REVIEW ARTICLE

PROTEOMIC PROFILING OF BLOOD BASED BIOMARKERS IN THE EARLY DETECTION OF ALZHEIMERS DISEASE

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ABSTRACT

Dementia is the massive public health issue affecting a large number of population and according to an estimate approximately 115.4 million people will be living with this neurodegenerative disorder by 2050. Such increasing numbers are alarming at individual level and also not sustainable for economy point of view. Dementia costs US$604 billion of global economy and it is estimated that by 2030, as the rate of prevalence is increased significantly the cost of this disorder is also increase by 85%. Alzheimer's disease (AD) is the mainstream of dementia, encompassing about 50-70% aged dementia population. AD is remarkable for the presence of large number of cognitive deficiencies which results in the notable impairment for the social and occupational function. Alzheimer's disease has now become one of the problematic and expensive health issues for the society. Now AD is considered as a chronic disease which progress slowly with consequences of various complex pathologies. AD is linked directly to the amyloid beta peptides. The monomeric forms of Aβ, aggregate to form fibril and variety of oligomers which later become neurotoxic. Alzheimer's disease is considered as the incurable, irreversible and progressive type of neurodegenerative disease, in which the symptoms of dementia become worse with time. It is essential to discover and validate the biomarkers for the diagnosis of AD and enhance the establishment of the new therapeutic strategies. Although the foundation of AD biomarkers from CSF and neuroimaging possess high degree of accuracy but there exist the barriers for the clinical prosecution. In past decades, much attention has been paid on blood-based biomarkers for AD. Thus, various proteomic tools can be utilized for the identification of biomarker from blood which can serve not only in early diagnosis but also for the development of new therapeutics targeting AD.

Keywords: Amyloid beta, Alzheimer's disease, Blood based biomarkers, Proteomics
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INTRODUCTION

Alzheimer's disease (AD) is the most frequently observed neurodegenerative disease which commonly causes dementia. Currently, the number of AD cases assessed to be greater than 5.4 million and it is anticipated that the prevalence will increase further in coming future. The characteristic features of AD are the loss of memory, cognitive decline and changes in behavior which notably hamper the occupational and social activities [1]. It is hypothesized that AD related neuro-degeneration emerge from the formation of amyloid beta (Aâ), which is an incomplete or miss folded structure of protein and tends to form the aggregates in the course of disease progression. Aβ play role in both pathological and physiological conditions. Studies have reported that the deprivation of this peptide causes synaptic dysfunction. Aβ is also involved in

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angiogenesis, tumor suppression and neurogenesis. On the other hand elevated levels of this peptide lead to the neuronal degeneration upon forming aggregates. Masters et al (1985) was the first who identified Aâ as aggregated form of protein which gets deposited in the plaque cores present in brain of AD patient [2]. The monomeric structure of this protein is not harmful but monomers of Aβ have ability to self-assemble to form amyloid-â oligomers, intermediates of amyloid-â aggregates and finally febrile aggregates which are accumulated in brain [3,4]. Blood-based tests are extensively utilized to aid in the diagnosis of metabolic, immunological, or cardiovascular diseases. The discovery of blood biomarkers for Alzheimer's disease is a viable answer for both initial illness risk and disease progression [5]. Biomarkers for the diagnosis of AD are of great value, since cognitive manifestations are quite diffuse type and tend to converge with other diseases. For the potent AD related biomarker, a guideline has been decided with agreement of all researchers working worldwide, which states that biomarker should reflect brain aging, explain the pathophysiological conditions, reflect pharmacological changes, highly specific and sensitive, reproducible with time change, cut-off values should be clear with minimum of two-fold changes and should be inexpensive [1]. Various biomarkers (e.g. genetic, biochemical and/or neuroimaging) have been reported, each highlighting the changes taking place in the brain in the course of disease spread. Despite, there exists the inadequate evidence of their diagnostic values. Additionally, other assays are difficult to employ and reagents are costly or hazardous (e.g. use of radioactive materials and extraction of CSF) [6]. In recent years, cerebrospinal fluid has imparted the promising origin for the validation of AD biomarkers. Deficiency of beta amyloid levels in CSF reported previously to discriminate between the normal individual and AD patient. As compared to the CSF, analysis of blood is of advantageous because sampling is relatively simple and less invasive [7]. Further 18 biomarkers by targeting a group of 120 proteins involved in various signaling processes have also been identified [8]. Likewise, O' Bryant and colleagues identified 30 biomarkers to investigate AD [9]. On the other hand, Soares and colleagues published a long list of AD biomarkers [10]. Therefore, there is immediate need for the development of approaches which are appropriate and convenient, like the evaluation of blood parameters which is relatively simple and can be repeated upon need with no contradictions. Algorithms can make the use of such data which merge the specific as well as non-specific changes so that the patients can be classify at different AD stages. In the proposed study, blood parameters taken into account are varied, these include the Apolipoproteins, feutin, fibrinogen and afamin. Newly developed proteomic techniques to investigate and quantify these molecules would be pivotal for early diagnosis of AD as well as to describe the new therapeutic targets [6].

The hallmark of AD is the deposition of amyloid β peptides. The aggregation of Aâ is the major cause of AD pathogenesis and it starts to occur about decade ago before the detection of clinical signs and symptoms [11]. The worldwide epidemic of AD is constantly increasing with the age of population. Till 2006, AD global prevalence was 26.6 million and it is believed that by 2050 its occurrence will be quadruple. Moreover, with the passage of time, cost of treatment is increasing globally with no noticeable amelioration in preventive and treatment strategies for AD. From the aforementioned fact, situation in the developing countries like Pakistan can be easily envisage. Likewise, it is not difficult to speculate that in near future, our system of socioeconomic and healthcare will be unable to convey the financial load of AD. Therefore, there is an urgent need for the development of reasonable strategies so that AD can be prevented and treated before the economy of nation becomes devastated by the financial load of this rapidly increasing epidemic [12]. For more than last three decades the enormous research attempts have provided knowledge about AD at molecular level. As mentioned above, AD is intricate progressive disease with highly interacting aggregates of amyloids with the occurrence of oxidative stress and inflammation due to which synaptic integrity, neural connectivity

is lost and neurodegeneration occurs. Research innovations from genetic, neurochemical and pathophysiological studies also providing the rising brace to amyloid cascade hypothesis [12, 13].

1. Processing of Amyloid Precursor Protein(APP)

Amyloid precursor protein (APP) is a glycoprotein, comprised of 695 to 770aa, present in the integral membrane of the neurons. Its function is to facilitate the neurogenesis along with the regeneration of the neurons, either in its intact form or by means of its breakdown product. The proteolysis of amyloid precursor protein is proceeding by the two distinct pathways. In non-amyloidogenic pathway α-secretase and γ-secretase act sequentially on APP, thereby releasing the soluble and degradable peptides. In amyloidogenic proteolytic pathway, β-secretase (or BACE 1) first act on the APP and produces sAPPβ and CTFγ. CTFγ later acted upon by the γ-secretase and thus form AICD (APP intracellular domain) and Aβ peptide. Amyloid β (a fragment of 40 or 42) either degraded or it is transported to the surface of the cell and released into extracellular space [6, 14]. From extracellular space these amyloids peptides are releases into CSF or degraded. If degradation or clearance fails, then Aβ possess the ability to form the ordered β-sheet structures which further aggregates to form oligomers as well as insoluble fibrils. Oligomer forms are the neurotoxins. Aggregates of Aβ42 specifically act on the synapses and impaired the neurotransmission and plasticity, and as a consequence the neurodegeneration spreads [6, 14].

2. Significance of Biomarkers

A biomarker or biological marker is a molecule, structure or process that can be accurately measured and examined, indicating the presence of pathogenic processes, normal biological processes and respond pharmacologically to any therapeutic involvement. Ideal AD associated biomarkers should match the criteria enlisted: 1) able to diagnose or identified AD with high degree of specificity and sensitivity, 2) it must be able to identify the initial disease stage, moreover monitors the disease progression, and 3) possess the ability to observe the therapeutic efficacy of given drugs [15]. Therefore, the development of the candidate biomarkers at early stage is crucial. It looks like that the combined examination of some of the target biomarkers listed below will help in evaluating the particular signature in AD patients. It is expected that the diagnostic markers will help in improving the patients follow up significantly.

3. Biomarkers Associated with Alzheimer's Disease

3.1 Tau

Tau-containing neurofibrillary tangles are the second hallmark of Alzheimer's disease. Increased tau phosphorylation, particularly on Ser/Thr-Pro motifs, is a recurrent characteristic event that impairs tau microtubule integrity and accompanies tangle formation and neurodegeneration. A 42-residue of Aβ (A42), tau, and phosphorylated tau at residue 181 (pTau181) measurements in human cerebrospinal fluids (CSF) has been shown as the most effective predictor of AD progression [5]. Both plasma Aâ phosphorylated tau have been linked to AD brain pathology, but their use in clinical settings requires additional validation. Other plasma proteins have been discovered to be associated with Alzheimer's disease and brain amyloid plaques [16].

3.2 Apolipoprotein A-1

ApoA 1 which includes ApoJ and ApoE, has ability to bind with Aβ. Expression of this protein has been reported in Alzheimer's patients. No significant difference in the expression of apoAI in CSF or brain of AD patients and non-AD patients has been found. However, in two independent studies low expression of ApoA 1 were reported in the plasma of Alzheimer's patients. Therefore, we decided to re-examine the function of ApoA 1 in plasma as a biological marker in AD patients and evaluates it association with the intensity of the disease [17]. Apo lipoproteins are involved in the lipid and cholesterol metabolism and current findings demonstrate that this group of protein may also take part in the neurodegeneration. The major constituents of HDL are ApoA1 and ApoA2 and their function is to transport cholesterol to liver [18]. Lewis et al (2010) reported that overexpression of this protein (ApoA1) in triple transgenic mouse prevented the occurrence of memory and learning deficits, in spite

of the amyloid deposition [19]. In contrast, Kawano et al (1995) reported the lower levels of ApoA1 and ApoA2 in plasma of Japanese patients suffering with late emergence of non-familial AD. It is demonstrated that ApoA1 attenuate the cytotoxicity induced by the Aβ [20]. Furthermore, it is reported that apoA-1 has ability to bind with the domain of APP protruding extra-cellularly and possess functional importance in controlling amyloid beta induced aggregation as well as toxicity [21].

4.3 Apolipoprotein A-4

Apolipoprotein A-4 is another type of apolipoprotein present in CSF and plasma and has role in metabolism of lipid. Studies have shown that the removal of this protein propagates the formation of amyloids and further ApoA-4 assist in the clearance of Aβ, which thus provides a mechanism distinct from that of apoE, albeit some of the characters are identical. In vivo clearance of Aβ is nexus process which occurs together with the effects of several factors, like different apolipoproteins thus such factors tend to maintain the metabolism of Aβ in brain [22]. Free radicals are produced by the redox metal ions, activation of microglia and process of inflammation, and these are involved in the pathological process of neurodegeneration. ApoA-4 is an antioxidant and was found to be increased in serum level of AD patients [23].

3.3 Fetuin-A

Fetuin-A also known as alpha-2 Heremans-Schmid glycoprotein was first identified in the fetus, therefore it is named as fetuin. It is mainly present in liver, brain, kidney, GIT and skin. Although, in adult liver is predominant in secreting this protein. This protein was known to be important in the inhibition of vascular calcification but biological diversity of this protein has been greatly extended. Fetuin-A known to possesses pro-inflammatory as well as anti-inflammatory functions [24]. Fetuin-A is abundantly present in plasma and its role to predict the presence of vascular disease is very well established [25]. Low level of fetuin-A concentration in serum corresponds to the presence of extreme cognitive deficiency in AD, thus emphasizing its neuroprotective and anti-inflammatory roles. Peripheral administration of fetuin-A has shown the attenuation of neuronal inflammation in rat having cerebral ischemia [24]. Smith et al (2011) first analyze the correlation between the concentration of fetuin-A in plasma and cognitive disablement in mild to moderate AD patients [25]. In comparison with healthy controls of identical age group, they found less amount of circulating fetuin-A in individuals with established AD.

3.4 Fibrinogen

Elevated fibrinogen levels lead to the changes in rheological characters of blood (e.g. changes in vascular reactivity and changes in the integrity of endothelial layer). Furthermore, deposition of fibrinogen either with or without its conversion into fibrin results in increase vascular permeability and increase in inflammation. After the damaged of blood brain barrier, fibrinogen accumulation occurs in CNS as a result inflammation increases by the activation of microglia and consequently outgrowth of neurite is suppressed. Therefore, fibrinogen elevated levels contribute in neuronal dysfunction and vascular pathology [27]. Van et al. (2005) also proposed that high levels of fibrinogen correspond to the high risk of vascular dementia and AD [28]. In AD, interaction occurs between the Aβ peptide and fibrinogen which results in oligomerization of fibrinogen itself. These oligomers of fibrinogen converted into the abnormal clots of fibrin which became resistant to cleavage by the fibrinolytic enzymes. Perseverance of fibrin clot causes decreased flow of blood, neuro inflammation and vascular insufficiencies, and thus all together contribute in pathogenesis of AD [27, 23].

3.5 Afamin

Alpha tocopherol (αTocH) belongs to the vitamin E family and it is required for the normal functioning of brain. It is accepted that preservation of αTocH level in brain is necessary for neurological function. Protein namely afamin work to transport the αTocH in CNS. Afamin belongs to the protein superfamily of albumin [29]. In AD brain inflammatory reactions results in oxidative stress and it is reported that

afamin protein associated with oxidative stress was down regulated in AD patient's plasma [30].

4. Proteomics and Alzheimer's Disease

Proteomic perspective is the noteworthy companion for the search of biomarkers related to neurodegeneration [31]. As proteins and peptides are the functional workhorses of the cell and thus their up and down regulations reflect about the biological state of the organism. A proteomic strategy permit the high throughput and differential identification of disease associated alterations occurring in body that cannot be detected by the strategies at transcript or genetic level and also assist in elucidating mechanisms of disease. Current innovations in proteomic approaches have help in providing the vast information of proteins and therefore, the crucial role of proteomics cannot be neglected [32]. Depleting the proteins present abundantly in sample permits the high resolution of proteins present in least amount (but significant) by means of 2D PAGE. Fluorescence staining of the gels governs the use of differential gel electrophoresis (i.e. 2D DIGE) that offers undoubtedly the notable advantages [33]. Mass spectroscopy allows identification as well as the quantification and characterization of target molecules simultaneously [34].

CONCLUSION

This disease is generally characterized by the systemic and progressive onset with continuous cognitive deterioration followed by duration of about 8.5 years from the progression till death. The intricate nature of AD and its prodromal period offers challenges for the diagnosis and demands for the evolution of treatment that can modify the disease for prevention. Therefore, it is very important to originate the biomarkers, particularly in the early stage so that the pathological conditions can be reflected and furthermore patients could be treated according to the severity of disease. However, immense research has endeavored the biomarkers for the purpose of diagnosis by comparative studies between the affected and unaffected people. Blood-based biomarkers provide the promising and less invasive ways for the early diagnosis, and additionally various omics technologies has helped to accomplish this.

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Author's Contribution

SUH: Concept and design, writing, edited and approved the manuscript

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