

Serum Levels of Mature Brain-Derived Neurotrophic Factor (BDNF) and Its Precursor proBDNF in Healthy Subjects

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Abstract: BACKGROUND: Accumulating evidence points to the brain-derived neurotrophic factor (BDNF) as a biomarker for neuropsychiatric diseases, such as major depression. Mature BDNF is synthesized from its precursor form, proBDNF. Although BDNF levels in human blood can be measured using commercially available human BDNF ELISA kits, due to limited specificity of the BDNF antibody, these kits are unable to distinguish between proBDNF and mature BDNF. In this study, we measured serum levels of proBDNF and mature BDNF in healthy subjects, using human proBDNF and BDNF ELISA kits, respectively.

METHODS: Serum levels of proBDNF and mature BDNF in healthy subjects (n = 40) were measured using the sandwich human proBDNF and BDNF ELISA kits.

RESULTS: In healthy subjects, serum levels of mature BDNF were 23.71 ± 5.61 ng/mL (mean \pm S.D., n=40). Serum levels of proBDNF in healthy subjects were 7.58 ± 7.68 ng/mL (mean \pm S.D., n=25). However in 15 subjects, serum levels of proBDNF were less than the minimum detectable concentration (0.5 ng/mL) of the kit.

CONCLUSIONS: This study shows that serum levels of proBDNF and mature BDNF are measurable using either the commercially available human proBDNF or BDNF ELISA kits, although the sensitivity of proBDNF kit was unacceptably low. These ELISA kits may be useful for measuring proBDNF and mature BDNF in the body fluids of patients with neuropsychiatric, cardiovascular and other diseases.

Keywords: Biomarker, brain-derived neurotrophic factor (BDNF), mature BDNF, proBDNF, ELISA, blood.

1. INTRODUCTION

At present, there are no clinical laboratory tests that can be used by doctors to assist with the diagnosis of patients with neuropsychiatric diseases. Identification of biomarkers in human body fluids such as blood, urine, and cerebral spinal fluid (CSF) would aid both in the diagnosis of neuropsychiatric diseases, and development of effective therapies [1].

Mature brain-derived neurotrophic factor (BDNF) is a 13 kDa polypeptide, known to play an important role in the survival, differentiation, and outgrowth of select peripheral and central neurons during development and adulthood [2,3]. Accumulating evidence suggests a pivotal role for BDNF in the pathophysiology of major depression, as well as in the therapeutic mechanisms of antidepressants [4-9]. Mature BDNF is initially synthesized as a precursor protein, prepro BDNF, in the endoplasmic reticulum. Following cleavage of the signal peptide, proBDNF (~32 kDa) is converted to mature BDNF (13 kDa) by extracellular proteases (Fig 1) [9-12]. It was initially thought that only secreted, mature BDNF was biologically active, and that proBDNF, localized intracellularly, serving as an inactive precursor. However,

recent studies show that proBDNF and mature BDNF elicit opposing effects *via* the p75^{NTR} and TrkB receptors, respectively, and that both proBDNF and mature BDNF play important roles in several physiological functions (Fig. 1) [9-12].

BDNF is present in human blood, although it is highly concentrated in brain tissue. Previously, we reported that serum BDNF levels were significantly lower in patients with neuropsychiatric diseases, such as major depression [13], eating disorders [14,15], pediatric depression [16], and high-functioning autism [17]. Subsequent meta-analyses confirmed our findings on major depression [18-21]. Therefore, it is likely that accurate measurement of blood BDNF levels could serve as a potential biomarker for major depression [9].

Considering the important role that both proBDNF and mature BDNF play in the physiological functioning of the brain, it would be valuable to measure individual levels of precursor and mature BDNF in the body fluids of human subjects [9]. In this study, we measured serum levels of proBDNF and mature BDNF in healthy subjects using the human either the proBDNF or BDNF ELISA kits (Adipo Bioscience) (Table 1). Furthermore, we also measured serum levels of total BDNF, including proBDNF and mature BDNF, using other commercially available human BDNF ELISA kits from Millipore, R&D Systems and Promega

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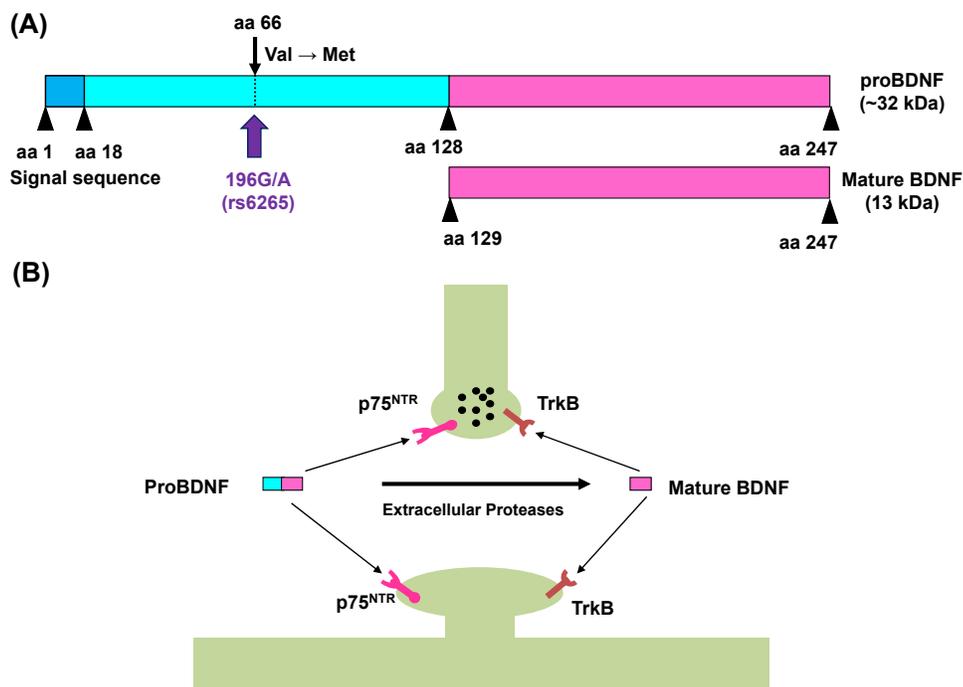


Fig. (1). (A) Structure of proBDNF and mature BDNF. Arrowheads indicate known protease cleavage sites involved in the processing of mature BDNF. The position of the single nucleotide polymorphism (rs6265, Val66Met) in the human *BDNF* gene is indicated by an arrow. (B) Extrasynaptic cleavage of proBDNF to mature BDNF. ProBDNF preferentially binds p75^{NTR}. ProBDNF is cleaved by extracellular proteases at the synapses and converted to mature BDNF. Mature BDNF preferentially binds the TrkB receptor. This figure is a modified version of previously published figures [9-11].

Table 1. Properties of Human proBDNF and BDNF ELISA Kits

	Human proBDNF ELISA Kit	Human BDNF ELISA Kit	Chem Kine BDNF Kit	Quantikine BDNF ELISA Kit	BDNF Emax ImmunoAssay System
Company	Adipo Bioscience	Adipo Bioscience	EMD Millipore Corporation	R&D Systems	Promega Corporation
Catalog No.	SK00752-06	SK00752-01	CYT306	DBD00	G7611
Sensitivity	0.5 ng/ml	5 - 8 pg/ml	7.8 pg/ml	20 pg/ml	15.6 pg/ml
Cross-reactivity	proBDNF	Mature BDNF	proBDNF and mature BDNF	proBDNF and mature BDNF	proBDNF and mature BDNF

(Table 1), since these kits are routinely used worldwide. It is known that the BDNF Emax ImmunoAssay System (Promega) is based on an antibody to the carboxy terminal region of BDNF, and recognizes both the precursor and mature forms of BDNF [22].

2. METHODS AND MATERIALS

2.1. Subjects

Forty healthy subjects (age: 30.7 ± 6.87 years old, range: 21-40 years old) participated in this study as normal controls (Table 2). The ethics committee of Chiba University Graduate School of Medicine approved the study protocol, and all subjects provided written, informed consent for participation in the study. Healthy subjects were recruited from the local Chiba area, by advertisement. Subjects were screened using the Structured Clinical Interview for DSM-IV Axis I Disor-

ders, Non-Patient Edition, to exclude Axis I disorders, according to DSM-IV criteria.

2.2. Procedures

Serum samples from normal control subjects were collected between 9:00 to 15:00, and stored at -80°C until use. Serum levels of proBDNF and mature BDNF were measured by using the human proBDNF ELISA Kit (Cat #: SK00752-06, Adipo Bioscience, Santa Clara, CA, USA) and the human BDNF ELISA Kit (Cat #: SK00752-01, Adipo Bioscience, Santa Clara, CA, USA), respectively (Table 1). Serum levels of total BDNF, including proBDNF and mature BDNF, were also measured using the BDNF Emax[®] ImmunoAssay System (Cat #: G7611, Promega Corporation, Madison, WI, USA), Quantikine[®] human BDNF Immunoassay (Cat #: DBD00, R&D Systems, Minneapolis, MN, USA), and ChemKine[™] BDNF Sandwich ELISA (Cat#:

Table 2. Serum Levels of proBDNF and BDNF in Healthy Subjects

Age (years old)	30.7 ± 6.87 (21-40)(n=40)
Gender (Male/Female)	19/21
proBDNF (Adipo Bioscience)	7.58 ± 7.68 ng/mL (0.656 – 31.85)(n=25) <0.5 ng/mL (n=15)
BDNF mature (Adipo Bioscience)	23.71 ± 5.61 ng/mL (12.56 – 36.64)(n=40)
BDNF (Millipore)	23.75 ± 16.82 ng/mL (5.80 – 79.19)(n=40)
BDNF (R&D Systems)	24.81 ± 5.87 ng/mL (11.34 – 36.62)(n=40)
BDNF (Promega)	16.50 ± 3.88 ng/mL (7.23 – 24.76)(n=40)

The values are the mean ± S.D.

The values in the parenthesis are the range.

CYT306, EMD Millipore Corporation, Billerica, MA, USA) (Table 1). To minimize assay variance, serum levels of proBDNF and mature BDNF from each subject were measured using the Adipo Bioscience kits, on the same day. Serum levels of total BDNF in each subject, using the three remaining kits were also measured on the same day. All experiments were performed in duplicate. Protocols were performed according to the manufacturer's instructions. The optical density of each well was measured using an automated microplate reader (Emax; Molecular Devices, Sunnyvale, CA, USA).

2.3. Statistical Analysis

The data were presented as the mean ± standard deviation (S.D.). Analysis of BDNF serum levels from four groups was performed using one-way analysis of variance (ANOVA), and the *post hoc* Dunnett test. The relationship between the two variables was ascertained using Pearson's correlation coefficients. Values of $p < 0.05$ were considered statistically significant.

3. RESULTS

Using the human proBDNF and BDNF ELISA kits (Adipo Bioscience), we measured serum levels of proBDNF and mature BDNF respectively, in healthy subjects. Serum levels of mature BDNF were 23.71 ± 5.61 ng/mL (n=40) (Table 2). Where proBDNF was measurable, serum levels were 7.58 ± 7.68 ng/mL (n=25). In 15 subjects, serum levels of proBDNF were below the minimum detectable concentration (0.5 ng/mL) of the proBDNF ELISA kit (Table 2). In 38 subjects, levels of proBDNF were lower than those of mature BDNF although, in two male subjects, this pattern was reversed.

Next, we measured total BDNF in the serum of healthy subjects using the three other ELISA kits. Total BDNF levels were 23.75 ± 16.82 ng/mL (Millipore, n=40), 24.81 ± 5.87 ng/mL (R&D Systems, n=40), and 16.50 ± 3.88 ng/mL (Promega, n=40) (Table 2). One-way ANOVA revealed significant differences [$F(3, 156) = 6.439, p < 0.001$] within the four groups, Adipo Bioscience, Millipore, R&D Systems and Promega, and *post hoc* analysis indicated that serum levels of total BDNF as measured by Promega were significantly ($p = 0.003$) lower than those of mature BDNF as measured by Adipo Bioscience. In contrast, there were no differences

among the three kits from Adipo Bioscience, Millipore and R&D Systems.

As shown in Fig. (2), there were significantly positive correlations between mature BDNF (Adipo Bioscience), and total BDNF serum levels (R&D Systems, $r = 0.701, p < 0.001$, Promega, $r = 0.673, p < 0.001$) (Figs. 2A and 2B). Furthermore, there was also a weak but significant correlation between mature BDNF (Adipo Bioscience) and total BDNF serum levels (Millipore, $r = 0.377, p = 0.016$) (Fig. 2C).

4. DISCUSSION

In this study, we measured serum levels of proBDNF and mature BDNF in human subjects, using either the human proBDNF or human BDNF ELISA kits. To the best of our knowledge, this is the first report demonstrating the measurement of proBDNF as well as mature BDNF from the serum of human subjects. According to the manufacturer's information, the human proBDNF ELISA kit (Adipo Bioscience: Cat No. SK00752-06) recognizes proBDNF, but not the mature form, while the human BDNF ELISA kit (Adipo Bioscience: Cat No. SK00752-01) recognizes mature BDNF, but not the precursor form (Table 1). In tests, we were unable to measure serum levels of proBDNF in some subjects, since the values fell below the minimum detectable threshold of the proBDNF ELISA kit. The manufacturer's instructions state that the minimum detectable concentrations of the proBDNF and BDNF ELISA kits are 0.5 ng/mL and 5-8 pg/mL, respectively (Table 1), indicating that the sensitivity of the proBDNF kit is markedly lower than that of the mature BDNF ELISA kit. Therefore, accurate measurements of low levels of proBDNF in human body fluids require the development of an ELISA kit of higher sensitivity than is currently available. Nonetheless, we were able to measure proBDNF and mature BDNF in the serum of human subjects using these ELISA kits.

In this study, we also measured serum levels of total BDNF, including proBDNF and mature BDNF, using the human BDNF ELISA kits from Millipore, R&D Systems and Promega, since these kits are commonly used worldwide. The manufacturer's instructions state that the Millipore *ChemiKine*TM BDNF ELISA kit is based on mouse monoclonal antibodies generated against human mature BDNF. The *Quantikine*[®] BDNF ELISA kit (R&D Systems) is based on monoclonal antibodies generated against human recombi-

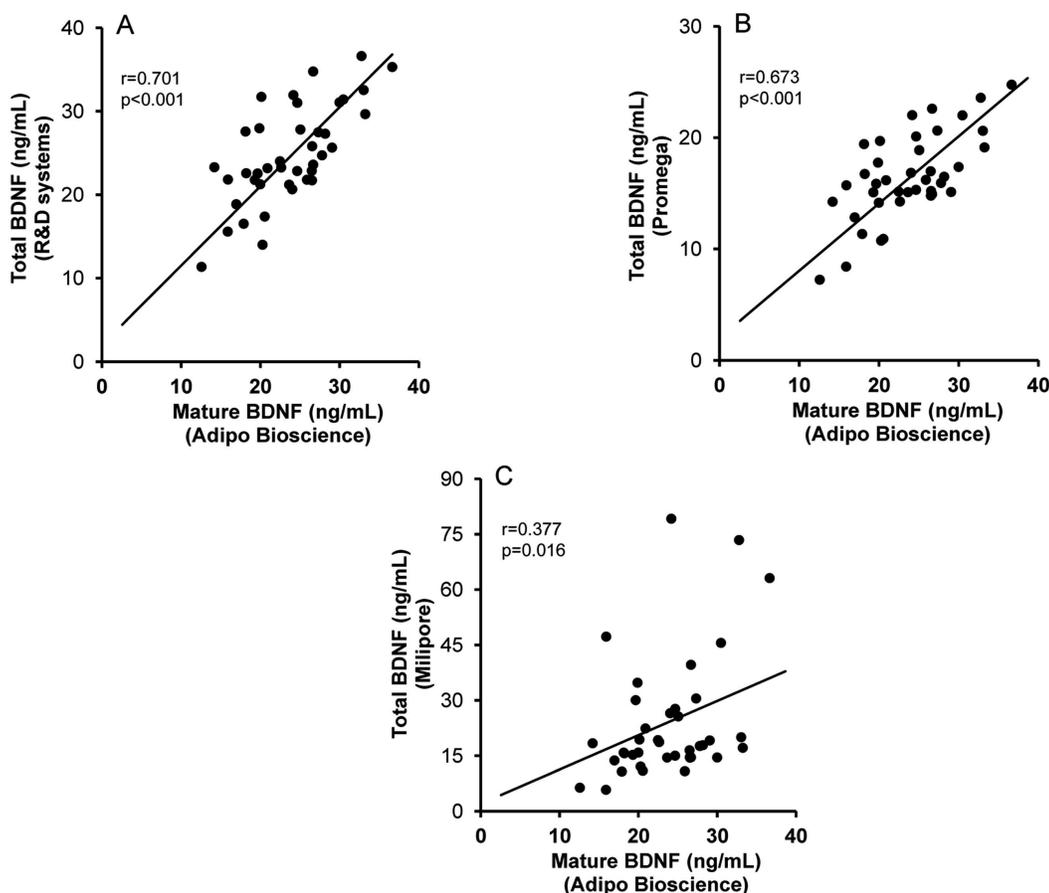


Fig. (2). Correlations between mature BDNF (Adipo Bioscience) and total BDNF (R&D System, Promega, Millipore) in human serum. (A) There was a significant correlation ($r=0.701$, $p<0.001$) between mature BDNF serum levels (Adipo Bioscience), and total BDNF serum levels (R&D Systems). (B) There was a significant correlation ($r=0.673$, $p<0.001$) between mature BDNF serum levels (Adipo Bioscience), and total BDNF serum levels (Promega). (C) There was also a significant correlation ($r=0.377$, $p=0.016$) between mature BDNF serum levels (Adipo Bioscience) and total BDNF serum levels (Millipore).

nant mature BDNF, and shows approximately 10% cross reaction against recombinant human proBDNF. The BDNF Emax[®] ImmunoAssay System (Promega Corporation) is based on an antibody to the carboxy terminal of mature BDNF [22], and also recognizes proBDNF. Each of these three kits recognizes proBDNF as well as mature BDNF, making them unsuitable for quantification of mature BDNF. In this study, we found positive correlations between the serum levels of mature BDNF (Adipo Bioscience) and the serum levels of total BDNF (R&D System and Promega) (Fig. 2A and 2B). It would therefore seem that serum levels of total BDNF using the R&D System and Promega ELISA kits correlate with serum levels of mature BDNF (Adipo Bioscience), although these two kits recognize both proBDNF and mature BDNF.

Alterations in the levels of total BDNF, including proBDNF, have been reported in the body fluids (e.g., blood) of patients with major depression [13, 18-21], schizophrenia [23], anorexia nervosa [14, 15, 24-26], bipolar disorders [27], and cardiovascular disease [28-30]. Furthermore, the presence of proBDNF and mature BDNF in human saliva has been reported by Western blotting analysis, but not ELISA method [31]. Given the opposing physiological roles of proBDNF and mature BDNF in the brain and peripheral organs, it would be of great interest to determine the exact

concentrations of precursor and mature BDNF in the body fluids (e.g., blood, CSF, saliva) of patients with these diseases, and healthy control subjects [9].

In this study, we found significant levels of proBDNF present in the serum of human subjects, although levels of proBDNF were lower than those of mature BDNF, with the exception of two subjects. ProBDNF is converted to mature BDNF by extracellular proteases (Fig. 1) [9-11]. Again, given the opposing biological effects of proBDNF and mature BDNF, it would be informative to study the precise mechanisms controlling the cleavage of proBDNF to mature BDNF [9]. To address these issues, it will first be necessary to develop highly sensitive ELISA systems that can differentiate between proBDNF and mature BDNF [9].

The Val66Met gene variant (196G/A: rs2665) of the human BDNF gene is thought to affect intracellular trafficking and mature BDNF secretion, as well as being associated with hippocampal volume and episodic memory in humans (Fig. 1) [8, 9, 32]. The frequency of this genotype is highest in Asian populations, including the Japanese [33]. It is predicted that both proBDNF-66Met and proBDNF-66Val could well exist in body fluids of subjects with the BDNF 196G/A genotype [9]. This raises the possibility that conversion of the two proBDNFs variants to mature BDNFs may differ between subjects who carry the BDNF 196G/A geno-

type, suggesting that in subjects with the BDNF 196G/A genotype, levels of proBDNF-66Met and proBDNF-66Val may vary [9,34]. If a highly sensitive ELISA system could be developed to distinguish between proBDNF-66Met and proBDNF-66Val, it would then be possible to quantify levels of the wild type and variant forms of proBDNF as well as the mature BDNF levels in body fluids of healthy subjects and patients with neuropsychiatric or other diseases [9,34].

CONCLUSION

This study shows that serum levels of proBDNF and mature BDNF in human subjects can be measured individually, using the human proBDNF and BDNF ELISA kits, although the sensitivity of the current proBDNF ELISA kit is currently unsatisfactory. Since it would be highly informative to measure serum levels of proBDNF and mature BDNF in patients with neuropsychiatric and other diseases, the development of a highly sensitive proBDNF ELISA system is a priority.

ACKNOWLEDGEMENT

The authors would like to thank Ms. Junko Gotoh and Mr. Kazushi Tsuru for recruiting human subjects.

CONFLICT OF INTEREST

None declared.

ABBREVIATIONS

BDNF	=	Brain-derived neurotrophic factor
CSF	=	Cerebral spinal fluid
ELISA	=	Enzyme-Linked ImmunoSorbent Assay
proBDNF	=	Precursor of brain-derived neurotrophic factor
SD	=	Standard deviation

ACKNOWLEDGEMENT OF FUNDING

A grant (to K.H.) from Comprehensive Research on Disability, Health and Welfare, Health and Labour Sciences Research Grants, Japan.

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Received: March 17, 2012

Revised: March 30, 2012

Accepted: March 30, 2012

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