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Comparison of Akt Expression in the Cerebrum and Cerebellum of Newborn *Mus musculus* Exposed to Physical Stress and Psychological Stress During Pregnancy

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Abstract

Introduction: Prenatal stress prevalence is almost half of the population of pregnant women worldwide. Stress will stimulate glucocorticoids which can disrupt the PI3K-Akt cascade. PI3K-Akt deficiency will cause impaired fetal brain growth and development.

Objective: To compare the Akt expression in the cerebrum and cerebellum of newborn *Mus musculus* exposed to physical stress, psychological stress, the combination of psychological and physical stress, and without stress exposure.

Method: This study was experimental laboratory research. Twenty-four female mice were used as samples and divided into four groups: physical stress exposure group, psychological stress exposure group, the combination of psychological and physical stress exposure group, and control group. Akt expression was tested by immunohistochemistry (IHC) staining. Statistical analysis used the One Way Anova and Kruskal Wallis test.

Results: There were significant differences in cerebrum Akt expression ($p = 0.008$) and cerebellum Akt expression ($p = 0.047$) between the control and stress exposure groups.

Conclusion: There were statistically significant decreases in mean Akt expression in the cerebrum and cerebellum of newborn *Mus musculus* exposed to stress during pregnancy.

***Keywords*:** *Stress, Akt, cerebrum, cerebellum, and Mus musculus.*

Introduction

The brain is an organ that controls all body functions. Intelligence, creativity, emotions, and memory are some of the many things regulated in the brain[8]. The brain is a stress adaptation center that triggers a behavioral and physiological response to stress[15]. Stress increases hormone secretion on the hypothalamus-pituitary-adrenal (HPA) axis. After acute experience stress, the HPA hormone axis quickly returns to pre-stress levels, but chronic stress triggers a sustained response that affects mental and physical distress[14].

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Depression and anxiety occurred in pregnancy, with estimated prevalence of 12% for depression and 15.2% for anxiety. Mild to moderate stress has been reported in half of the population of healthy pregnant women worldwide[1]. Prenatal stress increased glucocorticoids (cortisol) that can damage the brain[12].

High concentrations of glucocorticoids (GC) combined with glucocorticoid receptors (GR) will inhibit neurotrophic factor (NTF) signaling expressed by many tissues in the brain, especially in the cerebrum and cerebellum. NTF is known to increase cell survival by activating Akt/protein kinase B signaling[4]. Phosphatidylinositide 3 Kinase (PI3K)-Akt cascade dysfunction has been recognized as a cause of neurodevelopmental and neuropsychiatric diseases, such as autism, epilepsy, brain injury, and developing brain malformations[17].

Objectives

To compare the Akt expression in the cerebrum and cerebellum of newborn *Mus musculus* exposed to physical stress, psychological stress, the combination of psychological and physical stress, and without stress exposure.

Materials and Methods

This research is a study laboratory experiment with post-test only with a control group design. Samples were 24 female mice (*Mus musculus*), 2-2.5 months old, weighing 20-25 grams. The research was conducted in Faculty of Veterinary Medicine Universitas Airlangga, from January to March 2021.

After adaptation for a week, female mice were divided into four groups, which are: the physical stress exposure group (K1), the psychological stress exposure group (K2), the combination of psychological and physical stress exposure group (K3), and control group (K4). Below are some explanations:

1. K1 is the group with forced swimming for 5 minutes every day in a box measuring 50 cm x 30 cm x 25 cm with a water height of 18 cm. Water temperature ranging from 24ºC-28ºC and room temperature of 20ºC-25ºC.

2. K2 is the group with noise exposure with sound intensity 90 dB through TrueRTA software and measured by the real-time sound analyzer (TES 1358) given for 1 hour per day.

3. K3 is the combination of K2 and K1. Both types of treatment are given on the same day with noise exposure for 1 hour. 5 minutes after that, forced swimming for 5 minutes.

Stress exposure to K1, K2, and K3 is given simultaneously at 09.00. Stress exposure is given starting from day 6-15 of pregnancy.

At the end of the 16th day of the experiment, mice were anesthetized with ketamine (Ketamine Hydrochloride Pfizer®, New Jersey, USA) and acepromazine (Castran®, Venray, Netherlands). Delivery by sectio caesarea (SC). The newborn mice were sacrificed through neck decapitation, and the brain organs were dissected.

Akt expression was tested by immunohistochemistry (IHC) and analyzed using the Remmele semi-quantitative scoring system. This Immuno Reactive Score (IRS) is a multiplication between immunoreactive cells (A) and the color intensity score of immunoreactive cells (B).

The Shapiro Wilk test was used to determine the data's normality and the Levene test to determine its homogeneity. If the data's distribution and homogeneity were normal ($p > 0.05$), then the One Way Anova test was used, followed by Post Hoc LSD (Least Significant Difference). If the data distribution is not normal, then the Kruskal-Wallis test is used, followed by the Mann-Whitney U test to determine the difference between the two groups with abnormal data distribution. Meanwhile, to determine the difference between the two groups with normal distribution, it is necessary to use the T-test analysis. Data analysis using IBM SPSS Statistics version 26.00 (New York, USA).

Results

The research sample consisted of 24 female mice based on inclusion criteria and randomized into four groups.

The results showed the mean difference between K1, K2, K3, and K4, as shown in Table 1. Cerebrum Akt expression was higher in the control group (K4) than the stress exposure group (K1, K2, and K3).

Table 1. Akt expression in the cerebrum of newborn mice in each group.

	Mean ± SD			
	K1	**K2**	**K3**	**K4**
Akt expression	8,300 ± 0,726	6,616 ± 1,487	5,017 ± 1,038	9,617 ± 3,468

Statistical analysis used the Kruskal-Wallis test with the result p-value = 0.008. That means there was at least one significant difference between the two groups. The data were normally distributed, so the comparison between the two groups was analyzed using the T-test. T-Test showed significant differences between K1 and K2, K1 and K3, and K3 and K4.

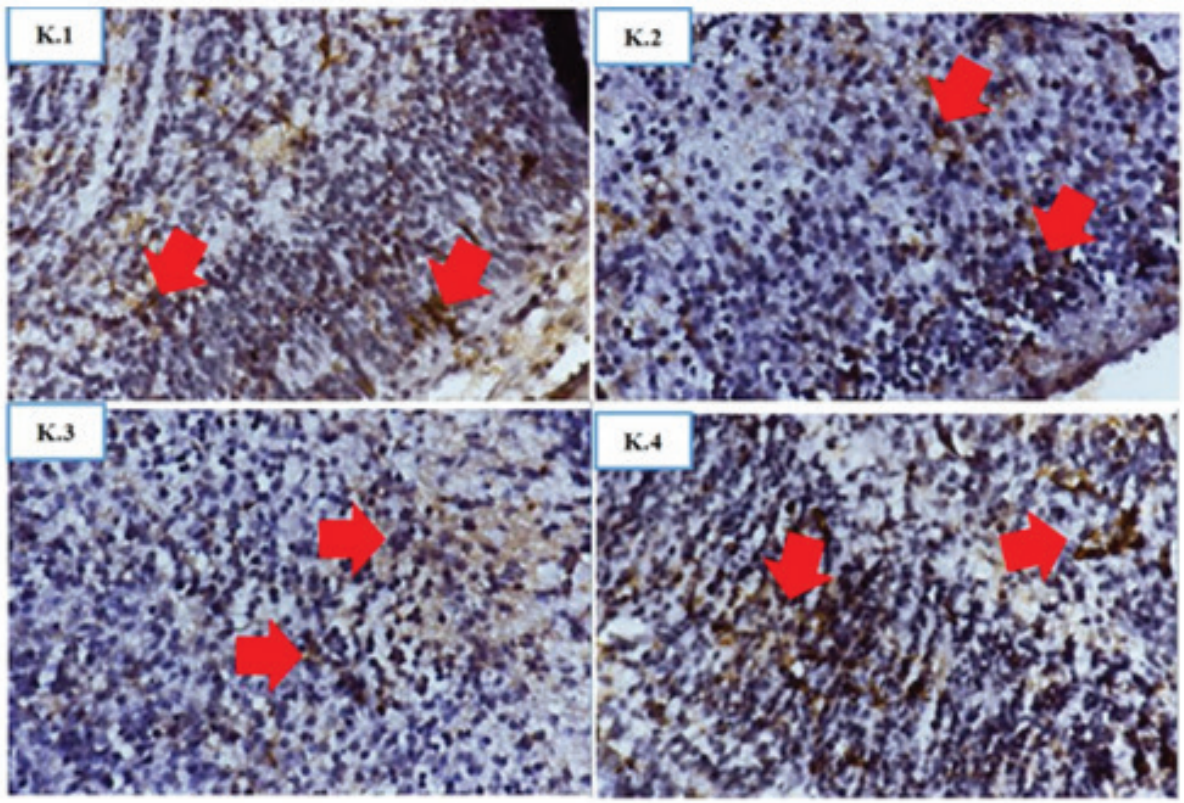

Figure 1. Akt expression (red arrow). Cerebrum Akt expression in the control group (K4) was strongest among the other groups (immunohistochemistry, 400x magnification, Nikon H600L microscope from Nikon Instruments Inc. ™, New York, USA).

Table 2. Akt expression in the cerebellum of newborn mice in each group.

	Mean ± SD			
	K1	K2	K3	K4
Akt expression	9,100 ± 2,767	7,200 ± 3,518	5,467 ± 1,880	9,917 ± 2,549

The results showed the mean difference between K1, K2, K3, and K4, as shown in Table 2. The decrease in Akt expression occurred in the stress exposure group. This Akt expression decrease was in line with the increased stress exposure. Statistical analysis used the One Way Anova test with p-value = 0.047, which means significant differences between groups. Post Hoc LSD test (Least Significant Difference) shows significant differences between K1 and K3 and K3 and K4.

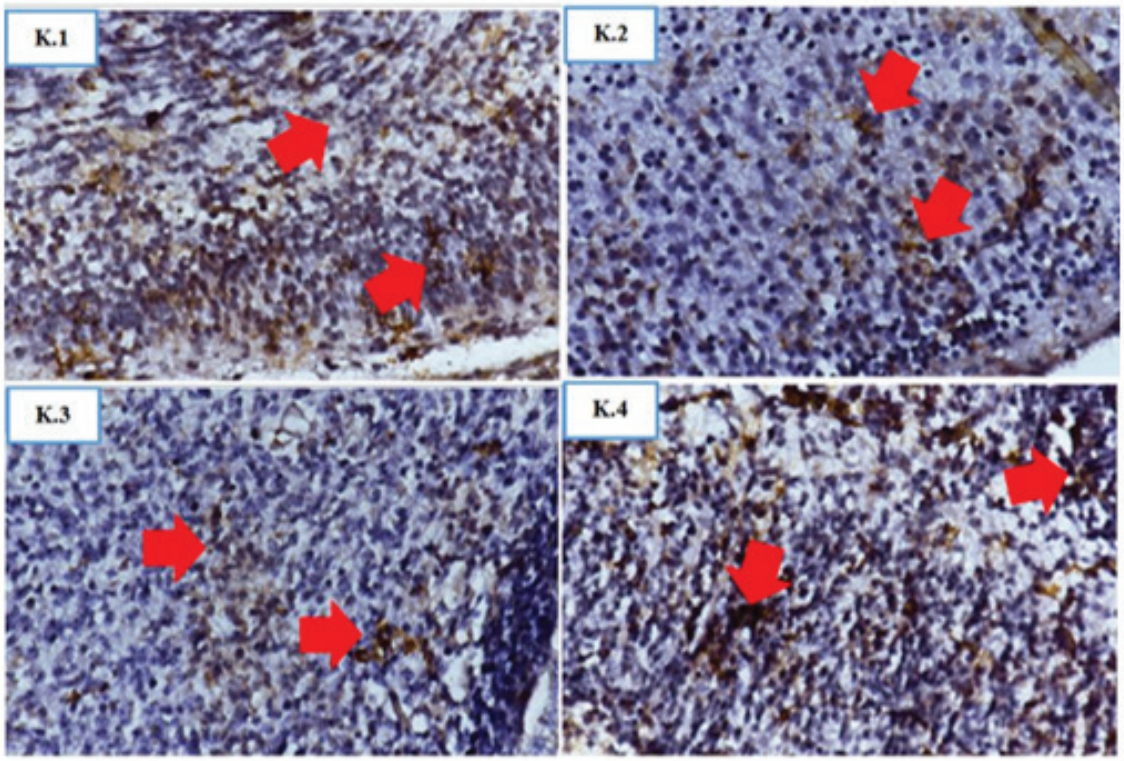

Figure 2. Akt expression (red arrow). Cerebellum Akt expression in the control group (K4) was strongest among the other groups (immunohistochemistry, 400x magnification, Nikon H600L microscope from Nikon Instruments Inc. ™, New York, USA).

Discussion

The results showed that stress exposure during pregnancy decrease Akt expression in the cerebrum and cerebellum newborn mice. Stress during pregnancy causes an increase in placental GC[2]. The fetal HPA axis is very sensitive to excess GC levels that can alter the regulation of HPA function[16]. High GC levels can suppress neurogenesis which endangers cell survival, causing an imbalance in several neurotransmitter systems[10].

The interaction of GC and active GR in adrenocortical cells causes the neurotrophic factor (NTF) signaling pathway to be suppressed. Several proteins have been

classified as NTF: BDNF (Brain-Derived Neurotrophic Factor), IGF-1 (Insulin-Like Growth Factor-1), and GDNF (Glial Cell Line Derived Neurotrophic Factor)[4]. The BDNF system is essential for neural survival and synaptic plasticity in the CNS[13]. BDNF has broad expression in the brain[18]. IGF-1 is an important growth factor in CNS (Central Nervous System) development. In early brain development, IGF-1 has an essential role as an autocrine, proliferative, and prosurvival factor[5]. GDNF activates the PI3K / Akt signaling cascades[4].

NTF plays a role in determining PI3K-Akt regulations. PI3K-Akt signaling activity triggers the catalytic lipid domain of PIP2 to become PIP3[4]. Inactive Akt binds to PIP3 in the plasma membrane, allowing PDK1(Phosphoinositide-Dependent Protein Kinase 1) to access phosphorylate T308 and mTORC2

(Mammalian Target of Rapamycin-2) to phosphorylate S473 as part of the activation of Akt[7].

The Akt signaling pathway has important biological effects on cells, such as increased survival, inhibition of aging, and physiological activity[19]. Akt is a growth factor-induced cell survival mediator and has been shown to suppress apoptotic death in several cell types[9].

The Akt pathways are negatively affected by glucocorticoid exposure[11]. PI3K deficiency causes a significant reduction in brain size during embryogenesis[3]. Many experiments have shown that glucocorticoids can inhibit IGF-1 expression in many tissues and cells[6]. It shows the importance of Akt in brain growth and development[3].

The results showed that Akt expression in the cerebrum and cerebellum was significantly higher in the control group than in the stress exposure group. Akt expression in the cerebrum and cerebellum of newborn mice decreased significantly in line with the stress exposure.

Conclusion

This study concluded that physical and psychological stress exposure during pregnancy could decrease the expression of Akt in the cerebrum and cerebellum of newborn mice.

Conflict of Interest: The authors state that there is no conflict of interest associated with this research.

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Ethical Clearance: This study was approved by the

Ethical Committee Faculty of Veterinary Medicine Universitas Airlangga with Number: 2.KE.002.01.2021.

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