological and behavioral changes that can interfere with hereditary care[4,9,13].

Initially, stress causes an increase in diurnal cortisol, facilitating the recruitment of energy and coping resources. However, repeated or sustained increases in cortisol can cause damage to the negative feedback system of cortisol secretion, resulting in flat slopes and negative clinical and health outcomes[13,20]. Heterogeneity in the relationship of chronic stress to diurnal slope indicates that some individuals are more susceptible than others to a disturbance in the healthy relationship between stress and cortisol secretion. One factor that might be a genetic risk. [21,22]

When cortisol levels begin to fall, the inhibitory effect of cortisol on the hypothalamus and anterior pituitary decreases so that the factors that stimulate increased cortisol secretion (CRH-ACTH) will increase[9]. This system is sensitive because cortisol production or excessive administration of cortisol or other synthetic glucocorticoids can quickly inhibit the hypothalamic-pituitary axis and stop the production of ACTH[23,24].

This study contradicts previous results, in which researchers investigated whether anxiety disorders were associated with the HPA axis by using a new method of assessing cortisol in hair for a method that was considered to provide a retrospective cortisol index of secretion over a long period of time. The results showed lower hair cortisol levels in anxiety disorder patients than in healthy controls. This interpretation tentatively indicates the condition of anxiety disorders is associated with hypocortisolism[3,25].

The hypoactive HPA axis model has been used to explain adrenocortical activity**R** in other psychiatric and psychosomatic disorders as well as in stress illustrating that participants who exhibit hypocortisolism often exhibit a series of symptoms characterized by increased sensitivity to stress, pain and fatigue[26]. which is characterized by increased sensitivity to stress, pain and fatigue. The pathway that leads to hypocortisolism is likely to result from exacerbated worries, higher tension and anxiety may lead to an initial increase in adrenocortical activity, namely hypercortisolism. With the increase in the mechanism of chronic compensation becomes activated and gradually produces a weakening of cortisol secretion, namely hypocortisolism. [16,20]

CONCLUSION

There was a significant difference ($p = 0.000 <\propto$), mean cortisol levels in the control group before observation (54.43 ± 25.86) and after observations (459.15 ± 28.08), whereas in the treatment group there were significant differences ($p = 0.004 <\propto$) cortisol levels before being treated (65.26 ± 28.74) and after being treated (53.65 ± 23.68), it can be concluded that prenatal yoga reduces cortisol levels before and after treatment.

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Conflict Of Interest- None of the authors has competing interests

Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University Makassar, (No. 839/UN4.6.4.5.31//PP36/2019), and all research subjects gave written informed consent.

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