Jurnal Kebidanan
UNIVERSITAS MUHAMMADIYAH SEMARANG

Jurnal Kebidanan – Vol 13, No 1 (2024), 35-40
ISSN 2301-8372 (print); ISSN 2549-7081 (online)
DOI: 10.26714/jk.13.1.2024.35-40

Number of Glia Cells in the Cerebrum and Cerebellum of Newborn Rats After Administration of Mackerel Fish Oil During Pregnancy

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Abstract

Omega 3 intake during pregnancy can support fetal brain growth and development. Omega 3 plays an important role in making neuron cell membranes and glia and protecting nerve cells. Mackerel is a source of omega 3 which is abundant and easily accessible to the people of Indonesia. The purpose of this study was to analyze the effect of mackerel oil intake on the number of glial cells (astrocytes, oligodendrocytes, and microglia) of the cerebrum and cerebellum of newborn rats. This research was a true experimental design with a post-test only control group. A total of 30 pregnant rats were randomized into 3 groups, namely the control group (K1), mackerel oil (K2), and omega 3 supplements (K3). Each group was given treatment on days 1-17 of pregnancy. On the 18th day, the termination was carried out. 3 newborn rats were taken from each parent and made preparations from brain tissue. Hematoxylin-Eosin test to assess the number of glial cells. The results showed the highest mean±SD number of glial cells (astrocytes, oligodendrocytes, and microglia) in the cerebrum and cerebellum was the highest in the mackerel oil group (174,460±33,777; 21,080±6,937; 11,300±2,090), and (156,280 ±34,980; 22,260±4,302; 11,060±2,383). ANOVA test showed a significant difference in the number of glial cells in the cerebrum and cerebellum between groups with a p-value <0.05. The results of this study can be concluded that the administration of mackerel oil during pregnancy can increase the number of glial cells (astrocytes, oligodendrocytes, and microglia) in the cerebrum and cerebellum of newborn rats.

Keywords

mackerel oil; omega 3; glial cells; newborn rat

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Introduction

Brain development begins when the baby is in the womb(Simarmata et al., 2012). Several factors that have a major effect on early brain development are the reduction of toxic stress and inflammation, the presence of strong social support and secure attachments, and the provision of optimal nutrition from the womb.(Cusick & Georgieff, 2016). However, in fact there are still pregnant women who experience nutritional problems. Riskesdas in 2018 recorded that the proportion of risk of chronic energy deficiency in pregnant women in Indonesia was 17.3%. This figure has decreased from 24.2% in 2013. Meanwhile the proportion in East Java was 29.8% in 2013 and 19.59% in 2018(Kemenkes, 2018).

Nutrition around the time of fertilization is important for gamete function and placental development. Starting 2-3 weeks after fertilization, the embryo undergoes a process of neuronal proliferation and migration, synapse formation, myelination and apoptosis to develop the fetal brain. One nutrient identified as having an important role in prenatal nerve development is polyunsaturated fatty acid (PUFA).(Li et al., 2019).

Omega 3 intake can be obtained by consuming sea fish. One of the marine products that is quite abundant in Indonesia is mackerel (Setyo Mahanani Nugroho et al., 2022). Mackerel is available in traditional markets and supermarkets at low prices so that it is affordable for the public(Indaryanto et al., 2014). The omega 3 fatty acid content in mackerel is DHA 10.62%, EPA 4.85% and AA 3.17%(Muhamad & Mohamad, 2012).

The peak of glial cell production occurs in the second half of pregnancy. Part of the glial cells undergo axonal myelination in the second trimester of pregnancy and the end of the first postnatal year(Hadders-Algra, 2010).Providing omega 3 fatty acids during pregnancy is an effort to optimize the growth and development of the fetal brain by increasing the number of nerve cells and glial cells by reducing apoptosis and increasing the glia-neuron ratio and increasing synaptogenesis.(Fauzi & Joewono, 2018). Docosahexaenoic acid has a significant effect on neuronal membrane dynamics, including transporter, receptor and neurotransmitter function.(Bernardi et al., 2012). Additionally, the presence of DHA in neonatal brain matter aids several brain development processes, including neurogenesis, synaptogenesis, brain plasticity, inflammatory signaling, neuroprotection, etc.(Basak et al., 2020).

This study aims to determine the effect of administering mackerel fish oil on the number of glial cells (astrocytes, oligodendrocytes and microglia) in the cerebrum and cerebellum of newborn Rattus norvegicus by using mackerel fish found in Indonesian waters.

Methods

This research is a pure experiment with a post-test only control group design which aims to analyze differences in the number of glial cells (astrocytes, oligodendrocytes and microglia) in the cerebrum and cerebellum of newborn Rattus norvegicus in the control group, mackerel oil and omega 3 supplements. This research carried out after obtaining ethical eligibility with ethical number 2.KE.056.04.2020.

The mackerel oil used is extracted directly from fresh mackerel fish. Soxhlet extraction was carried out using petroleum ether solvent. To determine the levels of fatty acids in mackerel oil, a GCMS test was carried out. In the GC-MS test, the results showed that mackerel oil contains many amino acid compounds, such as DHA, EPA, linoleic acid and arachidonic acid.

Acclimatization of Rattus norvegigus aged 2-3 months is carried out for 1 week. Next, superovulation is carried out with 10 IU of the hormone Pregnant More Serum Gonadhotropin (PMSG). After 48 hours, 10 IU Human Chorionic Gonadhotropin (hCG) was injected intraperitoneally and then Rattus norvegicus were mated by monomating. 30 pregnant Rattus norvegicus were randomized into 3 groups, then given treatment on days 1-17 of pregnancy. The control group (K1) was given ad libitum standard feed, the mackerel oil treatment group (K2) was given ad libitum standard feed plus 3.24 mg mackerel oil/ 120 gr BW/ day, the omega 3 supplement treatment group (K3) was given standard feed ad libitum plus omega 3 supplement 3.24 mg/ 120 gr BW/ day.

Surgery is performed on the 18th day of pregnancy. Three children were selected from each mother with the criteria of heaviest, medium and lightest weight to be sacrificed and brain tissue prepared. Hematoxylin-Eosin staining was carried out to count the number of glial cells under a microscope with 400x magnification in 5 fields of view. Data calculations used the ANOVA statistical test and to see the differences between each group, the BNT test was carried out.

Results and Discussion

The results showed that the mean number of glial cells (astrocytes, oligodendrocytes and microglia) in the cerebrum of newborn mice in the mackerel oil group was higher (174,460 ± 33.77; 21,080 ± 6,937; 11,300 ± 2,090) than the negative control and omega 3 supplement groups (Table 1). Then a normality test was carried out using the Shapiro-Wilk test and the results showed that the cerebral astrocyte cells of newborn mice in the control group had an abnormal

Table 1. Average ±SD and Further Testing of the Number of Glial Cells in the Cerebrum of Newborn Rats

Group	Number of Glial Cells		
	Mean ± Standard Deviation		
	Astrocytes	Oligodendrocytes	Microglia
Control	90,580 ± 23,994 a	9,100 ± 3,193 a	6,520 ± 2,134 a
Mackerel Oil	174,460 ± 33,777 c	21,080 ± 6,937 c	11,300 ± 2,090 c
Omega 3 Supplements	120,160 ± 34,358 b	15,780 ± 5,688 b	8,360 ± 1,066 b

Table 2. Results of Analysis of Differential Tests for the Number of Glia Cells in the Cerebrum of Newborn Rats

Variable	p value	Difference Test Analysis
Astrocytes	0,000*	Kruskal-Wallis
Oligodendrocytes	0,000*	ANOVA
Microglia	0,000*	ANOVA

Table 3. Average ±SD and Further Testing of the Number of Glial Cells in the Cerebellum of Newborn Rats

Group	Number of Glial Cells		
	Mean ± Standard Deviation		
	Astrocytes	Oligodendrocytes	Microglia
Control	95,480 ± 19,085 a	7,880 ± 2,925 a	6,460 ± 1,687 a
Mackerel Oil	156,280 ± 34,980 c	22,260 ± 4,302 c	11,060 ± 2,383 c
Omega 3 Supplements	124,860 ± 33,233 b	13,240 ± 1,733 b	8,540 ± 2,273 b

Table 4. Results of Analysis of Differential Tests for the Number of Glia Cells in the Cerebellum of Newborn Rats

Variable	p value	Difference Test Analysis
Astrocytes	0,000*	ANOVA
Oligodendrocytes	0,000*	ANOVA
Microglia	0,000*	ANOVA

distribution, whereas in the mackerel fish oil and omega 3 groups the distribution was normal, so the different test used was the Kruskal-Wallis nonparametric test. Tests for normality of the number of oligodendrocyte cells and cerebral microglia of newborn Rattus norvegicus in the three groups were all normally distributed, so to see the differences in each group the parametric Anova test was used.

Test *Kruskal-Wallis*on the number of astrocyte cells and the ANOVA test on the number of oligodendrocyte cells and microglia cells in the cerebrum of newborn mice, the results showed significant differences between groups with a value of p=0.000 (p<0.05) (Table 2). The results of the Mann-Whitney advanced test for astrocyte cells and the BNT advanced test for oligodendrocyte and microglia cells (Table 1) show that different superscripts in the same column are significantly different (p<0.05).

Based on table 3, it is known that the mean number of glial cells (Astrocytes, Oligodendrocytes and Microglia) in the cerebrum of newborn mice in the mackerel oil group was higher (174,460 ± 33.77; 21,080 ± 6,937; 11,300 ± 2,090) than the control and omega 3 supplement groups Testing the normality of the number of cerebellar glial cells in newborn mice using the Shapiro-Wilk test showed that the astrocytes, oligodendrocytes and microglia cells in all groups were normally distributed, so to see the differences in each group the ANOVA parametric test was used.

The ANOVA test showed significant differences between groups in the number of astrocytes, oligodendrocytes and microglia in the cerebellum of newborn Rattus norvegicus with a value of p=0.000 (p<0.05) (Table 4). The results of further BNT tests on the number of astrocytes, oligodendrocytes and microglia cells in the cerebellum of newborn mice showed that different superscripts in the same column were significantly different (p<0.05) (Table 3).

The results of this study showed significant differences in the number of glia cells in the cerebrum and cerebellum of newborn mice between the control group, mackerel oil and omega 3 supplements. The number of glia cells in the cerebrum and cerebellum of newborn mice given mackerel oil was shown to be higher than the control group and omega supplements. 3.

The content of DHA and EPA as a source of omega 3 in mackerel oil in this study given to pregnant Rattus norvegicus mothers is believed to be able to increase the number of glial cells in the brain of newborn Rattus norvegicus. Mice lacking endogenous DHA show changes in microglial architecture and cytokine factors without involving astrocytes. DHA replenishment restores the physiological expression of neuroinflammatory and neuroplasticity factors in the cerebral cortex. This suggests that DHA plays an important role in neuroimmune communication in brain function and synaptic plasticity(Basak et al., 2020).

A number of studies report that maternal DHA consumption contributes to the health and development of the baby (Sarih, 2020). In the early trimester of pregnancy, DHA plays a role in the initial process of placentation and is important for further placental development (Asifa & Rodiani, 2021). Docosahexaeonic acid rapidly accumulates during the third trimester of pregnancy and the first year after birth. DHA supplementation in the third trimester of pregnancy is very important because at this time the fetal brain is growing and requires DHA during this period, thus contributing to cognitive development and providing immune protective effects on the baby.(Zhang et al., 2018);(Basak et al., 2020).

Several observational and clinical tests have shown a risk of lower language development and visual acuity in babies if the mother does not consume enough DHA during pregnancy(Zhang et al., 2018). Inadequate DHA and EPA intake during pregnancy will disrupt optimal feto-placental growth and cause inflammatory disorders, behavioral changes and mental stress later in life.(Basak et al., 2020). Research conducted(Coti Bertrand et al., 2006)stated that the lack of omega 3 fatty acids in mouse mothers showed changes in the structure of the telencephalon of embryonic mice(Basak et al., 2020).

*Docosahexaeonic Acid*early in life is very important regarding the process of neurogenesis during perinatal and gliogenesis which continues until after birth. Gliogenesis is the formation of astrocytes, ologodendrodites and microglia. Astrocytes are important for neurotransmitter transduction, oligodendrocytes function to secrete myelin which insulates axons allowing signal transduction and microglia function to help remove cellular debris. In the process of gliogenesis DHA is very important, because it stimulates neurite growth (the growth of dendrites and axons of neurons) (Yunanto & Dwi Sanyoto, 2016). Additionally, the formation of synapses allowing one neuron to transmit signals to another neuron also depends on optimal DHA levels. Consuming foods rich in DHA is effective in increasing DHA levels in glial cells. Glial cells are described as the “glue of the nervous system” because they help form myelin and provide nutrients to neurons(DiNicolantonio & O’Keefe, 2020).

Mackerel oil contains omega 6 fatty acids (2.20% arachidonic acid and 1.86% linoleic acid) and omega 9 fatty acids (7.89% oleic acid). The content of omega 6 and omega 3 has an important role in normal brain formation and nerve myelination during nerve development(Cohen Kadosh et al., 2021). Oleic acid is the only fatty acid that can be synthesized by astrocytes. The single double bond of oleic acid can increase membrane fluid which is very important for neurons. In addition, oleic acid is preferentially incorporated into neurite bases, indicating that increased fluid is required at the growth sites of axons and dendrites.(Medina & Tabernero, 2002).

Several essential fatty acid contents other than omega 3 (DHA and EPA) are thought to be able to support the growth and development of the fetal brain and increase the number of glial cells more than the group that only received omega 3 supplements. Thus, fatty acid intake during pregnancy needs special attention. as an effort to prepare future generations. This fatty acid intake can be obtained from mackerel fish which is abundantly available in Indonesia.

Conclusion

Mackerel oil contains multiple components besides EPA and DHA, so that administration of mackerel oil during pregnancy can increase the number of glial cells (asrtocytes, oligodendrocytes and microglia) in the cerebrum and cerebellum of newborn mice.

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