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Expression of MRHR-CRHR1 (Type I CRH Receptor) and the Effects of Prenatal Yoga on Mother Primigravida Trimester III

Ruqaiyah^{1,2}, Nusratuddin Abdullah³, Mochammad Hatta⁴, Nasrudin A Mappeware⁵, Ayatullah Harun², Fatmawati Amir², Alfina Baharuddin⁶

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Abstract

Background: CRHR1 receptors are very important for determining the initial HPA axis response to stressful events. Changes in the CRH system and chronically elevated CRH levels are involved in stress-related affective disorders.

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 population in this study were all trimester III primigravida mothers who examined their pregnancy in PPK I ranks of the XIV / Hasanuddin Kesdam and several health centers in Makassar in 2019. The sample in this study amounted to 24 respondents

Results: the mean CRHR1 gene expression value after was greater than the mean CRHR1 gene expression before. So this means that in the control group CRHR1 gene expression in third trimester pregnant women there will be a significant increase.

Conclusion: an increase in CRHR1 gene expression means that it can be concluded that prenatal yoga decreases CRHR1 gene expression before and after treatment.

Keywords: *MRNA expression, CRHR1 gene, prenatal yoga, primigravida*

Introduction

In achieving the Sustainable Development Goals (SDGs) targets that are a continuation of the MDGs, particularly in the health sector, the participation of various community elements, including health / midwife institutions, is needed. In achieving the SDGs goals, midwives can play a role in achieving the third target of the SDGs, namely healthy and prosperous life, specifically related to maternal and infant health^{1,2,3}.

As a health service provider, midwives have a strategic and very unique role. Midwives as agents of reform are very close to the community and live in the midst of the community, and play a role in empowering women and the community^{4,5}.

Under physiological conditions, stress exposure activates the hypothalamus-pituitary-adrenal (HPA) axis. The HPA axis accepts a variety of inputs, including stressors that affect the medial parvocellular nucleus paraventricular hypothalamus (mpPVN) neurons. These neurons will synthesize corticotropin releasing hormone (CRH) and arginine vasopressin (AVP). CRH is secreted into the pituitary portal circulation and binds to the type 1 CRH receptor binding (CRH-R1) in the

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anterior pituitary and stimulates pro-opiomelanocortin (POMC) transcription through a process that involves the activation of adenylatsyclase^{16,7,8,9}..

CRH is a critical upstream effector of pituitary and adrenal hormone secretion into the circulating blood. CRH and AVP bind to each receptor, CRH receptor type 1 (CRHR1) and AVP 1B binding receptor (V1bR) located in the pituitary gland. The distribution of CRH receptors in the brain is consistent with the important role of CRH as a mediator of integral stress response. Encephalic structures express abundant CRHR1 including the dorsomedial hypothalamus nucleus, dorsolateral thalamic nucleus, supramammillary nucleus, anterior paraventricular thalamic nucleus and arcuate nucleus^{10,11,12}.

Acute stress causes the rapid activation of parvocellular CRH neurons as shown by the rapid increase in c-fos and CRH primary transcripts, the latter showing the activation of CRH gene transcription. 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 response. 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. 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^{13,14}..

CRHR1 receptors are very important for determining the initial HPA axis response to stressful events. Changes in the CRH system and elevated levels of CRH are chronically involved in affective disorders related to stress, especially anxiety and depression. Provision of central CRH in rodents raises phenotypic changes that lead to symptoms of affective disorders). Studies in animal models indicate that CRH's behavioral and hormone effects can be ascribed to CRHR-mediated actions^{15,16,17}

During the hypothalamic pituitary growth hormone (HPG) stress the axis is blocked at various levels. In addition to the direct inhibitory effect of glucocorticoids

on the HPG axis, increased CRH stimulates the release of somatostatin (SRIF) which is synthesized in PVN. SRIF further inhibits the secretion of growth hormone-releasing hormone (GHRH) in the arcuate nucleus (Arc) and growth hormone (GH) by the anterior pituitary somatotrope. Growth hormone exerts its anabolic action through the production of insulin-like growth factor 1 (IGF1) in the liver^{18,19}.

Stress besides pressing the HPA axis can also affect other neuroendocrine systems. Stress compresses the hypothalamic-pituitary-gonadal (HPG) axis on various species. The main mediator in this inhibition is stress-induced CRH stimulation. Increased CRH into the hypothalamic-pituitary portal blood flow system which successively stimulates the secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary and also interpreted as a holistic approach in the form of physical, mental and spiritual^{20,21}.

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 population in this study were all trimester III primigravida mothers who examined their pregnancy in KDP I ranks of the XIV / Hasanuddin Kesdam and several Puskesmas in Makassar in 2019. The sample in this study amounted to 24 respondents.

CRHR1 mRNA expression check with realtime PCR

How Realtime PCR works to determine the CRHR1 gene mRNA expression profile.

- 1) The process of primary oligonucleotide specific genes for the CRHR1 and b-actin genes as housekeeping gene (internal control).
- 2) Detect CRHR1 mRNA gene using forward and Reverse specific primers
- 3) PCR conditions with a temperature of 95oC for 10 seconds and 60oC for 30 seconds for 45 cycles.
- 4) And adapted to the Tomomi Yajima protocol where the qRT PCR uses the sybrgreen qRT-PCR master mix kit, one step. This protocol is optimized for real time PCR machine CFX Connect System (USA) instruments.

5) Reference to the passive coloring is included in the reaction, diluted 1: 500. Dyes containing dyes are kept away from light. Dilute 2 x SYBR Green QRT-PCR master mix and store on ice. Following the initial melting of the master mix, the unused portion is kept at 40C with a note, avoiding the repetitive freeze-liquid cycle.

6) The reaction of the experiment is prepared by adding the following components. Prepare a mixture of reagents for the reaction using several components as below.

7) Mixed reagents by taking a final volume of 25 µl including mRNA samples extracted according to the protocol Hatta, et al. 2017)

8) 12.5 µl of 2 x SYBR Green QRT-PCR master mix plus x µl from the initial primer (optimized concentration) plus Nuklease - free PCR - H2 level x µl final primer (optimized concentration) and also 0.375 µl reference dye solution from step 1 (optional) as well as 1.0 µl of RT / Rnase a mixture of enzyme blocks with 25 µl of the total reaction volume can also be used. The reaction is mixed slowly so as not to form bubbles (not rotated), then distribute the mixture to the test test tube by adding x µl of RNA to each test tube.

Result

Tabel 1: Test results for normality levels of

Group	Pengamatan	p-value	Distribusion
control	Before	0.087	Normal
	After	0.867	Normal
Treatment Yoga	Before	0.354	Normal
	After	0.276	Normal

In table 2, the comparison test of CRHR1 gene expression used paired sample t test.

Table 2 Results of comparison tests before CRHR1 gene expression and after treatment

Group treatment	Before Rerata ± SD	After Rerata ± SD	p-value
Control	5.79±0.51	8.58±0.6	T
Treatment yoga	6.24±0.52	5.71±0.46	0.015

Table 2 shows the mean value of CRHR1 gene expression after the greater value than the mean CRHR1 gene expression before. So this means that in the control group CRHR1 gene expression in third trimester pregnant women there will be a significant increase.

Table 3 Test results for comparing CRHR1 gene expression

Treatment	Control Rerata ± SD	Treatment Rerata ± SD	p-value
Before	5.79±0.51	6.24±0.52	0.045
After	8.58±0.6	5.7±0.45	0.000

Table 3 shows that there are significant differences ($p = 0.045 < \alpha$) on average CRHR1 gene expression. This means that the treatment of Yoga exercises in third trimester pregnant women can reduce CRHR1 gene expression.

Table 4 Correlation test results between variables

corelasi variable	N	coefisien korelasi (r)	p-value
Anxiety level with CRHR1	12	0.853	0.000
cortisol with gen CRHR1	12	0.956	0.000

Discussion

The mean CRHR1 gene expression results before treatment (6.24 ± 0.52) after treatment (5.71 ± 0.46). The average CRHR1 gene expression after the value was smaller than the average CRHR1 gene expression before being treated. This means that in the treatment group CRHR1 gene expression in third trimester pregnant women will be a significant decrease. In other words there is the effect of Yoga exercise treatment on CRHR1 gene expression in third trimester pregnant women,

There was a significant difference ($p = 0.045 < \alpha$) mean CRHR1 gene expression at 27-28 weeks gestation between the control group (before) (5.79 ± 0.51) and the treatment group (before) (6.24 ± 0.52). Based on the mean value of CRHR1 gene expression in the treatment group did not differ greatly with the mean value of CRHR1 gene expression in the control group. This means that it can be assumed that the mean CRHR1 gene expression initially did not differ greatly between the two groups.

Based on the mean value of CRHR1 gene expression in the treatment group the value is smaller when compared with the average value of CRHR1 gene expression in the control group. This means that third trimester pregnant women who did not receive yoga exercises showed a high CRHR1 gene expression when compared to third trimester pregnant women who received yoga exercises. In other words, the treatment of Yoga exercises in third trimester pregnant women can reduce CRHR1 gene expression. So the third minor hypothesis has been proven, that is, there is a difference in effect between non-action and prenatal Yoga exercises on CRHR1 gene expression in primigravida trimester III.

This study is in line with ⁶Four case control studies analyzing CRHR1 polymorphisms in 2336 Caucasian individuals, of which 1100 were suicidal individuals and 1236 were control subjects. analyzing Irish and Canadians with each attempted suicide, found a significant relationship between the CRHR1 gene and the severity of suicide behavior ($p > 0.05$). His study of 135 bipolar disorder patients was recruited for clinical assessment of childhood trauma and suicides through the childhood trauma questionnaire (CTQ) and the Columbia Suicide Severity Rating Scale (C-SSRS), investigating ⁸trauma in childhood and genetic variability on the HPA axis independently or interacting, in suicide attempts in bipolar disorder patients. Results of individuals with higher total CTQ scores, emotional abuse, sexual harassment and emotional neglect are more likely to indicate suicide^{22,23}.

An increase in antenatal pressure has been associated with negative outcomes in both the mother and fetus. One mechanism that is thought to underlie this relationship is the hypothalamic-pituitary-adrenal (HPA) axis. It is important to remember that the function of the HPA axis is not only modulated by environmental stimuli, but is also partly determined by genetic factors. in his research proved that the CRH-BP gene is marginally linked to suicidal behavior. The CRH-BP protein binds to CRH which limits its bioavailability and hence prevents activation of the CRH receptor and further signaling of the HPA axis. People carrying CRH-BP alleles rs7728378-C are more likely to report a history of suicidal behavior. It can be explained that abuse in childhood has been linked to permanent changes in the HPA axis, a stress management system with damaging

effects on the developing brain that can be linked to behavioral problems in life in adulthood^{18,16,20}.

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 adrenocorticotrophic 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. 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^{24,25}.

Conclusion

There was a significant difference in the mean CRHR1 gene expression in the control group before (5.79 ± 0.51) and after observation (8.58 ± 0.6), it could be interpreted that there was an increase in CRHR1 gene expression meaning it could be concluded that prenatal yoga decreased CRHR1 gene expression 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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