Brain-derived neurotrophic factor (BDNF) and the capute scales in offspring of mothers with normal and deficient vitamin D levels

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Brain-derived neurotrophic factor (BDNF) and the capute scales in offspring of mothers with normal and deficient vitamin D levels

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ABSTRACT

Background: Vitamin D deficiency during pregnancy is associated with a child's neurocognitive development. During the COVID-19 pandemic, the Capute Scales have become ineffective because requiring face-to-face interaction to examine children's neurocognitive development. Brain-Derived Neurotrophic Factor (BDNF) is an alternative method to determine children's neurocognitive development through blood sampling. This study aims to evaluate the relationships between BDNF and the Capute Scales in the offspring of mothers with vitamin D levels.

Methods: A diagnostic study from 14 community health centers in Semarang City, Central Java, Indonesia, was conducted. The study was performed from August 2017 until August 2018 and included vitamin D level records at 20–24 weeks of normal gestation in 2017 from all single births at these community health centers. The cases were divided into two groups: 30 children born to mothers with normal vitamin D levels (25(OH)D > 20 ng/mL) and 30 children born to mothers with vitamin D deficiency (25(OH)D < 20 ng/ml). This study aimed to assess the relationship between BDNF levels and the Capute Scales of children aged 2 years and to determine the cut-off value based on each group's examination of BDNF levels.

Results: The mean age of pregnant women whose vitamin D levels were checked was 29.32 years. The cut-off value of BDNF among the two groups was 7968 pg/mL. Of the 55 samples that met the Capute Scales criteria, 44 had a BDNF value less than the cut-off value, and 11 samples had a BDNF value more than the cut-off value. Meanwhile, of the five samples that met the Capute Scales criteria and were suspected of mental retardation, three had a BDNF value less than the cut-off value, and two had a BDNF value more than the cut-off value. The sensitivity, specificity, positive, and negative predictive values were 80%, 60%, 0.93, and 0.15, respectively.

Conclusions: BDNF levels can be an alternative neurocognitive examination in children born to mothers with normal and deficient vitamin D levels. BDNF is a growth factor that plays an important role in neurons' differentiation, growth, and survival in the postnatal phase.

Keywords: Brain-Derived Neurotrophic Factor, Capute Scales, Vitamin D.
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BACKGROUND

Vitamin D is a micronutrient that contributes to bone growth and calcium homeostasis regulation. Vitamin D has several effects on neurodevelopment, and it plays a role in the growth of bone.[1] However, vitamin D deficiency remains a major public health issue worldwide because it can affect people of various ages and socioeconomic backgrounds. Pregnant women are the most at risk for vitamin D deficiency. Based on the Endocrine Society, vitamin D deficiency is defined as <20 ng/mL (<50 nmol/L). In a study conducted in West Sumatra, Indonesia, vitamin D deficiency is prevalent in the first and third trimesters, accounting for 82.8% and 61.2%, respectively. Another study in West Java, Indonesia, found that 86.5% of pregnant women had vitamin D deficiency in the first trimester.[2–4] Vitamin D deficiency during pregnancy is due to lack of a vitamin D-rich diet, lack of mobility and exposure to sunlight, lack of vitamin D supplementation, and darker skin tones in some populations.

Fetuses are utterly dependent on their mother's vitamin D status.[2–12] Low levels of vitamin D during pregnancy have been correlated with a child's neurocognitive development, including signaling roles in cell differentiation and synaptogenesis, gene expression, neurotrophic and neurotoxic factor metabolic control, and a protective role during brain inflammation.[13–16] The Capute Scales assessment determines a child's neurocognitive status. The Capute Scales is a tool for assessing cognitive, verbal, and physical development in children under three years old. This evaluation takes only 6–15 min to complete.[17] However, this measure has some limitations because the COVID-19 epidemic limits regular doctor-patient meetings. This limitation has resulted in a search for other neurocognitive tests that

can be performed.

Neurotrophin, which is a growth factor, has been demonstrated to play a role in the differentiation, synthesis, and protection of neuronal cells in several recent studies.[18] It also leads to brain-derived neurotrophic factor (BDNF), which is involved in both prenatal and postnatal brain development.[19,20] BDNF has been linked to a variety of neurocognitive issues, including neurological and mental diseases, in multiple studies.[21] Therefore, this study aimed to determine the cut-off value of BDNF levels based on the Capute Scales examination in children aged 2 years who were born to mothers with normal pregnancies and vitamin D deficiency.

METHODS

Data collection

The design was cohort retrospective. This study collected data from 14 community health centers in Semarang City, Central Java, Indonesia. This study was performed from August 2017 until August 2018. In addition, this study included vitamin D levels recorded at 20–24 weeks of normal singleton gestation in 2017 at these community health centers. The inclusion criteria were singleton and normal pregnancy. The subjects will be excluded if they have congenital anomalies and refuse to join this research. It also included the BDNF of children from birth through blood sampling and the Capute Scales examination in children aged 2 years. During the first 1000 days of life, the research team extracted data using piloted data extraction forms. In total, 353 cases were collected, but only 60 were recruited for the pilot study because of the COVID-19 pandemic. All of the subjects used consecutive sampling.

Study design

The cases were divided into two groups: children born to mothers with normal vitamin D levels (25 (OH) D > 20 ng/mL) and children born to mothers with vitamin D deficiency (25 (OH) D 20 ng/mL). A total of 30 cases were included in the normal vitamin D group and another 30 cases in the vitamin D deficiency group. Information about pregnant women was collected, including age, education, body mass index (BMI) before pregnancy, and social-economic status. In addition, information about children, including sex, Z score, BDNF level, and the Capute Scales scores, was collected.

Plasma BDNF evaluation

Plasma BDNF contents were measured using a commercially available ELISA kit (Elabscience Biotech). Plasma was collected using EDTA or heparin as an anticoagulant. The samples were centrifuged for 15 minutes at 10000 × g and 2°C–8°C within 30 minutes of collection. Afterward, 100 µL of supernatant was added to the appropriate well and pre-coated with an antibody specific to BDNF, followed by incubation for 90 min at 37°C. Then, a biotinylated detection antibody specific to BDNF and HRP conjugate was added to each well and incubated for 1.5 h. The substrate solution was added to each well, and the enzyme-substrate reaction was terminated by adding sulfuric acid solution. The final absorbance was read at 450 nm using a microplate reader (Victor X5, Perkin Elmer).

Capute Scales examination

The Capute Scales consist of two types of examinations: the cognitive adaptive test (CAT) and the clinical linguistic and auditory milestone scale (CLAMS). A midwife in community health centers performed the Capute Scales examination. After the examination, the midwife, accompanied by a pediatrician, made a home visit and performed neurocognitive monitoring of the children. The CAT measurement also consists of 19 levels of testing age with 57 visual-motor milestones. The child should perform all of the milestones of the visual-motor scale (some spontaneous and some after being exemplified by the examiner). Each test should start at two age groups below the child's functional level and continue until the highest age group, where the child can complete the task. The CLAMS measures receptive and expressive language milestones. It has 26 expressive language milestones that cover 19 age levels of testing, namely, 1–12 months (1-month interval); 14, 16, and 18 months (2-month interval); 21 and 24 months (3-month interval); and 30 and 36 months (6-month interval). The Capute Scales were cumulative scores of CAT and CLAMPS.

Statistical analysis

Statistical analysis was performed using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA) for Windows. Normally distributed data were expressed as mean ± standard deviation. We analyzed the Independent-T Test to compare the parametric scale characteristics and the Chi-square test to compare the continuous and categorical data between the two groups. Pearson's correlation analysis was used to explore relationships. The cut-off value of BDNF in children born to mothers with normal vitamin D levels and children born to mothers with vitamin D deficiency was calculated using the Youden index of the receiver operating characteristic (ROC) curve. A p-value less than 0.05 was considered significant.

RESULTS

Sixty pregnant mothers and their offspring were included in this study. The characteristics of the samples are listed in Table 1. No significant difference in maternal age, weight, height, BMI, educational status, or socioeconomic level was observed between the two groups. The average vitamin D level in the normal group was 23.83 ± 4.39 ng/mL, whereas the average vitamin D level in the deficiency group was 15.57 ± 2.50 ng/mL. On the contrary, a mean difference in BDNF levels was observed in children

of pregnant women with normal vitamin D levels and the offspring of pregnant women with vitamin D deficiency, but this difference was not statistically significant. In addition, no differences were found between the two groups.

The optimal cut-off value was obtained using the Youden index J formula, the largest value (sensitivity + specificity − 1) of any one cut-off value. The ROC curve in Figure 1 shows an optimal cut-off value of 9,769.50 pg/mL. The cut-off sensitivity value was 80%, with a specificity of 60%. In addition, the positive predictive value was 0.93, and the negative predictive value was 0.15. The accuracy of the cut-off value was 76.67%, with a 95 % Area Under Curve (AUC) of 0.585.

Table 1. Characteristics of Samples.

Characteristics	All (N = 60)	Normal vitamin D level (n = 30)	Vitamin D deficiency (n = 30)	p
Pregnant Women				
Age (Year) (mean±SD)	29.32 ± 5.71	30 ± 5.93	28.63 ± 5.49	0.631
Weight (kg) (mean±SD)	53.72 ± 10.11	52.73 ± 9.69	54.70 ± 10.58	0.452
Height (m) (mean±SD)	1.52 ± 0.05	1.51 ± 0.05	1.52 ± 0.05	0.571
Body Mass Index before pregnancy (kg/m^2), n (%)				0.530
Underweight	6 (10.0)	3 (10.0)	3 (10.0)	
Normal	34 (56.7)	19 (63.3)	15 (50.0)	
Overweight	20 (33.3)	8 (26.7)	12 (40.0)	
Education Status, n (%)				0.136
Elementary School	4 (6.7)	3 (10.0)	1 (3.3)	
Junior High School	14 (23.3)	7 (23.3)	7 (23.3)	
Senior High School	36 (60.0)	18 (60.0)	18 (60.0)	
Diploma degree	4 (6.7)	0 (0.0)	4 (13.3)	
Bachelor degree	2 (3.3)	2 (6.7)	0 (0.0)	
Socioeconomic status (income), n (%)				0.500
Middle income	23 (38.3)	11 (36.7)	12 (40.0)	
Low income	37 (61.7)	19 (63.3)	18 (60.0)	
Vitamin D levels (ng/mL) (mean±SD)	19.70 ± 5.46	23.83 ± 4.39	15.57 ± 2.50	0.452
Offspring				
Sex, n (%)				0.060
Male	29 (48.3)	11 (36.7)	18 (60.0)	
Female	31 (51.7)	19 (63.3)	12 (38.7)	
Weight (kg) (mean±SD)	12.37 ± 1.56	12.25 ± 1.61	12.49 ± 1.53	0.869
Height (cm) (mean±SD)	89.41 ± 10.25	98.75 ± 14.12	91.08 ± 2.95	0.064
Head circumference (cm) (mean±SD)	47.68 ± 1.15	47.57 ± 1.17	41.74 ± 76.18	0.984
Upper arm circumference (cm) (mean±SD)	14.94 ± 1.07	14.94 ± 1.16	14.95 ± 1.14	0.111
BDNF level (pg/mL) (mean±SD)	7,605.55 ± 4,202.23	7,329.37 ± 3,791.60	7,881.73 ± 6,991.50	0.346

*p-values were provided by independent *t* test to compare the parametric scale characteristics and Chi-square and Fischer exact test to compare the continuous and categorical data between the two groups.

Table 2 shows the sample distribution with a comparison between the BDNF cut-off value and the Capute Scales criteria. Of the 55 samples that met the normal Capute Scales criteria, 44 had a BDNF value less than the cut-off value, and 11 samples had a BDNF value more than the cut-off value. Meanwhile, of the five samples that met the Capute Scales criteria and were suspected of mental retardation, three had a BDNF value less than the cut-off value, and two had a BDNF value more than the cut-off value. The Kappa value from the BDNF and Capute Scales was 0.116 (slight agreement) with a *p-value* of 0.299.

DISCUSSION

Vitamin D deficiency in pregnant women is a health issue faced by each country. Vitamin D can be obtained endogenously and exogenously and can be passed through the placenta to the fetus.[13–16] In general, vitamin D deficiency during pregnancy is due to a lack of a vitamin D-rich diet, lack of mobility and exposure to sunlight, lack of vitamin D supplementation, and darker skin tones in most Indonesians. Congenital rickets, osteopenia, asthma, rhinitis, and poor neurocognitive development are potential consequences in children born to mothers who are vitamin D deficient.[13–16] The effect of vitamin D levels in pregnant women and predictors of BDNF on the Capute scales, which measures the neurocognitive development of children of pregnant women with normal vitamin D levels and vitamin D deficiency, was the subject of this study.

To our knowledge, this study was the first to investigate plasma BDNF levels in children of pregnant women with normal vitamin D levels and vitamin D deficiency. Our retrospective cohort study included 60 women with singleton pregnancies: 30 had normal vitamin D levels, and another 30 women had vitamin D deficiency. The samples were collected from 14 public health centers in Semarang, Indonesia. Vitamin D levels were derived from data collected between 20 and 24 weeks of pregnancy, and each child was tested for BDNF at the age of 2 years. Based on the findings of our study, the cut-off value for BDNF levels in children born to mothers with normal vitamin D levels was 9653.50 pg/mL. Meanwhile, the cut-off value for BDNF levels among children born to mothers with vitamin D deficiency was 3611.00 pg/mL. A BDNF level greater than the appropriate cut-off value indicates a lower Capute Scales score. Our results are consistent with those of Subedi et al. They assessed the BDNF cut-off value in infants with neonatal abstinence syndrome (NAS)

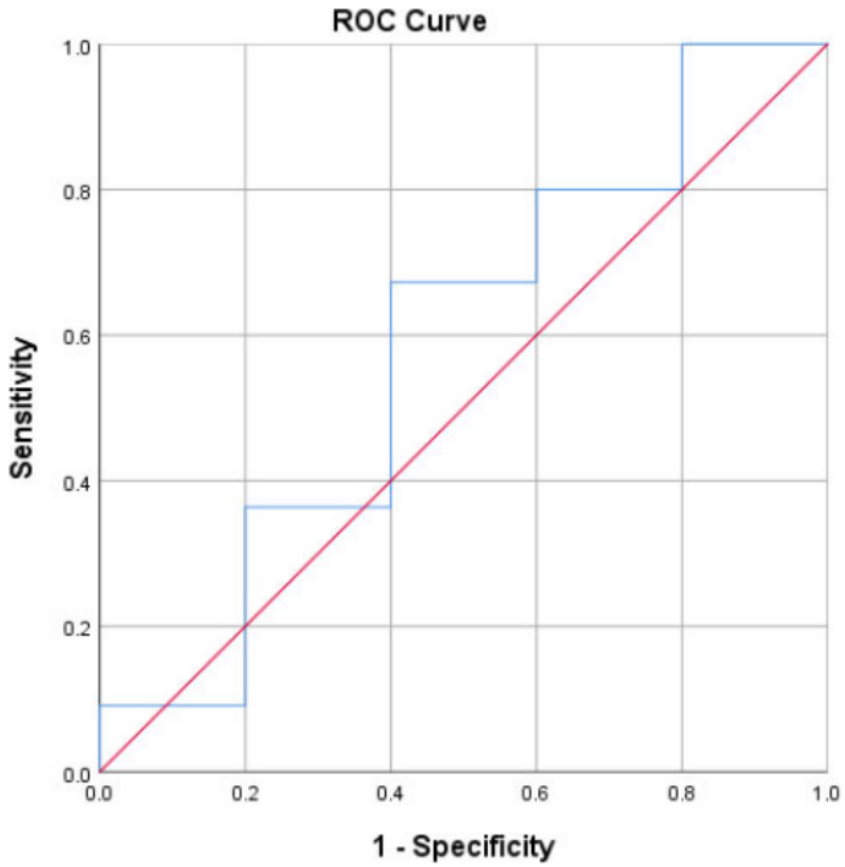

Figure 1. ROC curve addressing the association between BDNF levels and Capute Scales scores in offspring born to mothers with normal vitamin D levels and vitamin D deficiency. ROC, receiver operating characteristic.

Table 2. Comparisons between the BDNF and Capute Scales.

Variables	Normal	Suspected of mental retardation	Total
BDNF ≥ Cut-off	11	2	13
BDNF < Cut-off	44	3	47
Total	55	5	60

Sensitivity 80%; Specificity 60%; PPV 0.93; NPV 0.15; Cut-Off: 9,769.50 pg/ml
BDNF: Brain-derived neurotrophic factor, PPV: Positive predictive value, NPV: Negative predictive value

and found that in the early withdrawal phase, BDNF levels were high in infants with NAS.[22]

Several studies have associated Vitamin D deficiency with low levels of BDNF.[23,24] Previous studies have found an association between BDNF levels and acetylcholinesterase activity in those who were given D3 supplementation.[23,24] Therefore, vitamin D might indirectly affect the cholinergic system by enhancing BDNF levels. In addition, cholinergic neuronal maturation and survival were dependent on neurotrophins, including BDNF.[24,25]

In the present research, BDNF levels were found to be below the optimal cut-off value in 25 of 30 children of pregnant mothers with normal vitamin D levels. Meanwhile, BDNF levels were above the optimal cut-off value in 26 out of 30 pregnant women with vitamin D deficiency children. This finding demonstrates a significant difference in BDNF levels between children born to mothers with normal vitamin D levels and children born to mothers with vitamin D deficiency.

This study also has limitations. Many study participants refused to conduct home visits because of the COVID-19 pandemic; thus, the number of samples used in this study was limited. The effect of vitamin D levels in pregnant women on children's BDNF levels and their correlation with the Capute Scales score will be better described if the number of participants in this study is sufficient.

CONCLUSION

BDNF levels can be an alternative neurocognitive examination in children born to mothers with vitamin D deficiency. BDNF is a growth factor that plays an important role in neurons' differentiation, growth, and survival in the postnatal phase.

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CONFLICT OF INTEREST

The authors declared that they have no competing interests.

ETHICS APPROVAL

The study was approved by the Research Ethics Committee, Faculty of Medicine, Diponegoro University. All the study methods were conducted in accordance with the seven WHO 2011 standards. Written consent was obtained from participants of the study.

CONSENT TO PUBLICATION

The participants had oral consent that their data would be published in a journal article without mentioning their names.

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AUTHOR'S CONTRIBUTION

JD contributed to conceiving the study idea, writing the protocol, leading data collection and data analysis, interpreting data, and writing and reviewing the manuscript. FH contributed to writing a protocol and analyzing the result of the Capute Scales. MM contributed to developing a protocol, interpreting data, and revising the manuscript. AS contributed to creating a protocol and revising the manuscript. AE contributed to developing a protocol, interpreting data, and revising the manuscript. SH contributed to creating a design and revising the manuscript. DP contributed to writing a protocol, interpreting data, and reviewing the manuscript.

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