*Original article*

# The Analysis Effect Prenatal Yoga and the Expression gen MRHR-CRHR1 and mRNA FKBP5 with Real Time-PCR

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## Abstract

**Background:** Stress triggers the release of corticotrophic release factor (CRF) from the hypothalamus which in turn stimulates the anterior pituitary to release adrenocorticotrophic hormone (ACTH).

**Methods:** The design of this study is a quasi-experimental approach / quasi experimental and one group design pre and post test design with group control. The sample in this study amounted to 24 respondents

**Results**: Based on the results of the study showed that the sample LA24 (7,935) was the highest sample fold fold change and the lowest in the sample LA15 (6,194). Whereas in the post test results it was found that the LB18 sample (10,580) was the highest sample in fold change and the lowest was in the LB15 sample (8,472).

**Conclusion:** There is a significant relationship / correlation between CRHR1 levels and FKBP5 levels ($p = 0.001 <\propto$). This is indicated by the high / strong correlation coefficient value which is 0.846. A positive value at 0.846 indicates that there is a positive relationship or a concomitant relationship, namely the influence of yoga exercises will result in a decrease in CRHR1 levels and will further reduce the effect of FKBP5 levels in third trimester pregnant women.

***Keywords:*** *prenatal yoga, CRHR1 gene, FKBP5 gene , real time-PCR*

## Introduction

Pregnancy is a natural process, because it is natural for a woman to become pregnant and give birth, this will be a new experience for newly married women[1]. Pregnancy is a period when biomechanical and physiological changes occur quickly when the body adapts to support a developing fetus. Every woman experiences pregnancy with different experiences and with various physical and psychological symptoms that are interrelated[2].

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Mental disorders afflict about 450 million people worldwide Among mental disorders anxiety has as much as 31.2% in the general population: 36.4% for women and 25.4% for men. explained in his study that 54% of women reported anxiety in pregnancy. Anxiety in pregnancy not only affects the health of pregnant women but also affects the outcome of labor such as premature labor, prolonged labor, cesarean delivery, low birth weight, revealed the prevalence of pregnancy anxiety varies in different pregnancy trimesters with high levels in the first trimester and third trimester[3,4].

Stress is responded by activating the cardiorespiratory system, the locus ceruleus system (LC / norepinephrin (NE), the metabolic system and the HPA axis. hypothalamus-paryutary-adrenal Axis

(LHPA axis), then stimulates the hypothalamus and causes secretion of the hormone corticotrophin relesing hormone (CRH), stimulates the hypothalamus for ACTH secretion, increased ACTH secretion, causes increased secretion, cortisol. The hormone is released to maintain homeostasis in dealing with stress, both physical and psychological[5,6,7] Cortisol is assumed to be a stress biomarker, representing HPA system activity. Cortisol is a variable cause of stress conditions and will ultimately have a negative effect on immune function[8,9,10]..

Stress triggers the release of corticotrophic release factor (CRF) from the hypothalamus which in turn stimulates the anterior pituitary to release adrenocorticotrophic hormone (ACTH). ACTH then binds and stimulates the adrenal cortex to release glucocorticoids (cortisol in humans, corticosterone in animals)[11,12,13]. Glucocorticoids act on mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) located in several brain regions such as the hypothalamus and hippocampus to initiate negative feedback which weakens further release. Glucocorticoids can also inhibit telomerase activity (TEL) which can cause a reduction in telomere length (TL)[14,15].

The benefits of yoga for pregnancy can be categorized into physical, mental and spiritual benefits, among others, the physical benefits of pregnant yoga through yoga postures (asanas): Practicing good posture, robust and strong throughout pregnancy, Smoothing blood flow, smoothing the supply of oxygen, nutrients and vitamins from food to the fetus, Strengthens the back muscles, makes it stronger to support the burden of pregnancy and the pelvic floor-perineum which functions as a birth muscle, so that it strengthens the burden of pregnancy and supports the bladder and large intestine[16,17].

In this study, researchers focused on the molecular regulatory genes for the GR function, namely the FKBP5 and CRHR1 genes. FKBP5 and CRHR1 are the main proteins that regulate stress responses. Where FK506 binding protein 5 encoded by genes (FKBP5) and G-protein coupled type-I CRH receptors encoded by genes (CRHR1), has been linked to several neuropsychiatric disorders (Mahon.PB, et al., 2013). CRHR1 was chosen in this study for several reasons, where CRH is one of the main stress factors in the CNS. CRHR1 encodes the G-protein coupled type I CRH receptor (CRHR1), a protein that is essential for activation of signal transduction pathways that activate mesolimbic and HPA axis responses to various types of stress. The CRHR1 receptor is very important for determining the initial HPA axis response to stressful events.

## Materials and Methods

This study uses quantitative research with quasi experimental / quasi experimental approaches and one group design pre and post test design with group control. The sample in this study amounted to 24 respondents.

**Criteria objektif:**

a. Type I CRH receptor (CRHR1) gene expression is gene expression measured quantitatively using the real time PCR method which is assessed by the ratio of the CRHR1 gene mRNA to the mRNA of the control gene (housekeeping gene)

b. FK506-binding protein 51 (FKBP5) gene expression is gene expression that is measured quantitatively using real time PCR method which is assessed by the ratio of mRNA between FKBP5 gene and mRNA from control gene (housekeeping gene).

Examination ekspresi with real time-PCR

a. **Examination ekspresi mRNA CRHR1 with real-time PCR**

1) CRHR1 F: ACCTCATCACCGCCTTCATCC and

2) CRHR1R: AGCAGCCCTCGCCAAACAT (Gene bankXM_020113112.1)

3) House keeping gene:

(a) β-actin F: GGAAATCGTGCGTGACATTAAG

(b) R: CCTCTGGACAACGGAACCTCT (gene Bank:

(c) HQ386788.1 )

QPCR conditions with a temperature of 95oC for 10 seconds and 60oC for 30 seconds as many as 40 cycles

b. **FKPBP5 mRNA expression check with realtime PCR**

1) FKPB5F: 5′-AAAAGGCCAAGGAGCACAAC-3′ and

2) FKPB5R: and 5′-TTGAGGAGGGGCCGAGTTC-3′

3) *House keeping gene:*

(a) B2 microglobulin

(b) F: 5′-CCAGCAGAGAATGGAAAGTC-3′

(c) R: 5′-GATGCTGCTTACATGTCTCG-3′

QPCR conditions with a temperature of 95oC for 10 seconds and 60oC for 30 seconds as many as 40 cycles

**Data processing and analysis** Shapiro-Wilk test, this test is subject to ratio scale data that is age, anxiety level, cortisol level, CRHR1 level, and FKBP5 level. Decision criteria by looking at the Sig or p-value, if the p-value> □ = 0

## Result

**Table 1 : FKBP5 level Control group of pregnant women without yoga**

| NO | Sample | Ekspresi (Fold change) | NO | Sample | Ekspresi (Fold change) |
|---|---|---|---|---|---|
| 1 | LA13 | 6.706 | 13 | LB13 | 9.417 |
| 2 | LA14 | 7.022 | 14 | LB14 | 9.414 |
| 3 | LA15 | 6.194 | 15 | LB15 | 8.472 |
| 4 | LA16 | 6.588 | 16 | LB16 | 10.369 |
| 5 | LA17 | 7.416 | 17 | LB17 | 9.190 |
| 6 | LA18 | 7.531 | 18 | LB18 | 10.580 |
| 7 | LA19 | 6.997 | 19 | LB19 | 8.611 |
| 8 | LA20 | 6.228 | 20 | LB20 | 10.145 |
| 9 | LA21 | 7.286 | 21 | LB21 | 9.450 |
| 10 | LA22 | 7.931 | 22 | LB22 | 9.404 |
| 11 | LA23 | 7.389 | 23 | LB23 | 8.596 |
| 12 | LA24 | 7.935 | 24 | LB24 | 9.565 |

Based on the data in table 1 shows the sample LA24 (7,935) is the highest sample fold fold change and the lowest in the sample LA15 (6,194). While in the post test results it was found that the LB18 sample (10,580) was the highest sample in fold change and the lowest was in the LB15 sample (8,472).

**Table 2: Comparison of CRHR1 levels Pre and post test between control and Treatment**

| Level CRHR1 (pre) (control) | Level CRHR1 (post) (control) | Level CRHR1 (pre) (Treatment ) | Level CRHR1 (post) (Treatment ) |
|---|---|---|---|
| 5.333 | 8.835 | 6.854 | 5.204 |
| 6.183 | 8.235 | 5.613 | 5.141 |
| 5.401 | 7.560 | 6.408 | 5.324 |
| 5.409 | 9.457 | 5.515 | 5.434 |
| 5.530 | 9.020 | 5.854 | 5.599 |
| 5.658 | 8.178 | 6.774 | 5.485 |
| 6.351 | 9.212 | 6.351 | 5.648 |
| 5.826 | 8.439 | 6.300 | 5.697 |
| 6.633 | 7.800 | 6.165 | 5.784 |
| 6.565 | 9.321 | 6.617 | 6.230 |
| 5.173 | 8.327 | 5.467 | 6.349 |
| 5.399 | 8.603 | 6.949 | 6.583 |

Based on table 2 shows that CRHR1 levels in pre and post control have increased, as well as in CRHR1 levels both in pre and post test overall decreased means that there is a yoga effect on primagravidae mothers.

**Table 3: Comparison of CRHR1 levels Pre and post test between control and Treatment**

| Level FKBP5 (pre) (control) | Level FKBP5 (post) (control) | Level FKBP5 (pre) (Treatment) | Level FKBP5 (post) (treatment) |
|---|---|---|---|
| 6.706 | 9.417 | 6.367 | 6.105 |
| 7.022 | 9.414 | 7.743 | 6.173 |
| 6.194 | 8.472 | 7.898 | 6.576 |
| 6.588 | 10.369 | 6.877 | 6.399 |
| 7.416 | 9.190 | 7.323 | 6.484 |
| 7.531 | 10.580 | 7.438 | 7.188 |
| 6.997 | 8.611 | 7.782 | 6.622 |
| 6.228 | 10.145 | 7.400 | 7.277 |
| 7.286 | 9.450 | 7.502 | 7.331 |
| 7.931 | 9.404 | 7.903 | 7.454 |
| 7.389 | 8.596 | 7.916 | 7.406 |
| 7.935 | 9.565 | 8.105 | 7.564 |

**Table 4. Correlation Test Results Between Variables In The GroupTreatment After Yoga Exercises**

| Correlation variable | n | coefisien Corelasi (r) | p-value |
|---|---|---|---|
| Anxiety level with FKBP5 | 12 | 0.819 | 0.001 |
| Anxiety level with with kortisol | 12 | 0.853 | 0.000 |
| Anxiety level with dengan CRHR1 | 12 | 0.853 | 0.000 |
| Cortisol with FKBP5 | 12 | 0.937 | 0.000 |
| CRHR1 with KBP5 | 12 | 0.937 | 0.000 |
| Cortisol with CRHR1 | 12 | 0.956 | 0.000 |
| Cortisol with FKBP5 | 12 | 0.901 | 0.000 |
| CRHR1with FKBP5 | 12 | 0.846 | 0.001 |

According In Table 4. shows that there is a significant relationship / correlation between anxiety levels with FKBP5 levels ($p = 0.001 < \propto$). This is indicated by the high / strong correlation coefficient value which is 0.819. A positive value at 0.819 indicates a positive relationship or a concomitant relationship, namely the influence of yoga exercises will result in a decrease in anxiety levels and will further reduce the level of FKBP5 in third trimester pregnant women

## Discussion

The results of the study were between cortisol levels and FKBP5 levels ($p = 0.000 < \propto$). This is indicated by the high correlation coefficient value which is 0.937. A positive value at 0.937 indicates that there is a positive relationship or a concomitant relationship, namely the influence of yoga exercises will result in a decrease in cortisol levels and will further reduce the effect of FKBP5 levels in third trimester pregnant women. Furthermore, there was a significant relationship / correlation between CRHR1 levels and FKBP5 levels ($p = 0.000 < \propto$). This is indicated by the high correlation coefficient value which is 0.937. A positive value at 0.937 indicates that there is a positive relationship or a concomitant relationship, namely the influence of yoga exercises will result in a decrease in CRHR1 levels and subsequently will have an effect on reducing FKBP5 levels in third trimester pregnant women.

Stress induces activation of the HPA shaft, which is under the control of several neurotransmitter systems. corticotropin-releasing hormone (CRH) is released from the hypothalamus paraventricularis (PVN) nucleus into the portal venous system, where this hormone is transported to the anterior pituitary to stimulate the secretion of adrenocorticotropic hormone (ACTH) into the blood circulation. ACTH is transported to the adrenal gland cortex, where it activates the release of the primary stress hormone cortisol into the bloodstream[18.] Cortisol itself regulates the activation of the HPA axis through negative feedback by binding to glucocorticoid receptors (GR) in the anterior pituitary and in the hypothalamus. Chronic dysregulation of HPA axis activity in response to stress can be a risk factor for psychiatric illnesses such as depression, anxiety.[19]

In vitro experiments have shown that overexpression of human FKBP5 also reduces the hormone binding affinity and GR nuclear making it reasonable that changes in FKBP5 expression can also affect human GR function in vivo. FKBP5 expression is also induced by steroids, including glucocorticoids as part of an intracellular ultra-short negative feedback loop for GR activity. Increased transcription and translation of FKBP5 after activation of steroid receptors will then reduce the sensitivity of GR,[11,17,21.]

Given the fact that polymorphism is associated with higher levels of FKBP5 that lead to GR resistance

and negative feedback disturbances, one can speculate that FKBP5 alleles are associated with slower returns to baseline levels of stress-induced cortisol as well as increasing the risk of psychiatric related disorders with stress[5,8.,20].

Peripheral FKBP5 blood mRNA expression was also reduced in patients with PTSD, consistent with the increased GR response observed in this disorder and FKBP5 expression predicted by cortisol in studies of victims of the 9/11 attacks in New York City. Several studies have investigated the relationship between FKBP5 polymorphisms and mood disorders. Major depression has been associated with GR supersensitivity in high-induction allele carriers FKBP5. In studies in mice, prenatal stress has been associated with decreased FKBP5 expression in the prefrontal cortex and this is restored by administering antidepressant drugs[21].

FKBP5 mRNA levels in the ventral hippocampus are significantly regulated by stress. Indeed, Fkbp5 expression increases significantly after CMS (chronic mild stress). Research evidence suggests that animals exposed to CMS (chronic mild stress) show changes in the FKBP5-GR system that are important for HPA axis function and GR activity[5,18]. Changes produced by CMS, which reproduce phenotypes such as depression, show decreased translocation and GR function in the ventral and dorsal hippocampus as well as in the prefrontal cortex, and we show that chronic treatment with duloxetine antidepressants is able to normalize some of these changes, especially in the prefrontal cortex[9,10,22.]

CRH dysregulation that is induced nerve activity in the CNS may also be highly involved in the pathology of depressive disorders, Neuroendocrine findings in posttraumatic stress disorder (PTSD) reveal that PTSD patients are characterized by low adrenocortical activity and high CRH levels in the CNS CRH also inhibits eating behavior even in experimental animals that lack food[7,11]. It is possible that one of the peptides associated with CRH, such as urocortin I or III, mediates this gastrointestinal action. CRH is also a major inhibitor of reproductive function in both sexes. For example, intense or prolonged stress has been shown to inhibit gonadotropin secretion. Additional central effects of CRF include weakening of sexual behavior[3,8].

## Conclusion

There was a significant correlation / correlation between CRHR1 levels and FKBP5 levels ($p = 0.001 <\propto$). This is indicated by the high / strong correlation coefficient value which is 0.846. shows the influence of yoga exercises will result in a decrease in CRHR1 levels and FKBP5 levels in third trimester pregnant women.

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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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