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Comparative Investigation of the Levels of BDNF and NGF Genes Expressions in the Brains of Male and Female Newborn Methimazole-Induced hypothyroidism in NMRI mice

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ABSTRACT

One of the endocrine glands that play a significant role in the development of the prenatal brain is Thyroid. Therefore, the aim of this study is to compare the levels of BDNF and NGF gene expressions in the brains of male and female babies born on the first day methimazole-induced hypothyroidism Mice with NMRI race. In this study, 30 mature mice of Albino NMRI race were selected. Mice (Albino NMRI) were identified using after mating. In the next stage, we divided mice into three groups (high-dose intervention, low-dose intervention and control groups). Pure water was given during pregnancy to the control group. In the low dose group until the end of pregnancy given 20 mg of methimazole that dissolved in 100 cc of water. Also, in the high-dose group during period pregnancy until the end, they were given 100 mg of methimazole that dissolved in 100 cc of water. After the end of pregnancy, blood samples were taken from the mother's mice to determine the amount of T3 and T4 present in the bloodstream. In the end stage, the brain of one-day mice was removed and to determine the expression of BDNF, NGF we used to RT-PCR. According to the result this study, amount of T4 and T3 in the control group and low dose (27 and 1.59 ng/dl, and 8 and 0.87 ng/dl), respectively indicating a significant reduction in the expression of NT4, NT3, NGF and BDNF gene ($P<0.05$). Based on result of this study, between the high dose and control group there was significant relationship reduction in the expression ($P<0.05$).

INTRODUCTION

One of the most important endocrine glands that produce Triiodothyronine (T3) and Thyroxine T4 is Thyroid (Bunevičius et al, 1999; Baskin et al, 2002; Dayan and Panicker, 2018). Regulation of basal metabolism, calcitonin which plays a role in calcium homeostasis, development in utero and in the following stages of life are the most role T3 and T4 (Ben-Chioma et al., 2017). Based on report several studies iodine and tyrosine are the source of production T3 and T4 that this hormones can have adverse effects on many systems in the human body (Ben-Chioma et al., 2017; Moog et al., 2017; Hershman, 2017; Bifulco and Cavallo, 2007). Increased oxygen consumption in tissues and the regulation of the basal metabolic rate are the famous function of the T3 and T4 (Ben-Chioma et al., 2017; Moog et al., 2017; Hershman, 2017; Bifulco and Cavallo, 2007; Glinioer, 1997).

According to the result of the numerous studies, T3 thyroid hormones cause the release of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) neurotrophic factors through affecting astrocytes (Bathina and Das, 2015; Moore *et al.*, 2011).

The neurotrophic factor of BDNF plays an important role in neural guidance, transcription of certain genes and exiting the heterochromatic mode (Bathina and Das, 2015; Gilbert and Lasley, 2013; Kapoor *et al.*, 2015). Cooper studied Methyl Mercaptoimidazole (MMI) and Antithyroid drugs in Sinai Hospital of Baltimore, the Johns Hopkins University School of Medicine, Baltimore (Cooper, 2005). In 2002 Skaper evaluation of These components in mammals comprise NGF, BDNF, NT4, NT3, which was then called NT5 (Skaper, 2012). The aim of this study was to determine the levels of BDNF and NGF Genes Expressions in the Brains of male and female babies born on the first day methimazole-induced hypothyroidism Mice with NMRI race.

MATERIALS AND METHODS

This cross-sectional study was carried out on 30 mature mice of Albino NMRI race provided by Pasteur Institute of Iran during 2016-2017. Samples one week before the experiment in terms of environmental conditions, in particular temperature, light, food and animal room were completely under control.

Prepared for fertility, the female mice were mated with male mice. In the next stage, we divided mice into three groups (high-dose intervention, low-dose intervention and control groups). The control group received pure water during the pregnancy period, whereas the low-dose and high-dose intervention groups were administered with 20 mg and 100 mg of methimazole powder (provided by "Shimi Daroo Co."), completely dissolved in 100

mg of drinking water, respectively. During the pregnancy period, a suitable and controlled condition was provided for mice. After the end of pregnancy, blood samples were taken from mother's mice to determine the amount of T3 and T4 present in the bloodstream. In the end stage, the brain of one-day mice were removed and for determine the expression of BDNF, NGF we used to RT-PCR.

Measurement of T3 and T4:

One of the two measurement methods used in this study was ELISA kits. Inferior vena cava using syringe was used for blood samples obtained. In this method, the level of T3 and T4 measured after centrifuge and preparation of hematocrit from serum.

Dissection of Brains of One-Day Neonate Mice:

In this method, were used to cut the upper part of the heads after disconnection head. Then finally the precision should remove the upper part of the skull. To increase the accuracy of the results for each mouse were used new sterile sets of surgical tools. In the end stage, samples until performing the additional tests immediately transferred to liquid nitrogen and kept at -80°C.

Brain Preparation for Evaluation of BDNF and NGF Gene Expression:

First, the frozen brain tissue was taken out and turned into powder using liquid nitrogen. The obtained powder was put in 2ml micro tubes and applied to perform the RT-PCR process. In addition, specific primers were designed applying oligo7 software, followed by their synthesis in "Gen Fanavaran Co.". In the next stage Real-time PCR reaction used for properties of primers (Table 1). In the end stage, for the assessment of gene expression with respect to the normal group was analyzed Ct value by using $2^{-\Delta\Delta C_t}$ Levick model (Table 1).

Table 1 : Properties of used primers in Real-time PCR reaction

| Gene name | Accession number | Primer sequences |
|-----------|------------------|---|
| PKG1 | NM_008828 | F GGCATTCTGCACGCTTCAAA R AAGTCCACCCTCATCACGAC |
| NGF | NM_013609 | F AGCTTTCTATACTGGCCGCAG R AGGGCTGTGTCAAGGGAATG |
| BDNF | NM_007540 | F CTTTGCGGATATTGCGAAGGG R CTGGTGGAACATTGTGGCTTTG |

Data Analyze:

The coded data were entered in SPSS software version 13. Data analyses were performed using descriptive statistics (frequency, mean, and standard deviation for each variable) and one-way analysis of variance and Tukey's test and the values expressed Mean \pm SEM. In addition, P-value of less than 0.05 was considered statistically significant.

RESULTS

In the present study, 30 mature mice of Albino NMRI race were used for

determining the levels of BDNF and NGF genes expressions in the brains of male and female babies born on the first day methimazole-induced hypothyroidism. The difference between the mean changes in thyroid hormones, T3 and T4 in the control groups and hypothyroidism by one-way variance analysis and Tukey's test shows in Table 2. Based on The results the mean changes in thyroid hormones between test groups and the control group were a significant relationship ($P < 0.05$) (Table 2).

Table 2 ;The difference between the mean changes in thyroid hormones, T3 and T4

| parameter | | Control groups | low-dose intervention | high-dose intervention | control groups and high-dose intervention | control groups and low-dose intervention |
|------------|----------------|----------------|-----------------------|------------------------|---|--|
| T3 (ng/dl) | Mean | 1.590 | 0.870 | 0.7900 | P<0.05 | P<0.05 |
| | Standard error | 0.1170 | 0.038 | 0.0244 | | |
| T4 (ng/dl) | Mean | 27 | 8 | 6 | P<0.05 | P<0.05 |
| | Standard error | 1.333 | 0.816 | 1.054 | | |

According to the result of figure 1, the amount of T4 and T3 in the control group, low dose and high-dose were (27 and 1.59 ng/dl, 8 and 0.87 ng/dl and 6 and 0.79 ng/dl),

respectively. Based on the result of this study, between the high dose and control group there was significant relationship reduction in the expression ($P < 0.05$) (Fig. 1).

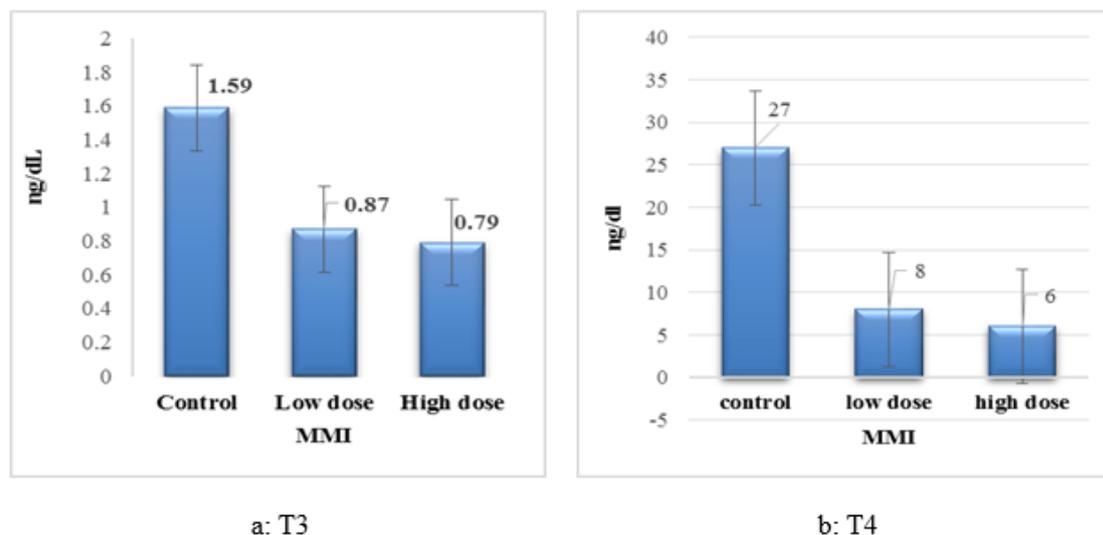


Fig.1 :The mean T4 and T3 of hormone in the serum of the mice in the test groups

By analyzing the RT-PCR expression of these genes, it was found that the expression of BDNF genes in the low dose drug group showed a significant decrease of more than 50% and in the high dose drug group, there was a significant decrease compared to the control group showed ($P < 0.05$) (Table 3). NGF also shows a significant decrease in the low dose of the drug compared to the control

group. The high dose group also showed a significant decrease compared to the control group, but there was no significant difference between males and females ($P > 0.05$) (Table 3).

According to the result of figure 2, amount of NGF and BDNF mRNA expression in the control group, low dose and high-dose showed.

Table 3.Anova analysis of NGF and BDNF mRNA expression in the test groups

| parameter | | Control groups | low-dose intervention | high-dose intervention | control groups and high-dose intervention | control groups and low-dose intervention |
|--------------|----------------|----------------|-----------------------|------------------------|---|--|
| BDNF Females | Mean | 1 | 0.574 | 0.553 | $P < 0.05$ | $P < 0.05$ |
| | Standard error | 0 | 0.029 | 0.027 | | |
| BDNF Males | Mean | 1 | 0.482 | 0.393 | $P < 0.05$ | $P < 0.05$ |
| | Standard error | 0 | 0.030 | 0.015 | | |
| NGF Females | Mean | 1 | 0.542 | 0.491 | 0 | 0 |
| | Standard error | 0 | 0.028 | 0.028 | | |
| NGF Males | Mean | 1 | 0.475 | 0.398 | 0 | 0 |
| | Standard error | 0 | 0.027 | 0.021 | | |

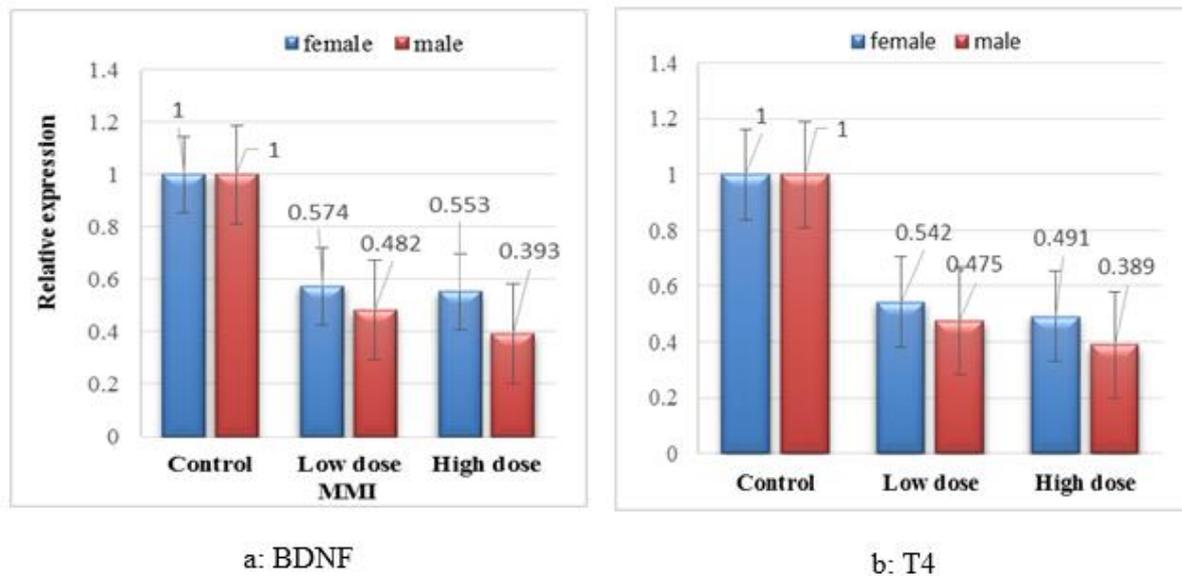


Fig.2 :Relative expression of NGF and BDNF mRNA in the brain of male and female neonates

DISCUSSION

In this study, we considered the role of thyroid hormones in regulating gene expression of the growth factors synthesized throughout the brain in vivo conditions and the results of this study have been studied at various levels. Based on our finding, in hypothyroid conditions, the number of newborns in each successful delivery period decreased significantly and the reduction in the severity of thyroid hormone decreased. Fauquier et al in 2014 studied Purkinje cells and Bergmann glia are primary targets of the TR α 1 thyroid hormone receptor during mouse cerebellum postnatal development (Fauquier *et al.*, 2014). Based on the result of their study thyroid hormone is involved in the early phases of cellular migration from the external germinal layer to the internal germinal layer, causing a delay in the migration of granular cells (Fauquier *et al.*, 2014). In this study was used to RT-PCR technique for describing the gene expression in the neonates' brain in the test groups. Regarding changes in thyroid hormones levels, the groups underwent severe hypothyroidism. Methidemazole drug substance due to the fact that the PTU drug substance has less teratogenic properties and fewer side effects to the fetus, it also has high power in hypothyroidism. This drug can

be transmitted through the bloodstream through the development of a developing fetus and will reduce the level of embryonic thyroid hormone. Therefore, maternal thyroid axis activity is delayed by the use of metha-methimazole and subsequently decreased in the mother and the thyroid hormones level. According to the result of this study, the amount of BDNF protein was measured on the morphology of neonatal and neonatal brain levels. The results show that BDNF levels in newborn and newborns do not differ significantly in neonatal zero, but the level decreases significantly with respect to the level of reduction of thyroid hormones compared to control status. In the cerebral cortex, thyroid hormones control the genes involved in cytoskeleton biogenesis, neural migration and growth and neuropathy sprouting (Navarro *et al.*, 2013). Thyroid hormones during development affect the level of expression of neurotrophins and other growth factors. Therefore, the hypothyroidism induced by methizomatium in pregnant women decreases the expression of NGF expression, BDNF and this decrease in the brain of neonates is somewhat higher but it does not have a significant effect; in newborns under developmental estrogen, the expression of NGF, BDNF is somewhat balanced. In these groups, the expression

levels of NGF, BDNF in both sexes the results showed a significant decrease compared to the control group but did not significantly affect the newborn's sex is done.

Conclusion

In the present study, we study of comparison the levels of BDNF and NGF gene expressions in the brains of male and female babies born on the first day methimazole-induced hypothyroidism mice with NMRI race. Based on the result of our study, demonstrated that those female pregnant mice with induced hypothyroidism by Methimazole, gene expression of BDNF significantly decreased in neonatal brain, compared to the control group. In addition, expression of NGF and T3 and T4 neurotrophins significantly reduced, compared to the control group. In conclusion, both low-dose and high-dose intervention groups experienced higher levels of reduction in T4 in compared to the control group. It could be suggested that thyroid hormones play a role in the expression of the mentioned gene, and any reduction in these hormones could reduce gene expression in the brain of infant mice with hypothyroidism.

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