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Brain-Derived Neurotrophic Factor: Concepts and Concerns in Clinical Chemistry: a Short Review

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Abstract: Brain-derived neurotrophic factor (BDNF) is a bioprotein, a member of the neurotrophic family of growth factors. It is associated with the canonical nerve growth factor. The protein has many roles in clinical disorders, including neurological, psychiatric, and other medical disorders. There are many concerns in the laboratory cycle for analyzing BDNF in clinical chemistry. Conclusions: In this review, the authors summarize insight to highlight the important details of the clinical chemistry laboratory diagnosis of BDNF.

Keywords: brain-derived neurotrophic factor; clinical chemistry; analysis.

1. Introduction

Brain-derived neurotrophic factor (BDNF) is a biomolecular in the human body, classified as a neurotrophin [1–4]. This bioprotein is in the neurotrophin family of growth factors related to the canonical nerve growth factor. The main biological process of BDNF is on neurons of the central nervous system (CNS) and the peripheral nervous system. BDNF is an important regulator in key brain circuits, and the protein was associated with cognitive function and emotion regulation. The BDNF abnormalities are observed in many psychological and neurological disorders [4].

BDNF is one of the most widely studied neurotrophins, and many ongoing types of research on this protein in healthy and diseased neuropathic subjects are presently performed [5]. The protein has many roles in clinical disorders, including neurological, psychiatric, and other medical disorders. Association between clinical disorders and BDNF are accepted. The abnormality of the biological process of BDNF is observable in several clinical disorders, especially neurological disorders, including depression, Huntington's disease, schizophrenia, Alzheimer's disease, and epilepsy. Similar to neuropsychiatric disease, there is a change of BDNF in the pathology of some tumors. The change of BDNF is reported in brain cancers. Similar to neuropsychiatric disease and malignancy, the abnormal BDNF level is observable in many infectious diseases. A significant change of BDNF is seen in a nervous system infection. There are many reports on the usefulness of BDNF determination in several medical disorders.

In this review, the authors summarize insight to highlight the important details of clinical chemistry laboratory diagnosis of BDNF.

2. General Concept for Clinical Chemistry Laboratory Testing for BDNF

BDNF is identified in blood. Standard venipuncture is basic specimen collection. A venous blood sample should be drawn and put into a collective blood tube and wait for coagulation for half an hour at room temperature. CSF is another clinical specimen for BDNF determination. CSF specimen collection is by a lumbar puncture procedure, which experienced licensed physicians must perform. Since lumbar puncture is considered more invasive than blood sample collection, CSF specimen is less commonly collected for BDNF determination in medical practice. Clinically, CSF specimen is indicated in the case of the patient with a neurological disorder, and BDNF determination is usually aimed at monitoring of therapeutic management of the patient.

In addition to blood and CSF, irine is another clinical specimen. Nevertheless, the Urinary BDNF is elevated in cases with benign prostatic hyperplasia [6]. The urinary BDNF test is also useful for monitoring enuresis [7] and neurogenic detrusor overactivity [8]. Regarding effusion specimen, measurement of BDNF is usually useful in case of malignant effusion diagnosis. The effusion BDNF analysis can help differentially diagnose benign and malignant effusion [9, 10]. According to the report by Duysinx *et al*., BDNF level was significantly higher in malignant effusions, and the best threshold cutting point BDNF level was 44 pg/ml [11]. Finally, saliva specimen is also usable. The detection limit of salivary BDNF determination is 62.5 pg/ml [11].

In clinical chemistry, the indication and application of BDNF test include these purposes: a) screening, b) diagnosis and c) following-up of therapy [1, 2, 12, 13]. Regarding screening, BDNF determination is not currently recommended as a basic laboratory screening test.

Nevertheless, there are some new BDNF related laboratory tests. In classical practice, the determination of BDNF is an analysis of the mature form of the protein. However, there is also an immature form of the protein. The immature form is named pro-brain-derived neurotrophic factor (pro-BDNF). Regarding the biosynthesis of BDNF, it is first synthesized as a precursor, namely pro-BDNF, before being further processed into mature. The pro- BDNF is also secreted and acts as a ligand for a receptor complex containing p75NTR and sortilin [14]. This receptor activation results in growth cone collapse, synaptic activity reduction, and developmental apoptosis of motor neurons [14]. The clinical usefulness of pro-brain BDNF is mentioned in several medical disorders. Similar to BDNF, pro-BDNF is reported for clinical usefulness in many neurological disorders such as epilepsy and Alzheimer's disease. Riffault *et al*. found that pro-BDNF- mediated p75NTR activation stimulated depolarizing action of GABA and increased Susceptibility to epilepsy [15]. Michalski and Fahnestock reported on decreased pro-BDNF in the parietal cortex in Alzheimer's disease [16]. At present, there are available laboratory assays for pro- BDNF, such as ELISA assays (detection limit 0.156-10ng/ml) (the cost of 48-strip wells ELISA kit is estimated 300 USD). The clinical specimen for pro-BDNF is similar to that of brain-derived neurotrophic factors. Blood or body fluid might be used. The specimen collection, storage, and preparation for pro-BDNF are similar to BDNF.

Molecular diagnosis Adding to a simple determination of BDNF, new molecular diagnoses are available for analyzing BDNF related parameters. Since BDNF is a bioprotein, there is a specific genetic process for protein coding. The abnormality of genetic protein-coding might be possible, and molecular diagnosis is useful for diagnosis those conditions. The

analysis of genetic polymorphism is an important BDNF related parameter test. The genetic polymorphism of BDNF is detectable by molecular diagnostic techniques [17]. An important new test is BDNF Val66Met polymorphism (rs6265 SNP). BDNF Val66Met polymorphism is a common genetic polymorphism of BDNF. There are many reports on the clinical association of the BDNF Val66Met polymorphism assay [18, 19]. There are some available assays, such as the TaqMan® SNP, for genotyping.

Point of care testing Point of care testing (POCT) is the contemporary concept of shortening the turnaround time for testing. The POCT analyzers for BDNF are available. Bockaj *et al*. recently developed a new POCT tool using the electrochemical principle [20]. The developed tool is ndoChip for the detection of plasma BDNF [20]. Bockaj *et al*. found that the new POCT tool gave a result that with a good correlation to standard ELISA [20]. Finally, nanodiagnosis is the application of nanotechnology in laboratory medicine. There are many ongoing types of research on new BDNF POCT for a rapid diagnosis for neurodegenerative disease.

3. Quality Control in Clinical Chemistry Analysis for BDNF

In laboratory medicine, quality management is necessary for any laboratory testing. Regarding BDNF test, quality management is necessary for all phases of the laboratory cycle. This means full quality management coverage on clinical chemistry analysis in all phases; pre-preanalytical, preanalytical, analytical, post-analytical, and post-post-analytical phases.

The pre-pre analytical phase is the first step in the laboratory cycle in laboratory medicine. If there is any problem in this step, no accuracy of the final laboratory result will be derived. It is commonly related to diagnostic test ordering and patient preparation. Regarding clinical chemistry test ordering for BDNF, the indication has to be fulfilled. There is no specific recommendation for patient preparation, but a good standard of patient preparation is required. No fasting or taking a specific substance is required. Additionally, fasting and exercise might stimulate the production of BDF and should be avoided. There are many effects of physiological background. In females, sex hormone has a significant effect [21]. The BDNF level in the luteal phase is significantly higher than in another phase [21]. The effects of exercise and diet in a woman are well demonstrated [22]. Finally, BDNF is an activity-dependent protein, and there are many different interference factors. An important key modulator is physical activity. Either electrical stimulation or physical activity can increase the BDNF expression in the skeletal muscles. Also, physical activity can cause an increased level of BDNF in the blood [23–24]. A high-intensity physical activity can cause increased serum BDNF level [23, 24]; hence, activity limitation is recommended in patient preparation [23, 24].

The pre-analytical phase is an important step in the laboratory cycle. This step in the laboratory cycle usually deals with specimen collection, specimen delivery and transportation, and specimen presentation to the clinical chemistry diagnostic unit within the medical laboratory. Since the pitfall is common in this phase, good quality management in the laboratory process is needed. The details of specimen collection are earlier mentioned and have to be followed. In brief, venous blood is required. Either EDTA blood or serum can be used. For specimen preparation, the sample received at the medical laboratory should be kept on ice, then centrifuged at 3,000 g for 10 minutes at 4°C within half an hour of collection [25–26]. Then plasms can be used for laboratory analysis or refrigerated at −80°C for analysis later [25,

26]. In the case of serum, after collection, allow samples to clot for 2 hours at room temperature before centrifugation for 15 minutes at 1000 × g. The collected serum might be kept overnight at 4°C [23 - 24,26]. Pre-analysis storage conditions can influence the determination of brain-derived neurotrophic factor levels in peripheral blood [29]. There are inter-individual variations in the plasma BDNF levels [29]. Also, there are inter-anticoagulant compound variations [2]. The plasma BDNF levels increase over time, whereas the serum brain-derived neurotrophic level does not [28]. According to the study by Tsuchimine *et al*., the plasma stored in heparin tubes at 4°C and in EDTA tubes at 25°C resulted in an increased BDNF level [27]. Effving *et al*. reported that the pre-analytical conditions were critical for plasma samples but less important for serum or whole blood samples [28].

There are many available diagnostic assays for analyzing the BDNF in the clinical laboratory. A widely used kind of essay is an enzyme-linked immunosorbent assay (ELISA) test, immunoaffinity, and Western blot test [26]. The efficacy and diagnostic properties of different assays are different. ELISA is the most widely used kind of assay at present. Its detection limit is about 0.3125 - 20 ng/ml. Recently, Effving *et al*. studied the intra- and inter-assay variation and the accuracy and yield of the BDNFELISA kits and found that inter-assay variation was low with a coefficient of variation (CV%) of 11 or less. Intra-assay CV% was 8 or less [26]. In another report by Trajkovska *et al*., the accuracy of the studied ELISA assay was 91.6 %, whereas inter-assay and intra-assay CVs were modest, 8.4 % and 17.5 %, respectively [29]. Regarding the chip-based immunoaffinity CE system, the intra- and inter-assay CVs are equal to 3.85% and 4.19%, respectively [30]. A proper laboratory test selection is very important. Since there are many assays for the analysis, it is necessary to have good clinical decision-making in selecting. There are many important points for consideration for a proper selection of the test assays for BDNF determination. Those points for consideration include a) efficacy of the test, b) cost (the cost of the 24-strip wells ELISA kit is about 265 USD) and cost-effectiveness of the test, and c) turnaround time of the test.

The post-analytical phase in laboratory medicine is also important. It usually deals with result validation and laboratory result reporting. The validation is also important in brain-derived neurotrophic determination [22]. Standard principles in laboratory medicine can be followed. The post-post analytical phase of the laboratory cycle usually deals with the interpretation of the laboratory results. A good interpretation must be based on the patient's history and condition. The interpretation of the reported result must also be based on the reference value of the local population in each setting. According to a recent study from the UK, the reference range of serum brain-derived neurotrophic value was 15.83 – 79.77 ng/ml [22]. There is an increase in serum level of 0.33% for every year of age [22].

4. Conclusions

In this review, the authors summarize insight to highlight the important details of clinical chemistry laboratory diagnosis of BDNF. Pre-analytical, analytical, and post-analytical quality controls are necessary for analyzing BDNF. The BDNF test has an advantage in the diagnosis and follow-up of the patient. Determining abnormal BDNF mRNA and protein expression can help probe the regional increases or decreases that are pathognomonic findings in each medical disorder. The peripheral BDNF test is a clinical chemistry diagnostic test that helps monitor disease progression and response to therapy because the changed expression level in disorder is generally related to disease progression in the course of the disease. In

clinical chemistry, there are many important considerations regarding brain-derived neurotrophic factors. Quality control in all phases, pre-analytical, analytical, and post-analytical phases, for BDNF is necessary. Continuous development in laboratory medicine leads to the new advancement in clinical chemistry diagnosis for BDNF. The new diagnostic technologies such as applied nanodiagnostics for the BDNF diagnostic test will be a future direction. The practitioner should recognize and update the data on clinical chemistry diagnostic issues regarding BDNF.

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Conflicts of Interest

The authors declare no conflict of interest.

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