

Novel Biomarkers and the Diagnosis of Prion Diseases

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Abstract

In fatal neurodegenerative diseases, prion diseases have high risk of transmission ability and no cure and effective treatment. This abnormal folded protein disease in brain pose a serious threat to public health and the development of early diagnostic markers and new therapeutic approaches is in pronounced plea. Prion disease show infectious and incurable irrepressible nature and long period of silent incubations and nature of prion diseases, development of early diagnostic markers is in great demand to prevent a potential spread of the disease and for early diagnosis of the disease given the long incubation periods of disease. Moreover discovery of novel biomarkers can lead to development of new therapeutic targets and better understanding of the underlying pathogenesis of the Prion diseases.

Keywords: Biomarkers; Novel; PrP; Proteome; Interactome; CSF; Exosome; RT-QuIC; TAU, Blood

Prion Diseases

Prion diseases or transmissible spongiform encephalopathies (TSEs) are included in neurodegenerative diseases, which are rare and fatal for both humans and animals [1]. Prions or “Proteinaceous infectious particle” are known to be the result of conversion of the cellular normal prion protein form (PrP^C) into its diseased aggregation-prone form called as prion (PrP^{Sc}) [2-5]. The only exact diagnosis is possible only after *post mortem*. The both diseased PrP^{Sc} and normal cellular PrP^C form consists of same amino acid sequence, but secondary, tertiary, and quaternary structures are differ [6-8]. Furthermore, different fragments of PrP^C as well as PrP^{Sc} can be found in various lengths [9-13] and the targeted detection could demonstrate difficulty in view of the complex biochemical nature. The normal function of these prion protein forms (PrP^C/PrP^{Sc}) are still not clear but there are some reports on the predictive role of (PrP) in the brain functions i.e. pro- and anti- inflammatory role [14-18], protection of mice in lipopolysaccharide induced infection in mice infection by regulating the process of inflammatory response [18].

Diagnosis of Prion Diseases

The usual way of prion diagnosis is by the clinical parameters and further confirmation with histopathological examination of brain tissue after post-mortem brain tissue. The reliable diagnosis of prion diseases relies on the ability to detect the pathological isoform of the host protein PrP^C (PrP^{Sc}) [19,20]. Immunological detection of PrP^{Sc} is sensitive and specific enough for postmortem testing of brain tissues; however, this is not the case for readily accessible body fluids or for the detection of recently identified novel prions with unique biochemical properties due to low concentration of PrP^{Sc} [21-23]. Several diagnostic kits are commercially available for the immunochemical detection of PrP^{Sc} in the post-mortem brain tissues.

Recent report demonstrated that the 83% of cases sCJD striatum and/or cortical regions of the brain showed high signal intensity in the fluid attenuated inversion recovery (FLAIR) and in the diffusion-weighted imaging (DWI) sequences [24]. Furthermore, for the specific and rapid diagnosis of sCJD real-time quaking-Induced conversion analysis (RT-QuIC) also a novel method for the ante-mortem diagnosis of human prion diseases [25-28].

To keep the human food chain clear, some quick screening tests are

also available for the infected cattle. But still in live animals or humans still there is a lack of detection of prion diseases. Novel approaches are under progress in body fluids, intended to increase sensitivity and specificity of PrP^{Sc} detection. In this report, we review the classical biomarkers as well as the possibility of novel biomarker discovery in prion diseases.

CSF Biomarkers

CSF proteome alterations reflect pathological changes in the brain, and could possibly be able to provide an early diagnostic tool in prion diseases. There are many protein based biomarkers in cerebrospinal fluid (CSF) that are used for diagnosis of human prion diseases. Frequently used biomarker proteins are 14-3-3, tau, phospho-tau/tau ratio, S100, and the neuron-specific enolase (Figure 1) [29-34]. The combination of elevated T-tau levels and increased T-tau/P-tau ratios in CJD patients has a very high sensitivity and specificity in comparison to differential diagnoses CJD [35,36]. A very recent study has described tau as a biomarker of neuronal damage with a significant negative correlation between CSF tau levels and the cognitive performance of the patients [37]. In addition, a significant positive correlation between tau levels and the clinical disease severity has been reported. This disease progression rate suggesting the notion that tau levels reflects the extent of neuronal damage, at least before the end stage of the disease [37,38]. Previously, an inverse correlation of CSF tau level to the disease duration has been reported but not with the disease progression stage [39]. Induced expressional levels of other neurodegenerative-related proteins such as beta-amyloid [27,40-42] and alpha-synuclein [37,43] have also been reported in the CSF of patients with sporadic CJD when compared to control samples. These CSF biomarkers have proven to be an extremely valuable in the confirmatory diagnosis of CJD cases. However, all the known biomarkers are sensitive only when the disease

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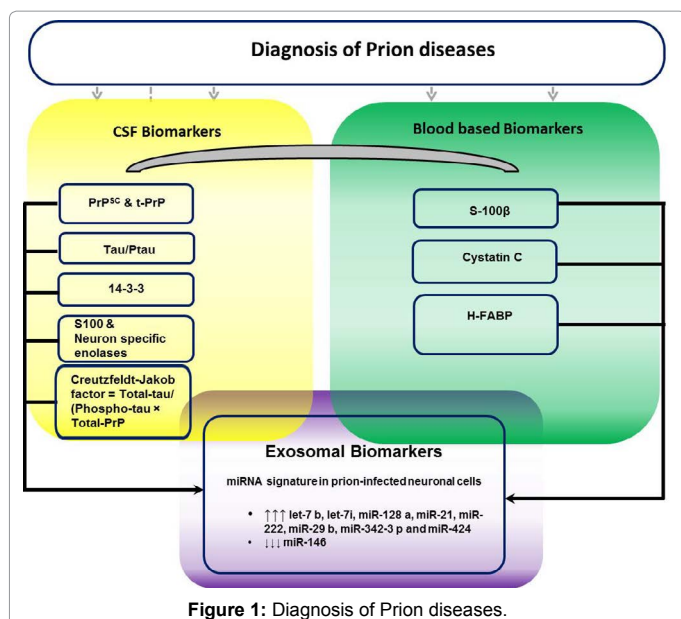


Figure 1: Diagnosis of Prion diseases.

is already at an advanced or terminal stage and there is no data available at preclinical stages of the prion disease.

In recent years, total PrP (t-PrP) level in CSF have been described as a new biomarker [44]. The specificity of (t-PrP) level could help the clinicians for differential diagnosis of atypical cases and profile ambiguity in between the CJD and Alzheimer diseases. Furthermore, the ratio of CSF t-PrP with tau proteins (Creutzfeldt-Jakob factor=Total-tau/ (Phospho-tau × Total-PrP) lead to differentiate CJD and atypical AD with 100% sensitivity and 95.7% specificity [44]. Previously we also reported differential regulatory response of PrP in CJD, Alzheimer disease, Parkinson disease, and dementia with Lewy Bodies disease and found slightly but significant decrease in comparison to age matched controls [45]. Another study also had shown decreased PrP levels in CSF of diseased patients as compared to control [46]. In CJD subtype (MM1 and VV2) cases showed reduced level of t-PrP in both subtypes but significantly more in CJD-MM1 subtype and decreased expressional regulation of t-PrP in CSF correlate brain at mRNA level [45-47]. Recent advancement and amalgamation of new techniques lead to improve the detection system by using CSF samples from Prion disease patients, showed sensitivity and specificity for seeding activity by using RT-QuIC in comparison to age matched healthy and non-demented controls [27,48-50]. Taken together, these studies indicate that PrP levels in CSF are on average lower level in individuals with prion disease. However, one study in sheep model reported that there is no significant difference in between CSF t-PrP expression as compared to controls [51]. In conclusion, t-PrP expression could be a specific marker in symptomatic prion disease patients than in controls [44-47].

Exosomal Biomarkers

Exosomes are nanoparticles, secreted into the extracellular environment during the transportation of vesicular factors [52] and are reported as carriers for PrP^C and PrP^{Sc} [53] For the diagnosis of prion disease, recently a group showed specific exosomal miRNA signature in prion-infected neuronal cells that can be utilized for specific diagnostic pattern. This signature consists of significant increases in let-7 b, let-7i, miR-128 a, miR-21, miR-222, miR-29 b, miR-342-3 p and miR-424 with decreased miR-146 [54]. Previous studies have also reported miRNA changes associated with exosomes detected in the brain of terminally infected mouse and primate

models of prion disease, and sporadic CJD samples [55,56] suggesting exosomal markers as a novel biomarkers for prion diseases and could possibly be therapeutic approaches in later stages.

Blood Based Biomarkers

The only possible confirmed tool for prion diagnosis was by postmortem brain tissue. But the need for early detection is still under consideration. Therefore, body fluids and blood could be used as an *ante mortem* test. The level of PrP^{Sc} is below the detection threshold, so PrP^{Sc} enrichment needs to be an additional step [57-59]. The major issue in blood detection system is high PrP^C background and extremely small quantity of prions, consequently, specificity and sensitivity is an essential mark up for the establishment of a blood test.

A number of studies have identified differential regulation of a handful of proteins in the blood of patients with CJD. One study has reported elevated levels of the S-100β protein and another, an increase in cystatin C levels [42]. Another study found elevated levels of heart fatty acid binding protein (H-FABP) in the serum of patients with CJD [60]. Fatty acid binding proteins are located within the cell and are responsible for the shuttling of fatty acids in the cytosol and are released from the cell in response to cell damage [60]. However, the elevated levels of heart fatty acid binding protein (H-FABP) have also been observed in Alzheimer disease, acute myocardial infarctions, and in stroke patients, implying that this is not a specific prion disease biomarker. Furthermore, murine scrapie showed erythroid-related genes named as (KEL, GYPA) differentially regulated in the spleens of infected [61]. However, these genes were found to be expressed at highly variable levels between individuals, thus prevent their usefulness as accurate markers for diagnosis.

Specificity of individual markers require careful analysis, So use of combination of multiple biomarkers rather than one single biomarker would be prudent in order to reach higher sensitivity and specificity for diagnosis of Prion diseases.

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