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Original Article

Serum Neudesin Levels in Patients with Congenital Hypothyroidism

Bahar S et al. Neudesin and Congenital Hypothyroidism

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What is already known on this topic?
Neudesin is a newly discovered protein; mainly secreted from adipose tissue and brain, which plays a role as a neurotrophic factor in the brain and as a negative regulator of energy expenditure. Neurodevelopmental delay and cognitive dysfunction are common features in cases with congenital hypothyroidism (CH) without treatment.

What this study adds?
This is the first study about the relationship of neudesin with congenital hypothyroidism.

Abstract
Objectives: Neudesin is a newly discovered protein mainly secreted from adipose tissue and the brain. It plays a role as a neurotrophic factor in the brain and a negative regulator of energy expenditure. Neurodevelopmental delay and cognitive dysfunction are common features in cases with congenital hypothyroidism (CH) without treatment. Considering neudesin's role in brain development and its contribution to the survival of mature neurons, any possible relationships between neudesin and thyroid hormone were evaluated.
Methods: A total of 52 patients (32 patients with CH, 14 females and 18 males, aged 19± 7 days; 20 healthy subjects for the control group; 7 females and 13 males, aged 22± 8 days) were included in the study. All patients were evaluated for thyroid hormones and plasma neudesin levels. The basal neudesin levels between the patient and control groups and the patients' neudesin levels before and after l-thyroxine treatment were compared.
Results: Regarding basal neudesin levels, there was no statistically significant difference (6.77±6.41 vs 7.93±7.04 ng/mL) (p=0.552) between the CH and control groups respectively. However, neudesin levels increased following one month of therapy (6.46±6.63 vs 12.85±18.74 ng/mL) in the CH group; this difference was statistically significant (p=0.019).
Conclusion: Although there was no difference in basal neudesin levels between the patient and control groups, neudesin levels increased with treatment. However, more extensive and different studies are needed to understand the pathophysiological role of this relationship in the disease or the recovery process.
Keywords: Congenital hypothyroidism, neudesin, levothyroxine sodium

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Introduction

Congenital hypothyroidism (CH) is defined as thyroid hormone deficiency at birth and occurs as a result of inadequate development or function of the thyroid gland (primary CH) or inadequate pituitary stimulation of the normal thyroid gland (central CH) [1]. CH has an incidence of 1:2000 to 1:4000 worldwide [2-4]. The CH is one of the common preventable causes of intellectual impairment [5]. Screening programs have assisted in the early detection and treatment of CH [5]. The thyroid gland produces and secretes thyroid hormones (thyroxine (T4) and triiodothyronine (T3)), which are necessary for growth, neurodevelopment, and the metabolism of energy. Thyroid hormones control the development of the central nervous system specifically by affecting myelination, synapsis development, and neuronal differentiation in the prenatal and neonatal periods (6-8).
Neudesin (Neuron-derived neurotrophic factor, NENF) is a secreted neuronal protein with 21 KDa and 171 amino acids [9]. The fundamental structure of neudesin exhibits a heme/steroid binding domain that resembles that of cytochrome b5, and it is categorized as a membrane-associated progesterone receptor [10]. NENF is highly expressed in neurons at embryonic stages, particularly in the brain and spinal cord [9]. Neudesin expression has been observed during embryonic brain cortical formation in the pre-plate region, in which post-mitotic neurons exist, but not in the regions of precursors that proliferate and migrate [11]. Neudesin improves the survival of mature neurons and the proliferation and differentiation of neural precursors into a neuronal lineage [11]. Moreover, it has also been shown that neudesin is a negative regulator of energy expenditure [12].
As neudesin and thyroid hormones have similar targets (brain development, energy expenditure), the primary focus was examining the association between hypothyroidism and serum neudesin levels in mature newborns referred to the hospital with thyroid dysfunction or clinical suspicion and diagnosed with CH.

Material and methods

Patients

Newborns who were found to have elevated thyroid stimulating hormone (TSH) in the screening program for CH and who presented to our pediatric endocrinology outpatient clinic were included in the study**.** Venous blood samples of 52 newborns were obtained at the first visit to confirm the CH diagnosis. In addition, blood samples were also centrifuged at 2500 rpm for 10 minutes (Nüve NF 1200 trademark was used for cooled centrifuge), and obtained serum samples were stored at -80 C° for the study of neudesin levels. The 32 newborns (14 female, 18 male) whose serum thyroid function tests were consistent with congenital hypothyroidism (TSH>20 IU/mL with low fT4 levels) were included in the patient group. In comparison, 20 newborns (7 female and 13 male) with regular thyroid function tests (TSH<6 IU/mL and fT4 levels in the normal range) were included in the control group [1]. Children with anatomical anomalies, small for gestational age, and premature children were excluded.

All patients' length standard deviation score (SDS), weight SDS, body mass index (BMI) SDS, head circumference SDS, gestational age, chronological age, gender, and mother's thyroid illness history were recorded in patient records. We also assessed the levels of anti-thyroid peroxidase antibody (anti-TPO), anti-thyroglobulin (anti-TG), thyroglobulin, and urinary iodine in the patient group.

Following the initial examination, L-T4 treatment (10-15 mcg/kg/day) was started for CH patients immediately. Initial L-T4 dosage was adjusted on the fifteenth day of treatment by clinical and biochemical status to preserve blood fT4 and TSH levels within the normal range. The etiology of CH was also investigated by thyroid ultrasonography. After the first month of the visit following L-T4 administration, fT4, and TSH levels were analyzed. Additionally, the second serum samples were collected and stored to assess the effect of L-T4 therapy on serum neudesin levels in 26/32 CH patients. At the end of the first month of treatment, 6 of 32 patients with CH did not visit the clinic. Therefore, their control blood could not be obtained.

Informed consent was obtained from the families of the participants, and the study protocol was approved by the Ethics Committee of Bezmialem Vakif University (by number 07.12.2021-E.42452) and supported by the scientific research project department of Bezmialem Vakif University.

Hormone Assays

Serum levels of TSH, fT3, fT4, anti-TG, TG, and anti-TPO were determined at the Bezmialem University Hospital, İstanbul, Turkey, by chemiluminescent enzyme immunometric assays [CLIA] using commercial kits (procured from Siemens Medical Atellica Solutions Diagnostics, USA). The reference intervals for our study were 13.02 to 25.86 pmol/L (1.02–2.01 ng/dL) for fT4, 4.3 to 8 pmol/L for fT3, and 0.420 to 7.55 uIU/ml for TSH (13). Spot urinary iodine was detected by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 770). Serum neudesin levels were measured with "Human Neudesin, NENF Elisa Kit (BT-LAB E4258hu)".

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences, SPSS ver. 23.0. Median and interquartile ranges were used for the metric variables. The baseline data of the control and patient groups were evaluated with the Mann-Whitney U test. In contrast, the pre- and post-treatment data in the patient group were analyzed using the Wilcoxon, nonparametric test. Spearman's bivariate correlation analysis method was used to evaluate the relationship between BMI SDS and neudesin.

Results

The average age of the patients was 19±7 days, and the control group was 22±8 days. Basal anthropometric measurements, laboratory results, and comparison of the patient and control groups are shown in Table 1.

Patients with CH who were treated with L-T4 and the control group were compared, and the two groups did not differ significantly concerning anthropometric measurements (height SDS, weight SDS, head circumference SDS) (Table 1).

The statistical comparison of fT4, fT3, TSH, TG, urinary iodine levels, and neudesin levels of the CH and control groups at admission are summarized in Table 1. All patients in the control group had TSH levels below 6 mU/L. The TSH levels in patient and control groups were 280.99±297.37 mU/L and 2.47±0.74 mU/L respectively ($p<0.001$), and the fT4 levels in patient and control groups were 6.31±2.64 pmol/L and 14.99±2.03 pmol/L respectively ($p<0.001$).

Regarding basal neudesin levels, there was no statistically significant difference (6.77±6.41 vs 7.93±7.04 ng/mL) ($p=0.552$) between the CH and control groups respectively (Table 1). However, neudesin levels increased following one month of treatment (6.46±6.63 vs. 12.85±18.74 ng/mL) in the CH group; this difference was statistically significant ($p=0.019$) (Table 2). After 30 days of L-T4 treatment in the CH group, as expected, there was an increase in fT4, whereas a decrease in TSH. The effect of L-T4 treatment on thyroid hormones and serum neudesin levels is shown in Table 2.

No correlation was found between serum neudesin levels and BMI SDS in bivariate correlation analysis (r:0.245, p:0.172)

Thyroid ultrasonography was performed on CH patients. Two patients had goiter, three had agenesis, and one had hemi-agenesis. The thyroid ultrasound of the remaining patients (26/32) was normal. In addition, iodine deficiency was found in 4 patients, iodine excess in 5 patients, and anti-thyroid peroxidase positivity in 2 patients.

Discussions

Neudesin is a membrane-associated progesterone receptor (MAPR) protein family member. It has been previously demonstrated that neudesin also plays different roles in energy metabolism, neural functions, and tumorigenesis [12]. Neudesin is primarily expressed in neurons. Moreover, neudesin exhibits significant neurotrophic activity in primary cultured neurons but not mitogenic activity in primary cultured astrocytes, indicating that it is a neurotrophic factor [9,12]. Neudesin is also expressed in neural precursor cells before the appearance of neurons in mice, indicating its potential role in neural development [11]. Neuronal differentiation may be promoted by neudesin, mediated through activation of the protein kinase A [PKA] and PI3K pathways in cultured neural precursor cells. Neural cell proliferation may also be promoted by neudesin in the developmental process. [11]. Thyroid hormones are also necessary for growth and neurodevelopment [8]. Moreover, although no molecular studies show the relationship between thyroid hormones and neudesin, a few studies demonstrate the relationship between thyroid hormones and other neurotrophic factors. Yajima et al. have reported that the lack of thyroid hormones induces a developmental delay in primary hippocampal neurons, likely caused by decreased brain-derived neurotrophic factor *(BDNF)* gene expression (14). Furthermore, in vivo experiments have shown that thyroid hormone administration increases BDNF expression in the brain tissue of young adult rats (15). In another study, Lesley et al. showed that BDNF expression decreased in adult rats with hypothyroidism, but this change was not detected in the neonatal period in the same study (16). As both thyroid hormones and neudesin affect neurodevelopment, we hypothesized that thyroid hormones might have a relationship with neudesin. Our results demonstrated an increase in neudesin levels after thyroid replacement therapy. However, this study could not explain the pathophysiological mechanisms of this relationship.

Activating the parasympathetic system leads to energy expenditure in white adipose tissue and increased heat in brown adipose tissue. A study in neudesin knock-out mice has shown that the parasympathetic nervous system is over-activated in these animals. Based on this finding, it has been suggested that neudesin has a negative regulatory role in energy balance [17]. Neudesin's role in different metabolic disorders such as obesity, polycystic ovary syndrome, and type 2 diabetes mellitus has been discussed [18-21]. Celikkol et al. have reported a negative correlation between neudesin levels and body mass index z-score [21]. Kratochvilova H et al. have also reported that neudesin levels change with chronic weight reduction or during prolonged fasting [18]. Moreover, Polkowska A. et al. have reported that neudesin levels were higher in diabetic patients compared to the control group. This finding has demonstrated that neudesin has a role in energy homeostasis. In light of these data, neudesin levels are expected to decrease with increasing BMI. However, in our study, although there was a small increase in BMI SDS levels, neudesin levels also increased, and no correlation was detected between BMI SDS and neudesin. Therefore, there is insufficient evidence to explain the increase in serum neudesin levels with changes in BMI in our cohort. Previously, it

has been shown that TRH/TSH is regulated by thyroid hormone feedback, and there is also central modulation by nutritional signals, such as leptin, and peptides regulating appetite [22]. The rapid recovery of the slow metabolism with the normalization of thyroid hormones can result in rapid energy consumption. One mechanism of adaptation to this rapid change in metabolism may be the increase in neudesin levels. Experimental studies are needed to evaluate the pathophysiological mechanisms between neudesin and thyroid hormones regarding energy homeostasis.

Although there are a few studies about neudesin levels in metabolic disorders, to our knowledge, there was not any clinical study about neudesin in patients with congenital hypothyroidism or other neurodevelopmental diseases. Therefore, the levels of neudesin in children with or without neurologic involvement in hypothyroidism are not yet known. The participants in our study were those with no neurological complaints, regular neurological examinations, and early hypothyroidism diagnoses as a result of the screening program. Our patient group was detected and treated earlier. Investigating the relationship between neurologic involvement and neudesin levels should be the subject of another study.

The most important limitation of our study was that, due to ethical reasons, second blood samples could not be taken from the group in which hypothyroidism was not detected. Therefore, we could not observe the change in the neudesin levels in the healthy control group. The second limitation was the low number of patients.

In conclusion, although there was no difference in basal neudesin levels between the patient and control groups, an increase in neudesin levels was observed with levothyroxine sodium treatment in children with CH. However, more extensive clinical or experimental studies are needed to investigate the pathophysiological role of this relationship in the disease or the recovery process.

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Table 1. Baseline data of 52 newborns (Patients group vs Control group)

Variables	Patients (n=32)	Control group (n=20)	P value
Gender M/F	18/14	13/7	0.371
Age (day)	19 ±7 (7-30)	22±8 (14-30)	0.136
Length SDS	-0.34±0.72	-0.51±0.75	0.26
Weight SDS	-0.041±0.77	-0.35±1.1	0.24
Body mass index (kg/m^2)	14.18±1.56	14.42±1.90	0,418
Head circumference SDS	0.043±0.80	-0.27±0.77	0.16
Mother's thyroid disorder history	7/32 (21%)	1/20 (%0.5)	**<0.001**
fT3 (pmol/L)	3.14±2.01	6.06±0.86	**<0.001**
fT4 (pmol/L)	6.31±2.64	14.99±2.03	**<0.001**
TSH (mU/L)	280.99±297.37	2.47±0.74	**<0.001**
Thyroglobulin (ng/mL)	1482,68±1351.97	67.74±39.90	**0,002**
Urinary iodine (mcg/L)	400.83±334.23	173.11±96.73	**<0,001**
Neudesin level*(ng/mL)	6.77±6.41	7.93±7.04	0.552
Thyroid USG	Agenesis: 3 Hemi-agenesis: 1 Goiter: 2 Normal: 26		

M: male; F: female; fT3: free triiodothyronine; fT4: free thyroxine; TSH: thyroid stimulating hormone; Tg: thyroglobulin; USG: ultrasonography; SD: standard deviation. *Baseline neudesin levels

Table 2: Effect of levothyroxine sodium treatment on anthropometric measures. thyroid hormone, and neudesin levels

	Patients before treatment	Patients after treatment	p
Length SDS	-0.03±.72	-0.15±0.66	0.41
Weight SDS	-0.42±0.77	-0.01±0.51	0.76
BMI	14.23±1.49	15.76±1.32	<0,001
BMISDS	-0.13±0.91	0.41±0.62	<0,001
Head circumference SDS	-0.43±0.80	-0.18±0.75	0.15
fT4 (pmol/L)	6.31±2.64	20.92±3.97	**<0.001**
TSH (mU/L)	280.99±297.37	4.89±16.07	**<0.001**
Neudesin level* (ng/mL)	6.46±6.63	12.85±18.74	**0.019**

fT4: free thyroxine; TSH: thyroid stimulating hormone; SD: standard deviation. *The second blood sample obtained only in 26 of 32 children.