

# Implication of preclinical biomarkers in identification of mild cognitive impairment (MCI) and Alzheimer's disease: Promises and future challenges

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## ABSTRACT

Mild cognitive impairment (MCI) has attracted central attraction by various researchers to diagnose prodromal stage of Alzheimer's disease (AD) by using different biological and imaging biomarkers. In evaluating disease changes, it is critical to have measurements that are sensitive, specific, and reliable. The purpose of this review is to bring a comprehensive overview about the recent studies done in development of biomarker for clinical diagnosis of MCI and pre-AD using different technological interventions. The current state of knowledge highlights the implication of certain proteins, transporter, enzymes and metabolic pathway related factors, radiotracer in identification of disease progression. However, all these studies done on biomarker development lack sensitivity and specificity to be classified as clinical marker for MCI and AD. The revised criteria of NIA-Alzheimer's association in diagnosis of MCI, pre-clinical AD and AD encompass use of biomarker which can detect pathological process and subsequent neurodegeneration. The present article highlights the significant development in field of plasma, cerebrospinal fluid, serum and neuro imaging biomarkers which have shown positive result in various studies and the future challenges in application of these biomarkers at clinical setting in early intervention of disease.

**KEYWORDS:** Mild cognitive impairment, Alzheimer's disease, Biomarker, Proteomics, Neuroimaging, Neurodegeneration

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## 1.0 INTRODUCTION

The increasing rate of people suffering from dementia worldwide is alarming, the number of people affected is projected to increase more than 80 million by 2040 (Ferri et. al, 2005). Alzheimer's disease (AD) comprises the most common form of dementia about 50% to 60% of all cases, followed by dementia with Lewy bodies combined with frontotemporal dementia (FTD) making up the other large segment (15%–25%) (Gauthier et. al., 2006). Mild cognitive impairment (MCI) is early phase of cognitive decline that precede dementia. The progression of MCI patient to AD, vascular disease and other kind of dementia is evident by various studies (Ganguli et al., 2004; Petersen et al., 1995; Rountree et al., 2007). The conversion of MCI to AD is 6.7 times more likely as compared with cognitive normal individuals (Boyle et. al., 2006). Therefore, MCI can be characterized as prodromal phase of AD. The loss of memory and cognitive function is major feature of AD and therefore it is difficult to differentiate it from other forms of dementia, especially in early clinical stages. Two major forms of AD have been recognized, a familial (genetic), early-onset AD (EOAD) form comprising a small percentage of those afflicted, and a sporadic late-onset AD (LOAD) variety affecting most AD patients. The amyloid hypothesis is closely associated with EOAD (Selkoe, 2000). The LOAD have genetic association in combination of environmental factors. The World Health Organization currently estimates that approximately 35.6 million people are afflicted by AD worldwide. In the United States, approximately 7 million people older than 65 years are known to suffer from AD, and this number is expected to be triple by 2050. According to the 2013 facts and figures from the Alzheimer's Association (AA), the number of deaths from major diseases such as cancer and cardiovascular disease has declined in the past decade, the number of deaths related to AD has increased 68% during the 2000 to 2010 period. The need of specific biomarker for preclinical AD is urgent

which can differentiate subject without risk of progression to dementia with those at risk of developing prodromal or actual AD. MCI will be useful AD prodromal phase to test putative biomarkers for their efficacy in early diseases detection. The pathobiology of AD begins much before the clinical symptoms are apparent, therefore the pre-clinical state consist of three distinct stages, Stage 1 is characterized by asymptomatic amyloidosis that begin as early childhood and evolve through midlife into old age, Stage 2, represent neurodegeneration due to deposition of CSF-tau or p-tau concentration that result in cortical thinning and hippocampal atrophy (HA); Stage 3 is evidenced by amyloidosis. The current status of biomarker development for preclinical AD is concentrated on cerebrospinal fluid (CSF), neuroimaging and peripheral blood methodologies. This article reviews the recent development in preclinical AD biomarkers and their implications in identification of subject at early stages and challenges in usage of these biosignatures for clinical assessment of AD.

## 2.0 CEREBROSPINAL FLUID BIOMARKERS-

The cerebrospinal fluid (CSF) is in direct contact with extracellular portion of brain and any biochemical changes in brain are reflected in CSF. Amyloid beta ( $A\beta$ ) and tau protein are primary protein found in CSF has shown positive potential biomarker for AD, recent studies have shown that  $A\beta$  load in AD subject can be assessed by using positron emission tomography (PET) with  $A\beta$  ligand. Further, Pittsburgh Compound B (PIB)-PET has shown lower level of  $A\beta$  in CSF (Fagan et al., 2009). Similarly another study have also shown lower level of CSF-  $A\beta$  binding with  $^{18}F$  FDDNP a PET ligand believed to label both plaques and tangles (Tolboom et al., 2009). The variability in the level of CSF-  $A\beta$  might be due to its aggregation in form of plaques and retention in brain parenchyma which result in decrease diffusion of  $A\beta$  in CSF (Tolboom et al., 2009). The level of CSF total tau (T-tau) is measure of

neuronal and axonal damage as evident by several studies (Hesse et al., 2001; Ost et al., 2006; Zetterberg et al., 2006). Recent studies have suggested that high CSF T-tau is associated with progression of mild cognitive impairment to AD which is released from degenerating tangle bearing neuron (Blom et al., 2009). The neocortical tangle pathology and increase in level of phosphorylated tau proteins mainly (P-tau 181, P-tau 231) is directly correlated (Buerger et al., 2006; Tapiola et al., 2009). The increase in the level of P-tau 181 in CSF of mild cognitive impaired individuals progressing to AD and very mild AD dementia is reported (Blom et al., 2009; Samgard et al., 2010; Snider et al., 2009). The specificity of 80% was achieved in several studies which has used CSF T-tau, P-tau and A $\beta$  42 as diagnostic marker for AD as compared with non-demented elderly population (Blennow and Hampel, 2003; Blennow, 2004). The levels of these markers are also normal in other disease such as depression and Parkinson's disease (Blennow, 2004). The limitation of CSF biomarker to differentiate AD from other form of dementia is not optimal. First, most studies of CSF biomarker is based on clinically diagnosed individuals, which involve large number of misdiagnosed cases, (Blennow, 2005; Forman et al., 2006) , second, large population of non-demented elderly have significant percentage of plaques and tangle warrant neuropathology to diagnosis of AD (Snowdon, 1997; Price and Morris, 1999). Third, there is a large overlap in pathology between AD and other dementias, such as Lewy body dementia and vascular dementia (Jellinger, 1996; Kotzbauer et al., 2001; Schneider et al., 2009). This overlap in pathology essentially precludes the possibility of finding any biomarkers that have close to 100% sensitivity and specificity for AD (Blennow, 2005).

## 2.1 sAPP $\beta$ and sAPP $\alpha$

The processing of amyloid precursor protein (APP) result in release of large amino terminal domain sAPP $\alpha$ , and sAPP $\beta$  which are secreted into extracellular space and reaches CSF. In sporadic

AD and MCI the level of these amino acid domains in CSF increases or slightly altered which can be used as promising tool in differentiating AD from non-demented subjects (Lewczuk et al., 2008; Olsson et al., 2003; Zetterberg et al. 2008).

## 2.2 BACE 1, A $\beta$ oligomers and A $\beta$ isoforms

The generation of A $\beta$  in brain occurs through APP-cleaving enzyme 1 (BACE 1). The expression and enzymatic activity of BACE 1 enhanced in postmortem brains of patients with AD (Fukumoto et al., 2002; Yang et al., 2003). The concentration of BACE 1 can be measured in CSF and it was found that its concentration and activity increases in AD, preferentially in MCI cases with prodromal AD (Holsinger et al., 2004; Zhong et al., 2007; Zetterberg et al., 2008). The deposition of A $\beta$  oligomers into insoluble fibrillar aggregates in brain inhibits long term potentiation, thereby play important role in pathogenesis of AD (Walsh and Selkoe, 2007). Some studies have published the usage of A $\beta$  oligomers in CSF for detection of prodromal AD (Georganopoulou et al., 2005). The presence of A $\beta$  oligomers in CSF has been done using CSF but the diagnostic utility of this assay is undermined (Santos et al., 2007). The level of A $\beta$  oligomers is very low as compared with A $\beta$  monomer, in CSF, therefore results gained from fluorescent methods has to be validated from mass spectrometry (Fagan et al., 2006). A $\beta$  40 is one of the major isoform found in CSF but no significant changes were found in the level of A $\beta$  40 as evidenced by PIB binding in CSF of AD and MCI (Fagan et al., 2006, 2009b). The diagnostic accuracy for identification of early onset of AD has further been worked by various researchers by estimating the CSF level of A $\beta$  37, A $\beta$  38, and A $\beta$  39 but the A $\beta$  isoform lack consistency in their level in CSF (Lewczuk et al., 2008; Schoonenboom et al., 2005; Fagan et al., 2009b). Several other truncated isoform of A $\beta$  1-14, A $\beta$ 1-15, and A $\beta$  1-16 are produced in CSF through novel pathway of APP processing, the concentration of these truncated isoforms has been estimated through immunoprecipitation and Matrix assisted laser

desorption ionization/Time of flight (MALDI-TOF) (Portelius et al., 2006a)

### 2.3 Neuronal, synaptic protein markers

The role of neuronal and synaptic protein is valuable because it provides information about cognitive function and disease progression. The diagnostic performance of visinin like protein-1 (VILIP-1) is similar to CSF-tau and A $\beta$  protein expression in AD patients (Lee et al., 2008). The role of neuro-filament proteins in the identification of neuronal sub-cortical disease is well evident (Agren-Wilson et al., 2007; Sjogren et al., 2001a). Therefore usage of neuro-filament protein is being done in differentiation of sub-cortical dementia, frontotemporal dementia from Alzheimer's disease (Sjogren et al., 2001b). The density of synaptic protein and cognitive performance is directly correlated, therefore, CSF level of these synaptic proteins can provide specific biomarker for AD. Recent studies have identified pre-and post synaptic proteins in CSF following liquid phase iso-electric focusing and western blotting. These protein include synaptotagmin, growth associated protein (GAP-43), synaptosomal associated protein (SNAP-25) and neurogranin (Davidsson et al., 1999)

### 2.4 Inflammation and oxidative stress markers

Neuroinflammation is non-specific feature of AD and many studies in last decades have identified various inflammatory and signaling molecules such as ( $\alpha$  1-antichymotrypsin, isoprostane, interleukins, TNF-  $\alpha$ , interferon- $\gamma$ , compliment C1q and TGF- $\beta$  in CSF of AD. However the results were very inconsistent due to methodological difference in CSF collection and processing assay, criteria used for subject ascertainment and method of diagnosis (Craig-Schapiro et al., 2009; Zetterberg et al., 2004). However recent studies have identified astrocyte marker YKL-40 by using proteomics based techniques, this marker is being extensively validated in large cohort of AD patient and normal non-demented individuals, the level of YKL-40 was

high in AD patients and also show increase level in cognitive decline progressing to mild dementia (Craig-Shapiro et al., 2010). Recently usage of multiplex assay system has added advantage over estimation of inflammatory marker along with CSF level of A $\beta$ , CSF-tau and P-tau which can be used for prognostic and diagnostic purpose for AD (Hu et al., 2010).

## 3.0 PLASMA AND SERUM BIOMARKER

CSF is good resource for research in neurodegenerative disease but its application in biomarker discovery is limited due to its invasive nature, requirement of trained personnel and routine application mainly in elderly population. Plasma is complex biological fluid which represents the physiology and pathology of various body organs including central nervous system (CNS). In human 500 ml, of CSF is absorbed into blood daily (Hye et al., 2006) making blood a suitable source for biomarker discovery in neurodegenerative diseases. Further, the venepuncture is comparatively more repeatable procedure as compared with lumbar puncture making it suitable for biomarker discovery and evaluating clinical trials for disease modifying treatments. The biochemical changes, neuropathological lesions during progression of MCI to AD are not always reflected in clinical diagnosis, degree of cognitive impairment or its rate of progression. In spite of all these limitations lot of emphasis is laid on plasma related biomarker discovery for MCI and AD.

### 3.1 Proteomics based plasma biomarker in MCI and AD

The exploration for unbiased marker for neurodegenerative disease can only be achieved by using complex mixture like plasma using proteomics tool. The latest techniques in protein separation includes two dimensional gel electrophoresis (2DGE), one dimensional gel electrophoresis (1 DGE), and gel-free methods, such as isotope-coded affinity tag (ICAT) and isobaric tag for relative and absolute quantization

(iTRAQ). One of the most powerful protein identification strategies is liquid chromatography (LC) separation followed by mass spectrometric analysis (usually electrospray ionization, ESI). Another commonly utilized protein identification method is peptide mass fingerprinting using matrix-assisted laser desorption/ionization (MALDI). Liao and colleagues identified six potential proteins from plasma with potential to be used as biomarkers from 900 differentially separated protein using 2DGE. These proteins includes  $\alpha$ -1-antitrypsin (AAT), vitamin D-binding protein, inter- $\alpha$ -trypsin inhibitor family heavy chain related protein, apolipoprotein J precursor (ApoJ), cAMP-dependent protein kinase catalytic subunit alpha1 and an orf. The role of some of these proteins found to play significant roles in CNS microglia activation, actin metabolism and fibrinolysis in periphery (Liao et al., 2007). The elevated level of AAT has been shown in CSF by using proteomics methods (Sihlbom et al., 2008)

and by other method such as rocket immunoelectrophoresis (Nielsen et al., 2007). Its oxidized form has also been detected in AD patients by several plasma proteomics studies (Choi et al., 2002; Yu et al., 2003). The increase expression of complement factor H (CFH) and  $\alpha$ -2-macroglobulin ( $\alpha$ -2M) in plasma of AD patients as compared with non-demented individuals using 2DGE (Hye et al., 2006). Similar finding has also been reported in amyloid plaques in AD (Bauer et al., 1991; Strohmeyer et al., 2000). Recent findings from Cutler and coworker reported serpin F1 (pigment epithelium derived factor) and complement C1 inhibitor are down regulated in plasma of AD patients, and these observations were confirmed by specific assays (Cutler et al., 2008). Similarly there are various proteins related to different pathways has been reported in plasma of MCI and AD patients as described in Table 1 by using different technological interventions.

Table 1. Protein identified in plasma and serum of MCI and AD subjects using different Techniques

Protein Identified (up/down regulated)	Sample	Function	Method	Reference
Apolipoprotein J precursor (up)	Plasma	Lipid transport	2-DGE LC/MS/MS.	Corder et al. (1994)
Inter-alpha-trypsin inhibitor family (down)	Plasma	Inflammation	2-DGE LC/MS/MS	Corder et al. (1994)
Alpha-1-antitrypsin (up)	Plasma	Proteolysis	2-DGE LC/MS/MS. Glycan affinity chromatography/MALDI-TOF 2-DGE/DNP-immunostaining/MALDI-TOF	Corder et al. (1994)
cAMP-dependent protein kinase catalytic (down)	Plasma	Blood coagulation	Heparin/1-DGE/MALDI.	Corder et al. (1994)
Unknown protein ORF (up)	Plasma	Unknown functions	2-DGE LC/MS/MS.	Corder et al. (1994)
Apolipoprotein B100 (up)	Serum	Lipid transport	1-DGE/MALDI.	German et al. (2007)
Apolipoprotein E (up)	Serum	Lipid transport	1-DGE/MALDI.	German et al. (2007)
Transthyretin (up)	Serum	Thyroxine transport	1-DGE/MALDI.	German et al. (2007)

Hemoglobin alpha chain (up)	Serum	Oxygen transport	DEAE/1-DGE/MALDI	German et al. (2007)
Factor H (up)	Serum	Inflammation	Heparin/1-DGE/MALDI	German et al. (2007)
Haptoglobin alpha 2 chain (up)	Serum	Inflammation	DEAE or Ni 2-DGE/MALDI.	German et al. (2007)
Alpha 1-acid glycoprotein (down)	Serum	Inflammation	ConA/LCQ	German et al. (2007)
Inter-alpha-trypsin inhibitor heavy chain H4 precursor (down)	Plasma	Immune regulation	1-DGE/MALDI.	Chen et al. (1995)
CD5 antigen-like precursor (down)	Plasma	Immune regulation	1-DGE/MALDI.	Chen et al. (1995)
Serum amyloid P-component precursor (up)	Plasma	Immune regulation	1-DGE/MALDI.	Chen et al. (1995)
Ceruloplasmin precursor (down)	plasma	Copper transport	1-DGE/MALDI.	Chen et al. (1995)
Hemopexin Plasma (up)	plasma	Heme transport oxidative stress protection	Glycan affinity chromatography/MALDI-TOF.	Ferri et al. (2005)
Transferrin (up)	Plasma	Iron carrier protein energy metabolism	Glycan affinity chromatography/MALDI-TOF.	Ferri et al. (2005)
Alpha-1-antitrypsin (up)	Plasma	Proteolysis	Glycan affinity chromatography/MALDI-TOF.	Ferri et al. (2005)

### 3.2 Oxidized plasma protein markers and AD

The role of oxidative stress in post-translation modification of protein in progression of cognitive decline is well evident by various studies (Choi et al., 2002; Yu et al., 2003). The elevated level of three oxidized glycoprotein in AD plasma mainly transferrin, hemopexin and AAT were identified using 2DGE, anti-carbonyl western blotting and MALDI-TOF (Yu et al., 2003). Similarly, Choi and coworkers identified seven oxidized protein spots in plasma of AD patients among which isoforms of fibrinogen c-chain precursor protein and AAT precursor were predominant proteins that have association with inflammation and senile plaques of AD (Choi et al., 2002). Some studies have only focused on qualitative change on protein

expression while other focused on differential regulation of protein. Once a panel of proteins is discovered, further validation is necessary to determine the specificity and sensitivity of detected proteins as biomarkers, and to establish their predictive value in large numbers of samples and in longitudinal studies

### 3.3 Plasma biomarker related to A $\beta$ metabolism

The levels of A $\beta$  peptide, total tau protein, phosphorylated tau have been estimated in CSF, but the level of tau protein in plasma is below the limit of detection by analytical methods in various studies (Tang and Kumar, 2008). A $\beta$  peptide is the main component of senile plaques which is the main pathological feature of AD, therefore, various studies has been carried out on A $\beta$  peptide as

effective biomarker in MCI and AD plasma (Biere et al., 1996; Selkoe, 1994a; Younkin, 1995). The large majority of A $\beta$  in plasma is bound to albumin, and very little A $\beta$  is free (Biere et al., 1996). The major two isoforms estimated in plasma as biomarker for AD is A $\beta$ 1-40, A $\beta$ 1-42, while six other isoforms A $\beta$ 1-37, A $\beta$ 1-38, A $\beta$ 1-39, A $\beta$ 1-41, and the N-truncated A $\beta$ 2-40, A $\beta$ 2-42 has been evaluated in plasma as biomarker for AD (Maler et al., 2007). A $\beta$  is generated in central nervous system and transported in peripheral vascular system across the blood-brain barrier (Zlokovic, 2004) and also secreted by platelets in blood (Chetelat et al., 2003). The level of A $\beta$  increases with age (Fukumoto et al., 2002; Mayeux et al., 2003), and their estimation in plasma is based on sandwich ELISA, which most of the times is influenced by lipoprotein and Fc binding proteins (Kawarabayashi and Shoji, 2008). The reports suggest that there is conflict in usage of A $\beta$  as diagnostic marker for AD, as early studies reported no significant difference in plasma A $\beta$  level of AD and control subjects (Fukumoto et al., 2002; Kosaka et al., 1997; Tamaoka et al., 1996). One study showed a significant increase of A $\beta$ 1-40 in AD, but with a substantial overlap between AD and control groups (Mehta et al., 2000). The association of progressive cognitive decline and decreasing level of A $\beta$ 1-40, A $\beta$ 1-42 and C reactive protein in plasma were reported in AD patient with follow up time of 4 years (Locascio et al., 2008). Although, Blasko and coworker reported increase in the plasma level of A $\beta$ 1-42 in cognitively healthy (CH) individual converted to MCI and CH-to- AD, on the other hand there is decrease in the level of A $\beta$ 1-42 in MCI-to-AD converter, this study also emphasized that plasma A $\beta$  are high at preclinical stages and drop as the disease progresses (Blasko et al., 2008).

In summary the work done on estimation of A $\beta$  and its isoforms in plasma for diagnostic biomarker of MCI and AD is conflicting. The changes in the level of plasma A $\beta$  peptide with progression of disease is not the true

representation of changing level of central amyloid deposition. Therefore, the research on usage of plasma A $\beta$  peptide as diagnostic marker will be useful when it is combined with some other plasma biomarker with high sensitivity and specificity.

### 3.4 Plasma homocysteine and AD

The risk for developing cognitive impairment and AD is significantly associated with homocysteine, a sulfur-containing amino acid derived from methionine (Sachdev, 2005). The level of homocysteine in plasma increase in subject having deficiency of Vitamin B12, Vitamin B6 or folate (Selhub et al., 1993). The longitudinal studies carried out by various research groups are in cohesion that homocysteine level increases with progression of cognitive impairment and conversion to AD as compared to non-converter (Blasko et al., 2008; Gabryelewicz et al., 2007; Seshadri, 2006; Tucker et al., 2005). Although, total homocysteine level in plasma represents the progression of cognitive decline and can be used as markers but on other hand lowering the level of homocysteine improves cognitive decline has to be further investigated and evaluated (Seshadri, 2006).

## 4.0 NEURO-IMAGING BIOMARKERS

The diagnosis of dementia clinically is based on clinical assessment, neuropsychological testing and exclusion of other etiologies. Although, the National Institute of Aging have issued new diagnostic criteria for AD and mild cognitive impairment (MCI) and suggested use of biomarkers and neuroimaging for better diagnostic efficiency (Albert et al., 2011; McKhann et al., 2011). The National Institute of Aging has defined preclinical stages of AD (Sperling et al., 2011). Preclinical AD stage I was defined as asymptomatic cerebral amyloidosis (the presence of increased amyloid binding on positron emission tomography [PET] scan or low amyloid concentrations on lumbar puncture). Stage II was

defined as stage I plus downstream neurodegeneration (the presence of elevated tau on lumbar puncture, abnormal fluorodeoxyglucose [FDG] metabolism on PET scan, or abnormal volumetric loss on structural magnetic resonance imaging [MRI] scan. Stage III was defined as stage II with the addition of subtle cognitive decline (O'Brien et al., 2010). An important concept introduced in these guidelines is the AD pathophysiological process (e.g., amyloid deposition in the brain), which includes preclinical disease before AD

The biomarker for preclinical AD and MCI is currently limited to research application but some studies translating these imaging biomarker for clinical diagnosis of dementia (McKhann et al., 2011).

#### 4.1 Structural biomarker of Imaging

The decrease in volumetric size of medial temporal lobe including hippocampus and deposition of A $\beta$ -associated memory decline by using magnetic resonance imaging (MRI) is well reported (Mormino et al., 2009; Schuff et al., 2009). Further, studies have also reported that rate of hippocampal atrophy in MCI has greater risk of conversion to AD. The MRI measurement requires careful method of computerized image processing methods for detecting the regions atrophy in brains. The variation within subject in structural change by MRI is quite high. In one study, predictive prognosis of MRI performed at one time point versus combining single time-point measures with 1-year change measures were compared, with the latter providing significantly improved discrimination in prediction of AD conversion (McEvoy et al., 2011). The recent development in neuro imaging is prediction of conversion of healthy controls to MCI. The change in parts of hippocampus mainly CA1 and subiculum was more closely associated with conversion to MCI than total hippocampal volume (Lazarczyk et al., 2012; Martin et al., 2010; Maruszak & Thuret, 2014; Smith et al., 2007).

Similarly, another study also created interest by finding that decreased gray matter volume in parietal lobe, especially in angular gyrus and prefrontal cortex lead to MCI (Burgmans et al., 2009; Westman et al., 2012). The combination of study using CSF protein biomarker and structural MRI changes yield 91% accuracy, 85% sensitivity and 96% specificity in prediction the conversion of MCI to AD (Douaud et al., 2013; Knopman et al., 2012).

Diffusion tensor imaging (DTI) has emerged as promising structural imaging technique which uses MRI to measure non-random movement of water molecules. The micro structural changes during early stage of AD can be detected using DTI imaging. The main parameters measured in DTI imaging are Fractional Anisotropy (FA), apparent diffusion coefficient (ADC) and mean diffusivity (MD). The fibre tract integrity can be used to determine the direct assessment of white matter fibre which could be potentially used as biomarker for AD. The importance of DTI in neuroimaging is to be able to provide ROI analysis, three dimensional visualization, color mapping, fiber tracking and computerized approach for tensor calculation (Chua et al., 2008; Sundgren et al., 2004). The clinical application of DTI could be to assess the differential diagnosis of dementia (AD and vascular dementia) using the DTI based tensor maps (Alves et al., 2012). Region based DTI analysis results shows that hippocampal microstructural changes could be a better predictor of risk of progression of MCI to AD (Clerx et al., 2012).

#### 4.2 Functional biomarker of imaging

Functional MRI (fMRI) is the latest technique which measure the blood flow in specific areas of brain due to blood oxygen level dependent (BOLD). This technique has high spatial and temporal resolutions and therefore can measure oxygen concentration of certain brain areas corresponding to particular stimuli or cognitive task (Tripoliti et al., 2010; Wagner, 2000). Recent report published has

shown that AD patients fMRI has less coordinated activity in hippocampus, inferior parietal lobes and cingulate cortex compared with healthy controls (Tripoliti et al., 2010; Wang et al., 2007). Recent advances in fMRI have helped to identify the neural substrate for cognitive behavioral functions in early phases of neurodegenerative disorder and correlated them with neuroanatomical network (He et al., 2007)

### 4.3 Molecular and beta-amyloid imaging

The use of radio-tracer in imaging technology has significant impact in detection of pre-dementia stage by assessing the deposition of beta-amyloids in brain. The results of several clinical trials indicate that PET imaging of amyloid plaques can identify patients destined to develop AD several years before the development of dementia (Bolmont et al., 2008; Ellis et al., 2011).

Molecular imaging technique aims to diagnose AD in its earliest stages. This technique determines the disease changes at molecular level in the brain. The results of several clinical trials indicate that PET imaging of amyloid plaques can identify patients destined to develop AD several years before the development of dementia (Bolmont et al., 2008; Ellis et al., 2011). Recently, radiotracers for amyloid plaques have shown the presence and extent of plaques in the brain (Mosconi et al., 2008). This could allow for early detection of AD. Direct imaging of amyloid load in a brain with AD would be useful for the early diagnosis of AD and the development and assessment of new treatment (Phelps, 2000). Currently used molecular imaging compounds in AD research include the following (Hefendehl et al., 2012). Pittsburgh compound B (PIB) was the first radiotracer used by Klunk and colleagues for highlighting deposits of beta amyloid plaques (Klunk et al., 2004). It helps to visualize the pathological hallmark of AD in living individuals during a PET scan. PIB is a fluorescent analog of thioflavin T, which can be used in PET scans to image beta amyloid plaques in neuronal tissue. PIB may be used in investigational studies

of AD. Florbetapir F 18 (18F-AV-45) is a novel radiotracer. It is a novel tracer for PET imaging of  $\beta$  amyloid plaques in the brain of AD patients (Villemagne et al., 2011). 18F flutemetamol (Flute) is a radiotracer for PET scan that is structurally identical to PIB except for one fluorine atom in place of a carbon atom. Amyloid imaging consists of an injection of a radiolabelled ligand targeting amyloid aggregates and use of PET technology to acquire images of the brain in order to display foci of abnormal amyloid accumulation. This technique will possibly provide an increase in the diagnostic accuracy of AD in the near future. In future, amyloid imaging will possibly be considered as a biomarker for AD.

The use of 18F-2fluoro-2-deoxy-D-glucose (FDG) as tracer of regional cerebral metabolism studies have established its usage in early diagnosis and preclinical detection of dementia. The FDG PET measures the glucose metabolism in different brain regions and help to predict the conversion of MCI to AD (Xiong et al., 2010). Several studies have shown the rate of glucose consumptions in AD patients as compared to normal elderly (Mistur et al., 2009). It has been assumed that metabolic change associated with neocortical dysfunctions may be detectable by FDG-PET before atrophy appears (Walhovd et al., 2010). FDG-PET provides closer relation to clinical symptoms, but it is less sensitive in preclinical disease. PIB-PET imaging seems more capable to detect early changes in progression of AD than FDG-PET imaging given its correlation with measures of cognitive testing and hippocampal atrophy by MRI. In future a combination of FDG and PIB-PET technique may be more useful in predicting short-term conversion to AD (Chetelat et al., 2003)

### 4.4 Single photon emission computed tomography (SPECT)

SPECT is molecular imaging technique that evaluate brain perfusion using rotating gamma camera and it help in differential diagnosis of patient with dementia (Stuhler et al., 2011). The

combination of PET and SPECT imaging could help to make accurate diagnosis and measure the progression of changes in brain (Stuhler et al., 2011). The combination of PET with SPECT can be better for diagnosis of early stages of dementia as compared to SPECT only because SPECT is cheaper and less specific.

#### 4.5 Magnetic resonance spectroscopy (MRS)

Magnetic resonance spectroscopy (MRS) is non-invasive technique for assessing metabolic and molecular correlates of dementia. The technique can be used to tracking the disease progression from mild cognitive impairment (MCI) to AD. H1 MRS has potential role in early and differential diagnosis of dementia (Kantarci et al., 2008). Proton MRS can detect normal metabolic pattern in patient with mild neurological impairment and severe brain abnormalities. Studies have suggested the MRS of cortical and hippocampus area can help to predict the conversion of MCI to AD (Dixon et al., 2002). Similarly, the H-MRS of occipital cortex may be valuable tool in predicting conversion from MCI to probable AD (Dixon et al., 2002).

#### 4.6 Magnetic encephalography (MEG)

Magnetic encephalography is imaging technique used to measure the magnetic field pattern generated by the brain. This technique can be used to understand the relationship between brain function and cognition (Berendse et al., 2000). The studies provide ample evidences that MEG can be used as powerful technique for the diagnosis of AD in near future (Zamrini et al., 2011). Some studies have shown MEG activity shows increased slow rhythms and reduced fast activity in AD patients compared with healthy subjects (Maestu et al., 2011). The presence of low frequency in temporal parietal regions plays key role in transition of MCI to AD. Studies estimated the MEG oscillations to detect changes in subject with MCI in the earliest preclinical stages of dementia. MEG is capable of detecting alterations in the

functional organizations of the central nervous system. MCI subject has showed decreased mean frequency MEG power spectrum as compared to healthy controls and AD patients (Fernandez et al., 2006)

#### CONCLUSION

The current review has emphasized the role of several plasma/ serum/ CSF biomarker in identification of AD and MCI. But most of these plasma/serum biomarker could not qualify NIA criteria especially in relation to specificity and sensitivity. However, a panel of plasma proteins achieved a higher level of specificity (Ray et al., 2007), and combination of plasma biomarkers increased sensitivity (91%) and specificity (71%) (Ait-ghezala et al., 2008). The diagnosis of MCI and AD may only be possible by combining multiple biomarkers from different metabolic pathway which can increase the sensitivity and specificity of diagnosis of cognitive decline, and is only way in near future (Solfrizzi et al., 2006). Some of identified plasma biomarker are still in preclinical testing stage and need much more validation in large population based longitudinal studies. Further development in neuroimaging techniques has the ability to detect clinical or pathological changes overtime. Advances in structural and functional neuroimaging techniques allow detection of AD, years before the symptoms of dementia develop. A recent major advance is the development of amyloid imaging techniques that allows in vivo identification of amyloid deposition in the brain. Longitudinal structural and functional imaging studies seem currently most robust to evaluate progressive impairment in MCI and AD. Therefore in near future the combination of neuroimaging based detection of cognitive decline along with biological fluid based biomarker can be useful in detection of MCI and AD.

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## CONFLICT OF INTEREST

Authors declares there is no conflict of interest.

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