REVIEW ARTICLE

Application of Proteomics in Alzheimer's Disease: A Mini Review

Pariveena Sukumaran[1], Ainun Aida Bahardin[1], Luqmanul Hakim Abdul Razak[1], Mohd Harizal Senik[1,2]

[1] Department of Neurosciences, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
[2] Brain and Behaviour Cluster, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

ABSTRACT

Alzheimer's disease (AD) is classified as one of neurodegenerative disease caused by neuronal death. It is characterized as memory impairment, including the inability to produce new memories. Since AD has low treatment effectiveness, proteomics research opens possibilities for advancement. Proteomics is the study of proteomes produced by the disease-bearing host to identify and understand diseases. In this case, to investigate the use of protein as a reliable molecular entity and their involvement in AD. Therefore, this review focused on three main applications of proteomics; the potential use of proteomics as a diagnostic tool for AD, the use of proteomics to assess the treatment progression of AD and the advancement in AD research. The review discussed three research areas utilizing the proteomics approach: ageing, behavioural, and demographic research of AD populations. Proteomic approaches have also been shown to be effective to discover the biomarkers for infectious diseases, cancers, heart diseases, and neurological disorders. Although much work remained to be done, the proteomics approach is an interesting method to be carried out in detecting AD at an earlier stage and will be very useful for AD treatment and management in the future. *Malaysian Journal of Medicine and Health Sciences* (2023) 19(5):317-330. doi:10.47836/mjmhs19.5.38

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Corresponding Author:
Mohd Harizal Senik, PhD
Email: mharizalsenik@usm.my
Tel: +609-7676439

INTRODUCTION

Alzheimer's Disease (AD)

Neurodegenerative disease covers many diseases caused by neuronal death (1), which can be inherited or sporadic. This disease leads to structural and functional loss of neurons, leading to other myriads of symptoms (2). Alzheimer's disease (AD) is one example of a typical neurodegenerative disease that affects around 46 million individuals globally (3, 4), especially among the elderly (5). AD usually manifests clinically with initial amnesia characterized by the inability to form new memories, reflecting dysfunction of the medial temporal lobe (MTL) episodic memory system (6). An individual with AD is not only unable to produce new memories (6) but also loses the ability to conduct daily activities independently due to the decline in the executive function of the brain (7). In Malaysia, the prevalence of dementia is around 0.126% in 2020 and is estimated to increase up to 0.454% in 2050 (3). The low prevalence is associated with the fact that most Malaysian families did not seek medical follow-up or diagnoses for their affected family members, contributing to the potential under-reporting of AD and dementia in Malaysia (3).

The progression of AD is proportionate to the age of individuals (8). AD can manifest differently in different individuals. Mild signs of AD include difficulties in retaining memory, challenges the cognitive and intellectual abilities, reflects the difficulty in managing money and judgment, which collectively resulted in poor quality of life (9, 10). In a later stage, AD patients require full-time assistance as they experience more severe cognitive alterations such as restlessness, confusion, obstacles in the control of movement and normal daily body functions as well as difficulty with the command of language and thoughts (4) which also affected the quality of life of family members and their carer. The discovery of neurodegenerative mechanisms that leads to AD pathology was able to outline the causes, mechanism, and plausible treatment. Nevertheless, researchers still have yet to identify the cure for this ailment (4).

Research on the pathogenesis of AD is still ongoing, as the cause of the disease development is still ambiguous (11). Some of the more established causes and pathogenesis of AD include the beta-amyloid (Aβ) plaques and the neurofibrillary tangles of tau protein (NFT). This pathogenesis ultimately leads to a

progressive loss of synapses and neuronal death (12). However, AD is not exclusively caused by plagues and tangles formation. Studies had also uncovered other pathways which are associated with the development of the disease, including disturbances in glucose regulation and lipid transport, as well as the progression of neuroinflammation (13). There are various protein formations and genes associated with these causes of disease progression, leading to various research approaches to study AD pathogenesis. In the study of proteins, proteomics is one of the promising approaches currently available.

Proteomics

The word protein was invented first in 1838 and since then, it had evolved from the study of a molecule to the study of the proteome (14). Proteomics emerged from the genomic knowledge of the Human Genome Project in 1997. The proteome is a whole collection of proteins created or changed by an organism or device. This formation and modification depend on the various internal and external factors that are experienced by the cell or organism (15). Interest in proteomes stems from the synthesis of intracellular protein, function, and specific patterns of behaviour (14).

According to Grant & Blackstock (16), it has been proposed that using proteomics to study protein complexes and signalling pathways provides a better way to explain the interaction of proteins to create cellular machines (17). Proteomics is typically considered to be much more complicated than genomics because, unlike the uniform nature of an organism's genome, its proteome varies according to cell types and development across time (14).

Proteomics is crucial in providing relevant single data set characterizing a biological system (14, 18). Although transcription of data from DNA provides information on gene expression at the mRNA level, it is still not sufficient to understand the finalization of these levels (19). Proteomic studies detect and identify diseases from the earlier stages, and provide prognosis, and assessments of the disease development. Proteomics also can be referred to as large-scale laboratory protein analysis and is used for protein purification and mass spectrometry (14).

Proteomics also plays an important role in novel drug development (14) through peptides and proteins identification, and posttranslational amendments, measurement of the amount of the protein, characterizations of proteins structures, and protein interactions (20). The research field of clinical proteomics aims to discover biomarkers panels that consist of multiple proteins to characterize diseases (21). Furthermore, the advancement in proteomic technologies has allowed this field of study to be integrated into pharmaceutical development and research in new drug discovery (22).

Proteomics can be classified into three subgroups as illustrated in Figure 1. Firstly, functional proteomics revolves around two key points: the explanation of the biological role of unknown proteins and the description of molecular cellular mechanisms (23). Secondly, expressional proteomics uses high throughput technologies to separate, identify and quantify protein expression levels (24). Thirdly, structural proteomic involves high throughput X-ray Crystallography/ Modelling or NMR Spectroscopy/Modelling based method (25). Two-dimensional Gel Electrophoresis (2-DE) is the oldest and most common method of protein detection, display, purification, recognition, and quantification used in proteomics research. Optimized protocols were able to isolate and identify more than 2,000 proteins and up to 10,000 proteins in a 20x25 cm gel. Nevertheless, the method can also be time-consuming and could only detect high protein abundance (usually the top 30%) (26).
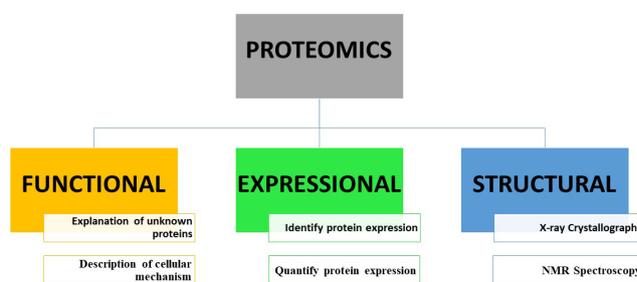

Figure 1: Proteomics classification

BASIC METHOD IN PROTEOMIC

Various methods are available in proteomics such as western blot, ELISA, flow cytometry, and immunohistochemistry. Out of all these methods, the western blot is the most used method in proteomic studies due to its ability to isolate, track proteins, and detect multiple proteins at once and its sensitivity compared to chemiluminescence. Additionally, this method allows the isolation of proteins from cells through size separation, solid support transition, and marking the target protein using antibodies (27). This technique uses electrophoresis gel to separate mixtures of proteins by creating a band for each protein. This is followed by the incubation of the membrane with primary antibodies and subsequently the secondary antibody. In this review, we are focusing on the mass spectrometry (MS) approach used in proteomic studies related to AD.

Mass Spectrometry as A Proteomic Molecular Approach Most of the current proteomic procedures use the MS technique in profiling the complex samples although more variety of MS-based proteomic methods has emerged to perform relative quantitation of many proteins (28). Ho et al. (29) carried out proteomics studies to

distinguish the proteomic patterns in post-mortem grey matter brain tissue of male bipolar disorder (BD) subjects with and without psychosis history. Identification and quantification analysis of the tissue proteome were done using mass spectrometry. They were able to find significance in 11 proteins and suggested that samples of BD with psychosis history showed an upregulation of neuronal processes and neuronal proliferation. On the other hand, the study was unable to detect any proteomic changes in subjects' mental states immediately prior to death.

Another proteomic study was carried out by Jacob et al. (30) to identify the early biomarkers of myocardial injury. They included patients who underwent planned myocardial injury (PMI) using septal alcohol ablation and patients who are undergoing emergency cardiac catheterization for spontaneous myocardial injury (SMI). The results of the proteomic profiling found changes in 376 target proteins in blood for PMI patients and 29 protein changes in SMI patients. They were also able to identify many novel markers that are intracellular protein that was not previously identified in the peripheral circulation. However, this expanded proteomics platform is uncertain where the exclusion proteins possibly altered both in myocardial injury and catheterization (30).

Similarly, the proteomic study is not only limited to humans but also to animal models to gain more understanding of various diseases. In clinical diagnosing, proteomics has been commonly applied in various models such as humans, rats, and mice (31). A chronic animal model study using rats was conducted by Zhang et al. (32) to investigate chronic pancreatitis-reported changes in urine proteins. They were able to identify fifty differential proteins as early as 2 weeks after injection when no specific pathological changes occurred. In week 3 and week 4, a few other proteins were differentially expressed and identified as abnormal in human chronic pancreatitis. Although they can detect proteins that act as biomarkers in the early phase of the disease, however, several proteins are associated with other diseases, such as pancreatic injury (32).

MS is the most basic technique used to study proteomics which could provide useful information in peptide identification (18). More advanced technologies are on the rise to collaborate with MS-based research in proteomics (20). The main purpose of proteomics is to analyse the protein spectrum, biological function, and detection of novel drug targets and diagnostic markers. Therefore, the proteomic study is widely used to investigate clinical diagnoses such as infectious diseases, cancers, heart diseases, and neurological disorders (33). It also shows diverse protein expression, role, connectivity, and localization in different neuron phenotypes. As proteomics are known to utilize a very complicated screening technology, it transitions from a

theoretical approach to functional practice throughout the year (34, 35, 36). In addition, proteomics is often capable of identifying various proteins along with specific cell components such as post-synaptic densities and membrane proteins which relate to the normal physiology of an organism (37, 38). Figure 2 shows the common workflow for proteomic analyses which involves different samples until bioinformatic analysis.

Figure 2: Proteomics analysis workflow

A review done by Huang et al. (28), discussed various approaches that use MS-proteomics to detect cancer markers in patients. Multiple MS methodologies such as 2-DE and chromatography-based proteomics, Shotgun based proteomics, Selected Reaction Monitoring (SRM)-based proteomics, Sequential window acquisition of all theoretical mass spectra (SWATH)-based proteomics, and Multiplexed MS/MS were discussed on the application method in cancer marker detection. These methods have been applied to discover the biomarkers for numerous types of cancers such as gastric, pancreatic, liver cancers, colorectal cancer, lung cancer, breast cancer, melanoma, and urinary cancer (28). In cognitive research, many studies were done specifically on neurodegenerative diseases such as AD and Parkinson's disease which include either the identification of biomarkers or a systemic review of previous studies (39, 40, 41).

In Malaysia for instance, proteomics studies had been carried out in various fields such as phytotherapy research (42), AD (43), breast cancer research (44), as well as research on snake venom proteomics (45). The main features of AD using molecular studies are Aβ and NFT containing hyperphosphorylated Tau. The existence of Aβ peptides and NFT are used to categorize the stage and intensity of the disease. With the advancements and innovations in both genomics and proteomics, fundamental insights into the pathogenesis of AD have been discovered. Many of the recent works and study of AD focuses on profiling deep brain proteomes (46), dissecting sub-proteomes and investigating complex protein post-translational modifications (PTM) patterns (47), as well as identifying new biomarkers (48). Proteomics study is helpful for early disease detection and to monitor the progress of AD in an individual. It also has a vital role in the drug and therapy development of AD as proteomic techniques can be used to characterize proteome, and proteins at any stage. This allows for further mapping of pathways and protein-protein interaction in AD. Advancement has been made towards profiling in AD and the discovery of potential biomarkers (refer to Figure

1 in 47), As the field of proteomics advances, it brings hope to further understanding of neurodegeneration in AD and the discovery and development of curative treatments and/or drugs (47).

PROTEOMICS APPLICATIONS IN AD RESEARCH

There are various practicalities of using the proteomics approach in research regarding AD, either globally or in Malaysia. In this review, we discussed three main potential applications of the proteomics approach in AD research. The first application is by using proteomics as a biomarker and a clinical tool to identify and diagnose individuals with AD. Secondly, the proteomic approach could also be useful to track the treatment progression of AD and to identify the effectiveness of any potential pharmaceutical or nutraceutical therapy for AD. Lastly, proteomics is a reliable method to further understand and uncover the pathogenesis of AD.

Biomarkers for AD Diagnosis
One potential application for the proteomics approach in AD is the use of certain proteins as biomarkers. Biomarkers are important in AD detection as they have the potential for a validated and high-accuracy AD diagnosis, disease tracing in AD patients, and act as avoid error in diagnosing a person with AD. AD biomarkers have several types, divided according to their interaction with diagnostic tools as well as the source of the bodily fluids from which the biomarkers are extracted. The former is the imaging biomarkers, which interacted with neuroimaging probes which then are detected by diagnostic neuroimaging devices such as positron emission tomography (PET). The latter is the fluid biomarker, which is further divided into 3 types: cerebrospinal fluid (CSF), urine, and blood biomarkers (49).

CSF and urine-based fluid biomarkers
The CSF-based biomarkers, as the name suggests, are extracted from the cerebrospinal fluid of an individual. The three main CSF biomarkers, the Aβ42, total tau and phosphorylated tau signify the most researched proteins expressed during AD pathogenesis (49). These 'established' CSF biomarkers had high sensitivity scores of more than 95% and high specificity scores of more than 85%, according to Sharma & Singh. (50). Other research, on the other hand, found the association of AD pathogenesis with other CSF biomarkers like YKL-40, interleukin (IL)–6, IL-7, IL-8, IL-15, IP-10, monocyte chemo-attractant protein 1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1), placental growth factor, and FMS-like tyrosine kinase 1 (FLT-1) (51).

According to Llano et al. (52), Vereinigte Glanzstoff-Fabriken (VGF or VGF nerve growth factor inducible) peptides are lowered in CSF of patients with AD which leads to cognitive impairment or a more advanced cognitive decline. On the other hand, high levels of VGF were associated with steeper subsequent longitudinal cognitive decline, especially in AD patients (53). VGF or VGF nerve growth factor inducible plays a vital role in synaptic function, synaptic plasticity, and hippocampal memory consolidation (54). Higher VGF protein level was associated with cognitive stability. Besides, the changes in the expression of discrete VGF fragments can be used as an indicator in different neurological and psychiatric conditions (55). Another study by Busse et al. (56) using the flow cytometry method has found an increased number of VGF-expressing T cells in AD patients compared to older healthy controls. Similarly, all VGF peptides using gel chromatography + immunohistochemistry methods were reduced in the AD samples (57). Together with other diagnostic tools such as PET (positron emission tomography), CSF biomarkers proved to be highly effective in AD detection and pathogenesis monitoring (38). However, the high cost and invasiveness of the procedures warrant a more effective strategy for AD detection and diagnosis (58).

Urine-based biomarkers are also one of the potential sources of AD biomarkers. For example, a urine-based assay for the detection of AD biomarkers, known as the AD reaction titre in urine, had been designed to be used in clinical settings. It detects a type of protein called the neural thread protein (NTP), which is expressed abundantly in AD compared to normal individuals (59). A study conducted by Ma et al. (60) found that the NTP level was higher in individuals with mild cognitive impairment (MCI), showing plausible early detection of MCI. The urine-based biomarker is advantageous in the sense that it is perhaps the least invasive and causes the least discomfort in terms of the sampling collection. However, it has some limitations where the variability of data in detection across multiple studies has not been standardised to be used in clinical settings. Despite this, some researchers suggested the use of urine-based biomarkers to be complemented with other approaches due to their advantages in AD diagnosis (61).

Blood-based AD biomarkers: A potential milestone for AD fluid biomarkers
Due to the cost-invasiveness issues of CSF-based biomarkers and data variability issues of urine-based biomarkers, research interest had taken a turn to another type of biomarker; the blood-based biomarkers. The attractive benefits of using blood-based biomarkers over the other types can be observed in terms of the cost, invasiveness, and reliability of the biomarkers. The cost-benefit analysis of blood is a frequent sampling of bodily fluid in a patient; hence a systematic medical follow-up could be established by periodical blood testing of AD biomarkers (49). However, the search to establish potential proteome biomarkers from blood present a huge challenge due to the variability of protein molecules in blood related to AD, as well as the complex dynamics of these molecules in their regulation in AD patients. Some molecules in the brain are too large to

cross the blood-brain barrier, leading to a decreased concentration in the blood (38). Some molecules such as multiple interleukins (IL), interferon-gamma (IFN-g), tumour necrosis factor-alpha (TNF-α), chemokines, selectins, integrins (62) S100B, neuron-specific enolase (NSE), protein breakdown products (protein BDPs), glial fibrillary acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase L1 (UCHL1) and myelin basic protein (MBP) (63) coincided with the pathogenesis of other disorders apart from AD, making a specific assay for AD biomarkers challenging (38).

A mere 13 years back, Thambisetty & Lovestone (64) pointed out the fact that there is a lack of proteomic approach in the identification of blood-based AD biomarkers. However, there was an increasing trend of identifying some viable proteins to be used for this purpose. Despite the obstacles highlighted previously, research had elucidated some candidate blood-based biomarkers that could be used for AD diagnosis. For example, several Malaysian researchers explored the potential use of blood-based biomarkers for AD detection in 2019 (43). The researchers conducted a meta-analysis across 22 papers and focused on six potential blood biomarkers which consistently showed a relationship with AD pathogenesis, whether in terms of upregulation or downregulation. They are the alpha-2-macroglobulin (α2M), pancreatic polypeptide (PP), apolipoprotein A-1 (ApoA-1), afamin, insulin growth factor binding protein-2 (IGFBP-2), and fibrinogen-γ-chain (Table I). Within the same year, the same team of researchers published another paper to uncover the potential use of fibrinogen

proteins for AD blood biomarkers. The study utilized the 2-DE method for the proteome's detection, and MS was used to analyse the blood samples taken from AD, non-AD, and mild cognitive impairment subjects. They found that two fibrinogen isoforms, fibrinogen-β-chain, and fibrinogen-γ-chain exhibited increased levels in AD compared to non-AD subjects (43). Fibrinogen-γ-chain was also found to have a significant negative correlation, though weak, with the level of cognitive decline, further making a case for the blood biomarkers.

Although there is a lot of potential for blood-based biomarkers for the diagnosis of AD, several obstacles need to be highlighted. Firstly, in some study like Rehiman et al. (65) utilized only a small sample size in the research, raising questions on how the result of this study would apply to a broader population of AD individuals. Secondly, the findings from different research laboratories also were not standardised, causing a problem of replicate of the results (66). Thus, like the urine-based biomarkers, more work needs to be done to establish a standard for using AD clinical blood-based biomarkers. This standard should address aspects like the type of blood biomarkers most reliable and suitable to be used and their ranges in AD patients and normal populations.

Treatment Progression of AD
Based on the proteomics study, many proteins have been discovered to play a role and are related to AD, as mentioned before. Even though current drugs or medications are not 100% effective (67, 68), that doesn't

Table I: Several potential blood-based biomarkers for early detection of AD

Protein Biomarker	Expression of the protein in AD patients	Research Team
Alpha-2-macroglobulin (α2M)	Increased	Thambisetty et al., 2011 (69)
Insulin growth factor binding protein-2 (IGFBP-2)	Increased	Doecke et al., 2012 (70)
	Decreased	Hertze et al., 2014 (71)
Apolipoprotein E (ApoE)	Decreased	Doecke et al., 2012 (70)
Afamin	Decreased	Kitamura et al., 2017 (72)
Pancreatic polypeptide (PP)	Increased	Gupta et al., 2017 (73)
Apolipoprotein A-1 (ApoA-1)	Decreased	Kitamura et al., 2017 (72)
	Increased	Rehiman et al., 2020 (43)
Fibrinogen β chain	Increased	Sun et al., 2020 (74)
Phosphorylated Tau-217 (P-tau217)	Increased	Palmqvist et al., 2020 (75)
	Increased	Barthĕlemy et al., 2020 (76)
Fibrinogen-γ-chain	Increased	Rehiman et al., 2020 (65)
	Decreased	Shi et al., 2021 (77)
Glial fibrillary acidic protein (GFAP)	Increased	Chatterjee et al., 2021 (78)
Neurofilament light chain (NFL)	Increased	Park et al., 2022 (79)

stop researchers to continue their research to find the best therapeutic agent to prevent, attenuate or stop AD (11). The currently available drugs in the treatment of AD do not slow down the progression but only temporarily alleviate the symptoms of this disease (80). Donepezil, galantamine, rivastigmine, tacrine, and memantine are commercial drugs that have been approved to treat AD but showed low efficiency in treating AD (81). New therapeutic intervention is needed to treat the effect of this disease. Various experiments using the proteomics approach have been carried out to study the effectiveness of novel therapeutic drugs. The increasing number of agents to be tested up to phase 3 in clinical trials can be seen over the years (82). A list of drugs that have been in phase 3 for clinical trials from 2016 to 2021 has been presented in table 1 in the review paper by Abubakar et al. (82) where the agents were divided into 3 categories: disease-modifying biologic, disease-modifying small molecule, and symptom-reducing small molecule.

Exploration for new therapeutic agents for AD not only from the existing and commercial drugs but also from natural substance (83, 84). A study by Hamezah et al. (83) has demonstrated the effect of tocotrienol-rich fraction (TRF) extracted from palm oil which represents vitamin E analogues provides a greater neuroprotective effect compared to other forms of vitamin E. APPswe/PS1dE9 double transgenic (TG) mice (AβPP/PS1), a mouse model of AD were used in this study. The researcher examines the effect of TRF on the proteome profile of wild type (WT) and TG mice brain regions (hippocampus, medial Prefrontal Cortex, and striatum) by using ultra-high-performance liquid chromatography (UHPLC) coupled to Q Exactive HF Orbitrap mass spectrometry. There were 4 groups of treatments including control which received only water to WT and TG mice, 1 group (TG mice) which received palm oil stropped of Vitamin E (PO) and 1 group received TRF (TG mice). All mice were supplemented daily with water, PO, and TRF for 10 months via oral lavage and were subjected to the behavioural test. Comparison of proteome profiling between WT control group and TG control group, and also between TG mice receiving PO and TG mice receiving TRF was done. Higher expression of amyloid beta A4 protein (APP) and receptor-type tyrosine-protein phosphatase alpha (PTPRA) was shown in TG mice hippocampus compared with the WT mice. While decreased expression of these proteins was found in the TG mice supplemented with TRF. The findings from this study demonstrated changes in proteins in specific area of the TG mice brain for groups supplemented with TRF and TRF also modulated APP and PTPRA protein expression in TG mice hippocampus. These findings suggested the potential use of TRF supplement in neuroprotection with further study on the effect of TRF associated with AD (83). Another study on a natural substance by Rahman et al., (84) suggested that Ganoderma lucidum, a medicinal mushroom, has a modulatory effect on AD. The researcher used AD Wistar male rats which were prepared by infusing with Aβ protein into the cerebral ventricles. The AD rats were fed with G. lucidum hot water extract and the brain samples were obtained for analysis. By using the proteomics approach, the protein was extracted from the hippocampi, and homogenization was done. This was followed by protein separation and quantification, statistical analysis, and bioinformatic analysis. The analysis of functional interaction among protein networks was done to obtain information in deciphering any bio-molecular system. The findings showed that modulatory effect of G. lucidum was via highly interactive differential protein expression, restoration process of the disrupted protein-protein interacting network and maintenance of integrated pathways. The proteins that involved are tubulin, β-actin, dihydropyrimidinase-related protein 2 (DRP-2), keratin, GFAP, Rho A proteins, septin, cofilin, gelsolin and dynamin. These findings highlighted the possibility of incorporating G. lucidum as an ameliorating AD agent by emphasizing the importance of determining the therapeutic dosage, toxicity, and safety (84).

Advancement of AD Research
Even though there are progressions in treatment, however, there is still no cure for AD (68, 85), and medications given to the patient now are not 100% effective (68). The main reason that contributes to the failure is due to the involvement of numerous proteins and various biological pathways (67). The proteomic analysis of the brain has limitations regarding the sample and the analytical approach (86), where there is still a considerable gap in the study of molecular changes for AD pathogenesis; therefore, this approach cannot stand alone. Other applications and approaches such as genetic, transcriptomic, proteomic (86) and even brain imaging (38, 49, 87) should be run in parallel for a better prognosis of AD (20). Currently, deep proteomic studies have focused on profiling the brain and biofluids. This will provide a deeper and enhanced understanding of molecular alterations and characteristics of AD. Studies via different pathways or mechanisms over the years which include the Aβ cleavage and degradation, apolipoprotein E (ApoE)-cholesterol pathway and NFT accumulation, acetylcholine production, Wnt signalling pathway, Ubiquitin mediated proteolysis, apoptosis, calcium signalling pathway, Notch signalling pathway, MAPK signalling pathway, abnormal ceramide accumulation, reactive oxidation process, neurotrophin signalling pathway, cell cycle, mTOR signalling pathway, lipid pathway, insulin pathway, inflammation pathway, FGF7/FRFR2/PI3K/AKT pathway, Janus kinase/ signal transducer and activator of the transcription pathway (JAK/STAT) and nerve growth factor metabolic pathway (NGF) (82) via the applications of proteomics methods in various factors of AD gives us further insight into the understanding of AD mechanisms (88).

Ageing Research in AD
Ageing is one of the most relevant biological processes

and significant risk factor for neurodegeneration in diseases like AD (89). Therefore, proteomic applications in investigating the effect and changes on the brain and also identifying factors and mechanisms of ageing should be considered (47). Each brain region carries a different function and role, therefore investigation at the proteome level of brain proteins changes in relations to age will provide essential data that will enable us to understand the interactions and conditions of these proteins in the ageing process (90, 91). Hamezah et al. (8) has conducted a study precisely in this regard to profile the proteomes in the brains of ageing rats. This study identified altered age-related proteins important for oxidative phosphorylation, glutathione metabolism, and calcium signalling pathway in the hippocampus, medial prefrontal cortex (mPFC), and striatum. These results showed that the changes mostly affected the hippocampus region, which is important in regulating learning and memory. The overall findings suggested that alterations in these processes and brain atrophy in the hippocampus, mPFC, and striatum may be involved in cognitive and locomotor impairments in aged rats (8). Besides ageing research on animal, studies have also been carried out at proteome level on human's ageing brain. Manavalan et al., (92) conducted a study to compare the proteome in the cerebellum with the proteome at the hippocampus and parietal cortex using aged human brain tissues (80 to 98 years old; control group) and from patient with AD. A total of 31 altered proteins were identified in the investigated brain area particularly Gelsolin (GSN), Tenascin-R (TNR) and AHNAK. These proteins could potentially act as novel biomarkers of ageing-related neurodegeneration. GSN is hypothesized to fight against neurodegeneration and downregulation of TNR expression might be one specific direct cause of impaired cognitive abilities in AD.

In other recent research, proteomic methods have been used by Duda et al., (93) to determine the quantitative analysis of protein expression in hippocampus, cortex and cerebellum using 1-month and 12-month-old mice. More than 1760 proteins were affected by ageing in hippocampus and various forms of hippocampal neuronal plasticity were also significantly altered in middle-aged animals.

Experiments conducted by Cabral-Miranda et al. (94) on different age of mice were to determine global proteomic changes in hippocampal tissue due to ageing factor. Quantitative proteomics were performed on tissues of ageing hippocampus and molecular alterations were studied; with neuronal plasticity-related proteins being the most affected. The proteomic data analysis found out that the overexpression of one particular transcription factor, called the spliced X-box Binding Protein 1 (XBP1s) change the expression of important proteins that are essential in the organization of synapse and regulation of neurotransmitters. This change of proteomes which occurs as an individual ages, concluded the study, ensure the preservation of the normal synapse and neurotransmitters (94).

Demographic Research of AD population
In present times, we can find individuals who have aged successfully (phenomenon known as 'successful ageing'), and this shows a promising strategy on controlling the ageing effect towards cognition (94). Ageing successfully depends highly on the various individuals' physiological, physical, and psychological characteristics (96). The condition of 'successfully ageing' brings about the definition of coming to age without impairment or deficiency in cognitive functions (97).

One of the reasons for the failure of AD drug treatment is that the genes involved in AD based on human genome studies have not yet been revealed (67) which we believed contributes to specific-demographic genes coded for AD. The study of the genetics of AD is very important to delineate the protein-product involvement at specific pathways or mechanisms to minimise the failure of drugs chosen (68).

Previous findings evaluating the role of cytokines inflammation might play a central role in the pathogenesis and progression of AD in Caucasian populations (98). A study in 2016 (85) found higher levels of cytokines and larger fold change in the AD patients of the Malaysian population. The levels of the non-classical pro-inflammatory, CXCL-10, and anti-inflammatory, IL-13, cytokines at appropriate cut-off points were also highly specific and sensitive for AD patients in Malaysia. However, in this study, they did not analyse the data based on ethnicity in Malaysia where out of 39 participants of the AD group, 5 are Malay, 26 are Chinese and 8 are Indian.

In 2019, another group of researchers in Malaysia run an experiment that only focus on the Malay population for the first time (95). They investigated the effect of age on the protein profile of 160 Malay individual plasma samples using several neuropsychological tests to assess their cognitive competencies. Most of the significantly expressed proteins were upregulated in Group 30 and Group 40 while Group 50 and Group >60 showed downregulation of those proteins, indicating a significant shift in protein expression with age, particularly between Group 40 and Group 50. The results signified a critical separation point in distinguishing the younger (Group 30 and 40) from the older Malay individuals (Group 50 and > 60), supporting the hypothesis that ageing contributes to cognitive competency, and that the degree of cognitive decline varies across different populations, with influence from environmental factors. However, since there are no experiments have been done on other ethnicities in Malaysia therefore no comparison can be made.

The various findings might be a result of ethnic differences, differences in age and sex, and variations in

lifestyle and diet (85). Understanding the differentially expressed proteins in different demographics will enable more defined therapeutic measures in improving cognitive competencies specific to the community. Data from the studies can also be referenced for biomarkers to create suitable interventions for individuals in that specific community and demographic.

Behavioural Research in AD

AD impairs memory and learning-related behavioural performances of the affected person as it involves degeneration of associated neurons in the hippocampus (99, 100). A study conducted by Rahman et al., 2019 (89) focused on memory and learning-related behavioural performances using an eight-armed radial maze and hippocampal proteomics of AD rats. AD rats took longer time to explore the maze than the control, indicating spatial learning and memory impairment. In AD brains, higher protein expression was found such as copper/zinc superoxide dismutase (SOD), glutathione peroxidase (GPX), peroxiredoxin, and glutathione-S-transferase (GST), causing disruption in energy production and oxidative stress (OS) management (101). This inter-relationship reinforces the OS and alters energy metabolism links of AD pathogenesis. AD rats also showed memory deficits when tested in a spatial water maze (WMZ) and proteomic differences in dorsal CA1 young AD rats. However, levels of phosphorylated tau, reactive astrocytes and microglia were significantly increased in aged AD rats and correlated with the WMZ learning index (LI) (102).

Since the aetiology of AD is multifactorial, other animal models with other symptoms, such as anxiety, depression (103) and diabetes related to AD (104) have also been studied. Triple transgenic mice of AD (3xTg-AD) treated with melatonin have been evaluated via open field test, elevated plus maze test, forced swimming test, and tail suspension test. The researchers discovered that glutathione S-transferase P 1 (GSTP1) (an anxiety-associated protein) and complexin-1 (CPLX1) (a depression-associated protein) which related to AD were significantly down-regulated in the hippocampus of 3xTg-AD mice (103). The role of methylglyoxal (MGO) in the pathogenesis of AD in a rat model has also been evaluated via behavioural and proteomic studies (104). MGO is a toxic by-product of glycolysis which is high in diabetes patients. The elevated plus-maze (EPM) behavioural study indicated that MGO induces anxiety. Hippocampal proteomics demonstrated that MGO-treated rats regulate proteins involved in calcium homeostasis, mitochondrial functioning, and apoptosis, which may affect neurotransmission and neuronal plasticity. The hippocampal tau phosphorylation level was increased in MGO-treated rats. This study provides insight into the role of MGO in the diabetes-associated development of AD.

CONCLUSION

This mini review summarized AD and how the proteomic approach plays an important role in understanding the disease at a molecular level. Based upon the review, different types of AD biomarkers discovery have led to a more improved understanding of the pathogenesis of AD. Proteomics studies allow researchers to identify and analyze the triggering protein pathways and thus discover an appropriate therapeutic intervention to overcome this disease (105). Besides that, the advancement of MS-based method has improved the analysis of proteome in AD. AD is a neurogenerative disease which developed from multifactorial components beyond genetics such as age, behavior, and demographic factors that either influence or are influenced by the proteome profile of an individual (106, 108, 108). However, more proteomic studies together with other approaches are needed in the future especially on cognitive perspective to deepen the understanding of AD.

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