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The Potential Use of Blood, Cerebrospinal Fluid, Saliva and Urine as Biological Samples for the Diagnosis of Alzheimer's Disease

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Authors' contributions

This work was carried out in collaboration among all authors. Author AZE designed the study, wrote the protocol, managed the literature searches, edited the manuscript and supervised the work. Author AAA collected data and wrote the first draft of the manuscript. Authors AZE and AIB revised the manuscript. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Background and Aim: Alzheimer's disease (AD) is the most common cause of dementia. 80% of all dementia is due to AD. Diagnosis of AD is a difficult task, as the accurate diagnosis requires post-mortem examination of brain autopsy samples. Diagnosis of AD in living individuals can be aided by the establishment of the clinical criteria, positron emission tomography (PET) examination, and biomarkers. The study of biomarkers for diagnosis of AD could help clinicians to evaluate individuals at risk, and confirm the occurrence as well as the progression of AD in a non-invasive manner. High sensitivity and high specificity of the used markers are mandatory criteria for these biomarkers to trusted for AD diagnosis and prognosis. So, this review article aims to focus on the potential use of body fluids as a source of the biomarkers that are used for investigating patients with AD.
Methodology: In the current study, we reviewed scientific articles that discuss AD pathogenesis and diagnosis of Google Scholar database, Pubmed, Pubmed Central, Cochrane Database of

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Systematic Reviews (CDSR), MEDLINE, and MedlinePlus with no time limitation. Moreover, we discussed the use of recently discovered biomarkers that are detected in blood, CSF, saliva, and urine.

Conclusion: In the current review, it could be concluded that in addition to the blood and cerebrospinal fluid as common biological samples for the diagnosis of AD, saliva and urine are useful potential biological samples. Moreover, both are noninvasive samples that give them priority to be used.

Keywords: Alzheimer's disease; biomarkers; biological samples; blood; CSF; saliva; urine.

1. INTRODUCTION

Dementia is defined as a clinical syndrome that is characterized by the progressive fall in two or more cognitive domains (as memory, language, visuospatial function, and others). There are also predominant abnormalities in the personality and behavior. Alzheimer's disease (AD) is the most common cause of dementia (up to 80% of all dementia are due to AD) [1].

The AD-related death rate increases progressively. It accounts for 89% in the period between 2000 and 2004 as reported in 2014 by Takizawa et al., [2].

Diagnosis of AD is a difficult task, as the accurate diagnosis requires post-mortem examination of brain autopsy samples. Diagnosis of AD in living individuals can be aided by the establishment of the clinical criteria, positron emission tomography (PET) examination, and biomarkers (blood, and cerebrospinal fluid, CSF) [3].

Pathologically, Alzheimer's disease is characterized by the accumulation and deposition of amyloid-β (Aβ) peptides, the formation of intracellular neurofibrillary tangles and consequently progressive neuronal cells loss due to mixed proteinopathy (Aβ and Tau proteins) [4].

Amyloid beta (Aβ, a 39–43-amino acid peptide) is found physiologically in the healthy brain of humans as Aβ fibrils. These fibrils are the cleavage product of a larger amyloid precursor protein precursor (APP). In AD, amyloid fibrils are accumulated as amyloid plaques (senile plaques) in the extracellular spaces of brain cells. It is associated with progressive loss of synapses. This leads to synaptic dysfunction, neuronal loss, and inflammatory reactions [5].

Tau protein (total-nonphosphorylated form) is located normally in the entorhinal cortex, hippocampus, and cortical areas of the central nervous system. Tau protein plays a pivotal role in microtubule stabilization. In AD, the tau protein is subjected to extensive hyperphosphorylation, leading to clumping of tau protein. This pathology leads to the formation of intracellular neurofibrillary tangles (NFT) [6]

Microtubule disassembly is the result of intracellular formation of neurofibrillary tangles (NFT) with subsequent collapse of the dendritic spinal, and axonal degeneration. The combination between NFT and senile plaques is the initial pathological event in AD pathogenesis [7].

The study of biomarkers for diagnosis of AD could help clinicians to evaluate individuals at risk, confirm the occurrence and progression of AD in an ease-of-use noninvasive manner. High sensitivity and high specificity are mandatory criteria for these biomarkers for AD diagnosis and prognosis. So, this review article aims to focus on the potential use of body fluid as a source of the biomarkers that are used for investigating patients with AD.

2. METHODS

In this study, we reviewed published articles of Pubmed, Pubmed central, Cochrane Database of Systematic Reviews (CDSR), MEDLINE, and Medline Plus as well as Google Scholar database and World Health Organization report. There is No time limitation of the articles that are used in this review

3. REVIEW

3.1 Biomarkers of Alzheimer's Disease

Biological biomarkers are any substances, metabolites, structural components or pathways, that can be investigated inside and/or outside the body that can reflect any deviation of the body homeostasis [8].

The importance of measuring biomarkers comes from its role in reflecting different types of pathophysiology mechanism that can be used for clinical diagnosis, especially in the early stages of the disease, to predict progression, to monitor effects of novel drug candidates in clinical trials, and lastly in clinical research to deepen our understanding of the pathogenesis of the disease [9].

The use of Biomarker of diagnosis of AD is of great importance because the AD- related cognitive symptoms are usually vague and confusing with other cognitive disorders, in addition to the slow and undefined rate of disease progression [10].

Keerthikumar et al., [10] and Sheinerman et al., [11] reported certain criteria to validate the potential use of a biomarker of AD as follow; it should be able to reflect the senility changes and explain pathophysiological processes in the brain. It should be also of high sensitivity and specificity. Reproducible results over time changes with clear cut-off values at least two-fold changes are available and easy collectible results and inexpensive tests.

3.2 Pathological Classification Biomarkers of Alzheimer's Disease

3.2.1 Biomarkers for β-amyloid pathology

β-amyloid (Aβ) plaque is a hallmark of Alzheimer's disease (AD) [12]. β-amyloid (Aβ42) is the major component of senile plaques and contributes to the pathogenesis of cerebral amyloid angiopathy in AD [13]. Aβ42(a 42-amino-acid isoform of β-amyloid) is the cleavage product of type I transmembrane precursor protein called amyloid precursor protein (APP) by β- and γ-secretases in synaptic vesicles. APP is metabolized by many cell types, but its secretion is mainly of neuron origin that is dependent on the synaptic activity [14].

3.2.2 Biomarkers for tau pathology

Hyperphosphorylated and truncated tau proteins are the major components of neurofibrillary tangles in AD and other tauopathies. The stabilizing function of non-phosphorylated Tau protein of microtubules in neuronal axons is inhibited when tau becomes phosphorylated. Its role in AD pathogenesis is directed related to the degree of phosphorylation [15].

3.2.3 Biomarkers for axonal degeneration

Axonal degeneration is a prominent feature of AD pathology. It is directly linked to the onset of cognitive impairment of Aβ. The onset of this axonal neurodegeneration coincides with the onset of the Aβ pathology in AD [16].

3.2.4 Fluid biomarkers for synaptic degeneration

Synaptic pathology is one of the earliest events in the development of AD. Cerebrospinal fluid(CSF) biomarkers that are related to synaptic damage are known to appear early in the disease process. The higher CSF levels of these biomarkers; neurogranin, T-tau, P-tau (181), and Aβ (42) were detected in mild cognitive disorders (MCI) as well as AD. So, they can be considered as established AD biomarkers in the early diagnosis of AD [17]. Neurogranin is a dendritic protein expressed by the excitatory neurons in the cerebral cortex and hippocampus. It is important for synaptic plasticity and induction of long-term potentiation (LTP) in the hippocampus [18].

3.2.5 Biomarkers for glial activation

Glial cells in the brain are star-shaped cells called astrocytes. They play roles in nutrient supply,repair after CNS injury ,and the active immune defense in the CNS [19].

Glial activation occurs in response to the disordered immune cytokine activities, this coincides with the increased inflammatory activity as a part of AD pathogenesis [20].It is reported that diminished clearance of Aβ clearance is related to the integrity and proper function of triggering receptor expressed on myeloid cells 2 (TREM2) and clusterin (Apo J) [19].Many genetic pieces of evidence support the pathophysiologic role of the impaired TREM2 activation, defective innate immunity, and disorders of microglial activity in the pathogenesis of AD [21] (Yeh et al., 2017).So, it is proved that TREM2 increases in parallel with amyloid deposition, which could lead to possible Aβ plaque-associated pathology [22].

So, Nordengen et al. [23] enumerated some of the glial activation markers that have roles in AD pathogenesis and could be of diagnostic importance. These markers include TREM2 (sTREM2), clusterin, and chitinase-3-like protein 1 (YKL-40) which are markers of astroglial activation. chemokine ligand 1(CX3CL1;

fractalkine) is a marker for neuron-microglia communication. In addition to the monocyte chemoattractant protein 1, (MCP-1). It is a well-established marker for microglial mobilization and inflammatory reaction.

3.2.6 Biomarkers for TDP-43 pathology

Hyperphosphorylated TDP-43 proteinopathy is detected in approximately half of the patients with Frontotemporal dementia (FTD). It has been found as a pathophysiologic mechanism in aging as well as cognitive impairment. Its level is directly related to tau and Aβ pathology [24]. This could prove its role in AD pathogenesis.

3.2.7 Biomarkers for α-synuclein pathology

Misfolding and aggregation of α-synuclein is the principle of formation of inclusions called Lewy bodies. Lewy bodies are characteristic pathologic findings in neurodegenerative diseases especially AD [25] as well as Parkinson's disease (PD) [26].

4. BLOOD BIOMARKERS OF AD

4.1 Markers Related to APP and Aβ Metabolism

4.1.1 Amyloid β-protein

The total concentration of Aβ and Aβ 42 increased in plasma of patients with familial AD regardless the cause as in APP, PSEN1, and PSEN2 mutations [27]. Moreover, a detectable change in plasma Aβ in patients with nonfamilial type is also documented in spite of that it is not of diagnostic value [28].

The clinical importance of Aβ measurement could be of prediction of AD, disease progression, and monitoring of the successfulness of therapy. The therapeutic success is verified by a significant reduction of its plasma level with treatment. This also is of value as it asserts the mechanism of action of the used drug (the drugs that inhibit β and γ secretases enzymes [28].

The increased Aβ level in plasma could be explained also by an imbalance between its production and the rate of its degradation which mostly observed in elderly and consequently participate in AD pathogenesis [29]. But unfortunately, plasma Aβ lacks the sensitivity and specificity as a biomarker for diagnosis of AD [30].

4.1.2 Brain to plasma Aβ flux

The measurement of brain-to-plasma efflux of Aβ can be used as an indicator of the degree and severity of β amyloid deposition in the brain of patients with AD, even in the early onset non-symptomatic individuals, but this finding is still under debates. Aβ peptide is produced centrally by CNS neuron and also by the peripheral tissue. In AD, several mutations lead to over production of central Aβ42, then induction of aggregation and plaque formation is the early pathogenic mechanism of early onset AD [31]. The imbalance between Aβ production and clearance from CNS leads to amyloid pathology and development of AD. The clearance is mediated by many mechanisms that include transport out to CSF, reabsorption to venous blood and direct transport to the venous blood though the blood brain barrier(BBB) [32].

So, it is concluded that Aβ concentration in the CSF correlated directly with the concentration in the interstitial fluid (ISF) of the brain and consequently used as a biomarker of AD [33], then transport from the CSF to the venous blood is a complementary mechanism for clearance of CNS-derived Aβ as demonstrated in an animal model of AD, in the human study it is not confirmed yet. So, the use of brain-to-plasma Aβ efflux could be a peripheral an indicator biomarker of AD but needs more study to confirm [30].

4.1.3 Aβ autoantibodies

It is postulated that individuals with a considerable level of Aβ autoantibodies are less vulnerable to develop AD. This could be explained and evidenced by the result of a study done on APP transgenic mice in which immunization against Aβ42 led to reduced amyloid deposition in the cerebrum and hippocampus [34].

Moreover, it is documented in human study that presence of autoantibodies against Aβ is associated with decreased deposition of amyloid plaques in cerebral tissues (Hock et al., 2003). The development of immunity is monitored by presence of plasma Aβ titers while those with AD have very low or even absent titer. At the biochemical level, it is also can be

used as biomarker of the effectiveness of therapy [30].

4.2 Markers Related to Cholesterol Metabolism and Vascular Disease

High plasma total cholesterol level is reported to be associated with a high risk of developing AD and other forms of cognitive impairment in the elderly [35]. The role of hypercholesterolemia in AD pathogenesis is evidenced by that statins drug medication decreased the risk of development of elderly associated dementia including AD [36], so measurement of plasma cholesterol level could be considered as an indirect biomarker for AD susceptibility rather than the diagnosis. Similarly, Lipoprotein Lp(a) is associated with various forms of cerebrovascular diseases and other forms of cognitive impairment in the elderly [37]. The association of hyperhomocysteinemia with AD pathogenesis could be explained by the same mechanism that postulated its pathogenic role based on its role in generalized atherosclerosis pathogenesis and its sequelae including AD [38]. The apolipoprotein E (apoE) phenotype of patients with APOE ε4 allele genotype is of higher risk to develop AD than non-APOE ε4 allele carriers. So, Plasma measurement and its correlation to its genotype is a biomarker of risk factor assessment for AD [30].

Plasma 24S-hydroxycholesterol is a biomarker for cerebral cholesterol metabolism specifically than the total plasma cholesterol.Its plasma level is an indicator of the metabolic balance of its cerebral production and hepatic clearance [39]. AD patients usually showed a high 24S-hydroxycholesterolin CSF and plasma which diminished by the use of nystatin preparation. SO it is considered as a prognostic risk factor biomarker rather than diagnostic biomarker [40].

4.3 Markers Related to Oxidative Stress

Oxidative stress resulted from an imbalance between oxidant production and the antagonizing scavenging system plays an important proved role in the pathogenesis of neurode generation and AD [41]. The mechanism involved the peroxidation of cerebral protein, lipids, and nucleic acids with marked deficient brain antioxidant enzyme activity [42].

Peroxidation of cerebral proteins and polyunsaturated fatty acid resulted in production of soluble biomarkers that could be used as indicators of AD development [43].Isoprostanes is a peroxidation products that elevated in the brain in AD and appear in CSF and plasma [44]. Measurement of plasma antioxidant level is also of importance.these antioxidants include carotene, lycopene, vitamin A, vitamin C, vitamin E, urate, and bilirubin [45]. Deficient plasma levels of these antioxidants is remarkable sign for AD and other neurodegenerative diseases [46].

4.4 Markers Related to Inflammation

Inflammation plays a very important role in the pathogenesis of AD, so evaluation of soluble serum, plasma, and CSF markers of inflammation may help in the prediction or diagnosis of AD and mild cognitive impairment as well as disease progression [47].

Inflammation induces AD of inflammation is a result of AD hidden pathology is a controversy as Amyloid beta (Aβ) and amyloid precursor protein (APP) may have a role in induction of biosynthesis and secretion of cytokine and chemokine by microglia, astrocytes, and neurons. On the other hand, these chemokine and cytokines could induce the gene expression of amyloid beta, facilitate its deposition and vice versa [48].

These inflammatory biomarkers include interferon γ, interleukins, tumor necrosis factor (TNF)-α, eotaxin-1, macrophage inflammatory protein (MIP)-1β, macrophage-derived chemokine, and MCP-4 [49].

The importance of gut microbiota composition in AD pathogenesis is recently discussed in the mediation of AD-related neurode generation. Scientists suggested that brain neuroinflammation is linked to systemic inflammation with an evident role of the brain-gut axis in AD pathogenesis [50,51].

4.5 Metabolomics

Metabolomics includes several methodologies of assessment including untargeted metabolomics, targeted metabolomics, lipidomics, and fluxomics [52]. Untargeted metabolomics study aims to measure the metabolites that have a metabolic identity, related to a specific disease state and help to identify phenotype analysis. The non-targeted approach provides an idea about the relative changes in metabolites where these metabolic pathways are not fully understood. While, targeted metabolomics provides the tools

of measurements of the metabolites of a particular pathway (e.g., glycolysis or TCA cycle) in a quantitative manner. Lipidomics is a term that is used to estimate changes in lipid profiles and analysis of hydrophobic/lipophilic metabolites. Fluxomics is an approache that is used to assess the rates of metabolic reactions within a biological system (*in vivo*) so, it is used to determine the metabolic fluxes in living cells. cells, tissue, and biofluids are media that can be used for omics study [53].

Metabolites are small molecules of less than 1,500 Da in molecular weight. They are implicated in the major cellular biological functions. There are nearly 150,000 or more metabolites. The Human Metabolome Database reported more than 100,000 metabolites until 2017 [54].

The most commonly used analytical methodologies that are mostly used for characterization and quantification of metabolites are Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy [53].

Early metabolic disorders are associated with reduced glucose utilization are detected in patients with MCI, and AD. This indicates a state of brain hypometabolism. This occurs approximately 20 years prior to the definite manifestation of AD. Brain hypometabolism proves that metabolic dysfunction is a contributing factor in the pathogenesis of AD [55].

Disturbed energy homeostasis is an evident feature in patients with AD [56]. Many metabolic alterations including neurotransmission and inflammation were detected in both CSF and plasma of patients with AD. Metabolomics study has the ability to enable monitoring of these cellular metabolic changes, so its application in AD research became of importance [53].

The integrated metabolomics approaches are not only for the diagnosis of AD but also, could help to understand the pathophysiologic underlying mechanism. It is found in animal studies that abnormalities of membrane phosphatidylcholines and sphingomyelins are characteristic events in MCD and early-onset AD pathology. They are associated with an abnormality in CSF Aβ42 levels. Moreover, tau-pathy in long-chain acylcarnitines and sphingomyelins are implicated in lipid metabolism disorders and involved in the neurodegeneration that starts early in AD. This AD-associated shift in energy metabolites could explain the metabolic evident alterations that occur in the late stage of AD pathogenesis [57].

5. CSF BIOMARKERS OF AD

Apart from brain biopsy, CSF is considered the most specific and accurate biological sample for diagnosis and monitoring of the cerebral condition.CSF is in direct contact with the cerebral interstitial space of brain tissue. So any cerebral change is reflected in CSF at the biochemical, molecular, cellular, and microbiology level [58].

CSF markers of amyloid and tau pathology are the major protein constituents of the pathology of AD [59]. CSF levels of Aβ1-42, t-Tau, p-Tau, and the combined ratios are considered the best-studied CSF biomarkers. Aβ1-42 is the marker of amyloid deposition, and Tau biomarkers either total or phosphorylated are the biomarkers of neuronal injury [60].

5.1 Amyloid Pathology-Related CSF Biomarkers

Aβ is the proteolytic product of the amyloid precursor protein (APP). The expression level of APP could be of value as a diagnostic marker of AD but the exact contribution of CSF-APP is under debate [61]. APP is expressed in the brain and other body tissues. Then it is cleavaged by either α-secretase or β-secretase to produce sAPP-α or sAPP-β, respectively. The modification of APP by α-secretase occurs in non-amyloidogenic tissue, thus the CSF level of sAPPα is decreased in AD patients. However, cleavage of APP by β-secretase with subsequent degradation by γ-secretase leads to the production of Aβ (38–43 residues) peptides [62].

Aβ42 is the 42-residue-long Aβ isoform. It is hydrophobic and aggregates as extracellular plaques [4] and represents the prevailing component of the AD plaques [63]. So, a decrease of CSF Aβ42 in AD patients is reported [64]. The deposition of Aβ in plaques could explain the decreased CSF-Aβ42 levels in AD, a phenomenon called amyloid sinks [63]. It is reported that CSF Aβ42 is considered a reliable biomarker for diagnosis, prognosis, and plaque extent especially in preclinical AD and mild cognitive impairment. Surprisingly, CSF Aβ40 shows no change in its level in AD patients [64].

A decreased Aβ42/Aβ40 ratio is of importance in the diagnosis of AD than the reported reduction of CSF Aβ42 alone. Moreover, other forms of APP cleavage products as Aβ37, Aβ38, and Aβ39 are found in CSF of AD patients. For example, an increased CSF Aβ38 levels is detected in a combination with a decrease in CSF Aβ42 levels in AD [65]. Moreover, a number of short truncated A*β* isoforms (A*β*14, A*β*15, and A*β*16) are found in the CSF of AD patients which are produced from APP by an alternative *β* and *α* secretase actions [66].

5.2 Tau Pathology-Related CSF Biomarkers

Tau protein is an intracellular protein, that provides the stability of neuronal microtubules with a low concentration of tau is in CSF. However, an increased CSF level of tau and hyper-phosphorylated tau is reported in AD patients and correlates with the onset and degree of neurodegeneration in AD [63].

Although the CSF total tau (t-tau) estimation is considered a very sensitive biomarker for AD diagnosis, it has limited ability to distinguish AD from other major causes of dementia. It is reported that CSF t-tau increased in vascular dementia (VAD) and frontotemporal dementia (FTD) [67].

CSF t-tau level depends on the degree of neuronal degeneration and consequently, it gives an idea about its intensity. The highest CSF level is recorded in severe neurodegenerative disorders like Creutzfeldt-Jakob disease (CJD), the moderate increased level is present in AD while normal CSF level is found in patients with depression where there is a minimal degree of neurodegeneration [68].

The CSF Phosphorylated-tau (p-tau) is a potential biomarker for AD diagnosis as it is the master component of neurofibrillary tangles (NFT), a hallmark of DA pathology. CSF concentrations of p-tau in AD have been detected with the other distinct epitopes of p-tau such as (Thr181 + Thr231), (Thr231 + Ser235), Ser199, Thr231, (Ser396 + Ser404), and Thr181 [69].

CSF P-tau is more specific than CSF t-tau in the diagnosis and monitoring of AD. CSF P-tau could differentiate the neurodegeneration of AD from other forms of neurodegeneration. Its level gives an approximately accurate idea about the degree of tau phosphorylation. Other forms of neurodegeneration disorders are associated with a change in its level or minimal change [70]. For example, no change in CSF P-tau after acute stroke is reported in spite of the marked increase in T-tau and nearly similar finding regarding Creutzfeldt-Jakob disease (CJD) [71].

Interestingly, it is documented that the combination between markers of tau and amyloid pathology markers has a better diagnostic value than measuring each of them alone as follow; The sensitivity and specificity for the combination of CSF T-tau and Ab42 (89% and 90%, respectively), for T-tau (81% and 91%, respectively) and for Ab42 (86% and 89%, respectively) alone. This reflects the priority of the use of combined CSF biomarkers rather than each one alone [72].

5.3 Other Novel CSF Protein of Potential Diagnostic Importance of AD

In addition to the amyloid plaque and neurofibrillary tangles formation as the main pathophysiologic events in AD pathogenesis, there are other mechanisms are involved such as gliosis, neuronal loss, synaptic disorders, and neuroinflammation. All of these mechanisms could be presented in CSF by the release of mediators or metabolites,so, many molecules can be detected in CSF and become of diagnostic or prognostic importance of AD [73].

5.3.1 CSF biomarkers of APP processing

5.3.1.1 sAPP-β& sAPP-α

The primary constituent of amyloid plaques is Aβ peptide. Itis generated by cleavage of APP by two secretases (β and γ) enzymes. β-secretase enzymes as BACE1 produces APP protein product that is termed sAPP-β. sAPP-β is released in a soluble form the brain cells to the interstitial fluid and then into the CSF. α-secretase enzyme is another type of secretases that cleaves APP producing another peptide called sAPP-α [74]. CSF levels of sAPP-β and sAPP-α are of low diagnostic value as it mildly elevated only in the sporadic type of AD and MCI [73].

5.3.1.2 SorLA/sLR11

SorLA/sLR11 are sorting protein-related receptor with A-type repeats (SorLA, SorL1 or LR11). This type of protein is embroiled in AD. It acts as a

neuronal receptor for APP and controls its intra-cellular transport and processing (*Schmidt* et al., 2007). It is reported that SorLA is downregulated in the brain cells of AD patients [75]. The soluble forms of SorLA (sLR11) are released in CSF, and its CSF level decreased in AD patients [76].

5.3.1.3 Neuroserpin

Neuroserpin (NS) is a serine protease inhibitor. Neuroserpin involved in Aβ metabolism, and implicated in other pathophysiologic pathways as neuroinflammation, so it may have an impact AD pathogenesis in addition to its role on Aβ metabolism. Moreover, NS is associated with amyloid plaques in the AD brain [77]. It is reported that its CSF levels are higher in AD in comparison to age-matched controls. So, NS is considered an important CSF candidate biomarker for AD diagnosis [78].

5.3.1.4 Cystatin C

AD-related changes in CSF cystatin C is under debate, but it is documented in a large study that cystatin C is a complementary to the tau:Aβ42 ratio in differentiating AD from normal age matched controls [79]. Moreover, the measurement of C-terminally truncated form of cystatin C is found to be increased in AD CSF [80]. Consequently, it could be a CSF candidate for AD prediction and diagnosis.

5.3.2 Biomarkers of Synapse loss/neurodegeneration

Many proposed CSF indicators of synapse loss and neuronal injury/degeneration have arisen as CSF candidates for AD diagnosis. These biomarkers include Calbindin, Calsyntenin 1, N-cadherin/cadherin-2, Neurogranin, Secretogranin (I, II & III), and Chromogranin(A& B) [73]. Moreover, levels of synaptic adhesion molecules such as NrCAM, NCAM-120, neuronal pentraxin receptor, N-cadherin and nectin-like molecule-1/TSSLL-1/SynCam3 are reported to be changed in AD [81].

5.3.3 Biomarkers of neuroinflammation

The molecules of neuroinflammation are considered as important CSF candidate biomarkers that are involved in AD diagnosis and/or follow up. These biomarkers included cytokines, chemokines, complement proteins, proteases, protease substrates [79].

High CSF levels of these neuroinflammatory biomarkers are directly correlated with the degree of cortical thinning in AD. Moreover, higher CSF levels of YKL-40, ICAM-1, VCAM-1, and IL-15 are directedly associated with the rapid progression of MCI to AD [82].

Correlating the biomarkers of neuroinflammation with Aβ status is discussed by Janelidze et al., [83]. They reported a negative association between the higher levels of YKL-40, ICAM-I, VCAM-1, IL-15, and Flt-1 with Aβ status in MCI and AD.

6. POTENTIAL BIOLOGICAL SAMPLES FOR THE DIAGNOSIS OF AD

6.1 Saliva

The potential use of salivary metabolites and biomarkers for the prediction, diagnosis or follow up of AD is a new research area that needs more work to establish and verify its sensitivity and specificity [84].

In a review published in 2018 by Hartmann & Ledur Kist [85], the possible relation between the biochemical components of saliva and AD had been discussed. Moreover, in 2019 another systematic review article was published by Gleerup et al., [84].

6.1.1 Salivary estimation of Aβ40 and Aβ42

It is reported that the salivary level of Aβ42 is higher AD patients especially in early stages with non-recorded increased level in the healthy individuals or even other forms of neurodegenerative disorders as Parkinsonian Disease(PD). This finding proved its potential use as a biomarker for AD with higher specificity. Measurement of salivary levels of Aβ40 gave no similar result [86].

6.1.2 Salivary Tau proteins

Salivary levels tau proteins were detected using mass spectrometry with no difference between AD patients and individuals with no neurodegenerative disease. However, the saliva level of the phosphorylated form (p-tau) is much higher in saliva of AD patients than age-matched normal controls [87].

6.1.3 Saliva metabolomics

It is proved that metabolomics analysis of saliva from AD patient is different from age matched

healthy individuals. The documented metabolomics that showed a significant increase in AD saliva samples are sphinganine-1-phosphate, ornithine and phenyllactic acid while inosine, 3-dehydrocarnitine and hypoxanthine showed a significant decrease in comparison to the healthy individuals. The method used for metabolomics analysis is fast ultra-HPLC coupled with TOF-MS [88].

6.1.4 Saliva cortisol

It was found that the cortisol level in the morning and evening salivary samples of AD patients increased than their corresponding controls. These levels are correlated with Mini-Mental State Examination and Global Deterioration Score and negative correlation is the results which prove its potential use as a biomarker for AD prediction and/or diagnosis [89].

6.1.5 Other potential salivary biomarkers

Lactoferrin and trehalose were investigated in a previous study, a decreased level of lactoferrin was detected in MCI and AD patients than healthy controls [90] while increased saliva level of trehalose is the finding of Lau et al., [91] in their study.

Regarding some selected salivary metabolites, increased levels of propionate, spinganine-1-phosphate, ornithine, and phenyllactic acid and decreased levels of inosine, 3-dehydrocarnithine, and hypoxanthine were reported by Liang et al., [92]. A statistically significant difference between AD patients and the healthy control group in methylguanosine, histidylphenylalanine, choline-cytidine, phenylalanylproline, phenylalanylphenylalanine, and urocanic acid were documented but need more investigation to confirm.Moreover, a statistically significant difference between AD patients and MCI patients in the metabolites: amino-dihydroxybenzene, glucosyl- galactosyl -hydroxylysine-H2O, aminobytyric acid + H2, alanylphenylalanine, and phenylalanylproline was also reported by Huan et al., [93] and presented in the systemic review of Gleerup et al., [84].

6.2 Platelets

Based on the fact that platelets are considered one the best biological sample for the investigation of metabolic pathways that are implicated in the pathogenesis of AD, especially the amyloid cascade and the oxidative stress-related pathways [94]. So, platelets are an important source of circulating forms of the APP, Tau and glycogen synthase kinase-3B, all of these molecules have potential diagnostic value in AD. Moreover, platelets express phospholipase A2, biomarkers of the inflammation, and oxidative stress well as it contains the APP-cleaving enzymes [94,95]. These platelets specific properties gave the priority to be a useful biological sample to understand the pathological changes that occur in the brain of AD patients.

6.3 Urine

The analysis of urine could provide a good diagnostic tool for prediction and diagnosis of AD as well as could be used to differentiate AD from MCI [96]. Many urinary biomarkers are investigated as follow:

AD-associated neuronal thread protein(AD7c-NTP), AD patients exhibited a higher urinary level of AD7c-NTP that is directly related to the degree of cognitive impairment and dementia. So AD7c-NTP could be used as a screening metabolite in the elderly at high risk of AD and MCI as well [97].

Oxidative stress plays a role in the pathogenesis of AD either as a cause of an effect. Several oxidative stress-related urinary metabolites have been identified as 8-hydroxy-2′-deoxyguanosine (8-OHdG) and paraoxonase 1 (PON1) as AD urine examination showed a significantly higher level of 8-OHdG and a lower level of the antioxidant enzyme PON1 in AD patients [98].

Isoprostanes, the peroxidation product of arachidonic acid, the urinary level of F2-isoprostanes is significantly higher in AD subjects than their matched healthy volunteers while, 3-hydroxypropyl mercapturic acid/creatinine reduction is an ideal urinary biomarker that succeeded to differentiate patients with AD from those with MCI [96].

Some urinary biomarkers have prognostic value as it can differentiate between early-onset DA and MCI from late-stage AD. These urinary metabolites include homogentisic acid, tyrosine amino acid, 3-Hydroxykynurenine which appear in the urine of patients very early even before the start of clinically detected dementia. While, trimethylamine, urea, trigonelline, and oxoglutarate are urinary metabolites that are predominant in late stage-AD [99].

7. CONCLUSION

In the current review, it could be concluded that in addition to the blood and cerebrospinal fluid as common biological samples for the diagnosis of AD, saliva and urine are useful potential biological samples. Moreover, both are noninvasive samples that give them priority to be used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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