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Prevalence, distribution, treatment, and modern methods for *in vitro* diagnosis of Alzheimer's disease in India: Challenges and future prospective

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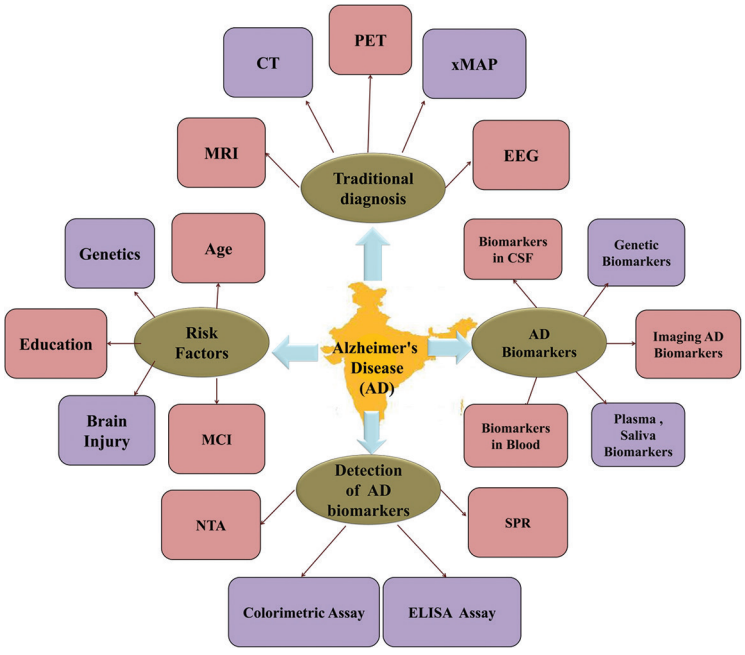
ABSTRACT

In India, the plethora of evidence indicates that neurodegenerative conditions are a significant public health issue wherein over 4 million Indian peoples have been affected. For the past two decades, the prevalence of Alzheimer's disease (AD) is growing rapidly in India that may due to the significant lack of health-care services and poor knowledge of AD and another form of dementia. Therefore, this in turn to develop ultramodern techniques for the efficient detection of AD biomarkers that helps to the prognosis and diagnosis of AD. Recently, significant progress has been observed in the area of AD that includes prognosis, and diagnosis of AD. This review article discussed different risk factors associated with AD, data on the dissemination of AD in India according to different virtues and socio-economic categories. The different standard diagnostic techniques commonly used for the identification of AD biomarkers are mentioned. This review also focuses on the new techniques established by Indian researchers such as surface plasmon resonance centered biosensors, and fluorescence-based probes that offer the enormous potential of highly sensitive and selective detection AD biomarkers. In conclusion, the present review article is providing a short overview of AD prevalence and AD-centered research in India.

Keywords: Alzheimer's disease, biomarkers, biosensing, *in vitro* diagnosis, India



Graphical Abstract: Alzheimer's disease friendly India: Prevalence, distribution, treatment, and modern methods for *in vitro* diagnosis of AD

INTRODUCTION TO ALZHEIMER DISEASE

Background of Alzheimer's Disease (AD)

Neurodegenerative disorders are a kind of condition that involving neuronal degeneration in some circumstances.[1] Normally, this disease category affects normal behaviors connected with a healthy neuron system, including movement, memory, respiration, and speech. In this background, AD is an acknowledged example of neurodegenerative diseases.[2] Particularly, AD is one of the widely well-known forms of dementia observed in people aged 65 years and older. Herein, people's mental capacity gets declines gradually and it becomes too challenging for them to maintain an ordinary life.[3] At the last stage, patients become more reliant for survival on their immediate family members as the AD continues to increase.[3] As per published data, Dr. Emil Kraepelin was introduced a new phrase, namely, AD in 1910. In this shadow, 1st time (in 1906) Dr. Aloysius Alzheimer (the German neuropathologist) were identified the pathology following his extensive observations on brain anomalies with his patient Auguste Deter.[4] There are plenty of symptoms of AD that includes forgetfulness, difficulty in concentration, language difficulties, issues related to planning and problem solving, difficulty in performing previously familiar tasks, problems in social behavior, and complexity in spatial relationships.[5] Notably, it is categorized into various stages based on its development in patients. In brief, the first stage of AD includes pre-dementia/mild cognitive impairment (MCI)/mild neurocognitive disorder owing to AD. Principally, it is distinguished by a decline in cognition, which involves offsetting interventions and steps to retain autonomy and to maintain normal tasks. The second stage of AD is mild Alzheimer's dementia or major mild neurocognitive disorder due to AD-mild that shows symptoms, which slightly affect daily activity so that patients have to be supervised on challenging operations such as financial management, etc. The third stage of AD is called moderate Alzheimer's dementia or major neurocognitive disorder due to the AD moderate. Mainly, it is associated with symptoms that moderately influence everyday activities such that AD patients need the help of a third person for those activities. The last stage of AD is called severe Alzheimer's dementia that commonly known as a major neurocognitive disorder due to AD (a severe case). In particular, the symptoms are characterized by such a weakness that the everyday life of patients is entirely reliant on another person for their essential requirements. In serious dementia, AD patients cannot able to communicate and walk. Besides, AD patients can require a special caregiver for prerequisites of the patient including food, and taking a shower. In addition, naming of respective items or finding the right words for particulars are very challenging to communicate in the case of AD patients.[6]

Diagnosis of AD

The published literature mentioned that AD is linked with a brain that covers brain cell death, brain shrinkage, and accumulation of abnormal protein such as tau protein and beta-amyloid (Aβ).[2] Unfortunately, notwithstanding huge advancements in the science and technology field, the researchers have not recognized the precise origin of AD yet. For this context, multifactorial etiology is supposed to be a genetic factor, environmental factor, and lifestyle factor. Concisely, the recent most interesting research area in AD is to identify the respective genetic factor that responsible for AD development. Recently published AD-related literature divulged the role of the gene namely Apolipoprotein E (ApoE) in AD development. It has been reported that the ApoE gene appears in various forms. Out of that, if only one or more than one form of ApoE-4 appears in the test, then it lifts the probability of an individual being affected by AD.[2] Furthermore, other imperative factors such as obesity, diabetes, stroke, heart disease, hyperlipidemia, and high blood pressure are associated with AD. It is worthy to mention that the accurate and early recognition of disease/disorder biomarkers is a decisive step in the biomedical meadow for prognosis and diagnosis. Interestingly, this could help to inform a respective person regarding signs that are attributed to AD or other health causes such as stroke, sleeping disruption, Parkinson's disease, tumor, and side effects of medicines. In addition, it also assists their families for their better future, quality livelihoods, and supportive relationships. In this vein, early diagnosis of AD gives additional chances to the patients to engage in clinical trials. Initially, AD diagnosis is possible only after the death of the patient. In this line, only expert doctors can able diagnose AD using several related facts that include past medical history along with the existing health condition of the AD patient. Notably, the significant change in the patient's ordinary actions and character often leads to AD diagnosis. In addition, the assessment of AD is often supported by performing cognitive tests involving memory, analytical thinking, and language.[6] Nowadays, regular diagnostic tests such as blood/urine tests of a person are administered to rule out other factors. In addition, brain examinations and computed tomography (CT)/magnetic resonance imaging (MRI) scans are commonly used for AD diagnosis in India.[2]

Treatment of AD

At present, medical treatment, psychosocial treatment, and proper care are used for the effective management of AD in patients. Notably, the medical treatment for AD includes cholinesterase inhibitors (e.g., donepezil, rivastigmine, and galantamine) and N-methyl-D-aspartate receptor (NMDA) receptor blocker (e.g., memantine). Herein, the different cholinesterase inhibitors are preferred for the management of mild to moderate AD whereas the NMDA receptor blockers are used for the management of moderate to severe AD. Despite huge advancements in science and technology, AD management is still challenging to researchers. Although AD can be accompanied by symptomatic improvement, still there is a need to investigate the total cure for AD.[5] Out of different treatments, psychosocial management in AD is used as a combination of medication management that can be categorized as a supportive approach, cognitive approach, and behavioral approach. Finally, yet importantly, the proper care of AD patients is playing an imperative role in the effective management of AD. As patients with AD have no care, definitely they can increasingly stop themselves from being cared from AD. In conclusion, medication and proper care are necessary and the patient must be carefully monitored throughout the AD.[6] Lately, different standard clinical procedures

are routine for the effective diagnosis of AD. It covers the physical examination, spectroscopic detection, radiologic imaging, etc. It confirmed that the earlier diagnosis of AD is a crucial factor that can avoid the physical, psychological, and financial losses of a person. Fascinatingly, the clear-cut early diagnosis of AD can guide to prompt and appropriate care of the patient, which helps to increase the survival rate of the AD patient. Recently, in contrast to existing traditional diagnostic methodologies, several innovative methods have been implemented with magnificent advances in science and technology for AD diagnosis.[2] The analysis of AD patients also highlights numerous commonly used AD detection diagnostic strategies and those are simplistic, speedy, and painless in the development process. The existing AD diagnosis paradigms in India along with the latest statistics have been mentioned in brief. Besides, Indian researchers' contributions in terms of research and development efforts to detect AD are also outlined here. We have discussed some important observations made in the year 2011 Population Census. Besides, 2001–2026 population projections for India along with different states of India have been overviewed in brief. Therefore, this review article will assist Indians investigators as well as other researchers working on the diagnosis and management of AD. In addition, it will help to focus on the current health issues in India for future research work.

ALZHEIMER DISEASE FRIENDLY INDIA

Prevalence of AD in India

Surprisingly, it has been estimated that more than 4 million peoples in India have suffered from dementia from different studies.[7] Furthermore, India is expected to have about 7.5 million dementia and AD burden by the end of 2030.[8] According to the study "Dementia India" released by the Indian Alzheimer's and Related Disorders Society (ARDSI), India is the number second largest in the world that projected to be 4.1 million people who experience dementia. As per the particulars, India's near about 104 million populations are elder (<60 years), wherein 53 million females and 51 million males. It has been mentioned that over 73 million elderly peoples (71%) of India are living in rural areas. Notably, the proportion of elderly peoples is rose from 1961 to 2011. In the case of rural areas, it was 5.8–8.8% whereas it was 4.7–8.1% in urban areas. As of 2019, India had 1.38 billion people wherein the elderly population was 140 million.[7] In this streak, it is reported that about 70% of the global population aged 60 and over will be living in developed nations by 2020, whereas 14.2% aged 60 population will be present in India only.[9] In 2011, 8.6% of the total population was over 60-years-old. This share is anticipated to rise in the coming decades due to declining population growth rates.[10] Globally, at least 44 million patients are presently living with dementia, whereas greater than 4 million Indian peoples are suffering from different types of dementia.[11] As we know, the burden of geriatric diseases will begin to rise with the aging of the population. In the future, India may be the least equipped to cope with the burden of degenerative diseases such as dementia and other geriatric conditions (like memory loss).[10,11] This is because there is insufficient knowledge of geriatric disease experts.[10] Remarkably, AD is an extremely frequent type of dementia distinguished by the gradual degeneration of cognitive ability.[12] In its initial stages, its symptoms result from clumsiness to loss of speech and lack of mobility in its later phases.[2] Furthermore, AD distinguishes it from other geriatric disorders because its early signs are frequently mistaken with those of old age and its onset is often ignored.[10] As per the ARDSI dementia India study 2010, there have been approximately 3.7 million Indians with dementia in 2010, with the figure expected to increase to 7.6 million by 2030. In addition, the overall costs of AD patient care would increase 3 folds.[13]

Based on the statistics provided by Statista Research Department (October 16, 2020), the number of cases of AD in senior citizens across India forecasted to be about 4.6 million in 2050. Figure 1 statistic represents the number of cases of AD in senior citizens across India from 2011 to 2050.[14] Further, the relative change in the prevalence of AD is nearly constant with dementia. Therefore, the AD prevalence and dementia prevalence were found exponential in India from 1990 to 2010. The relative change in the prevalence rate of AD in India is depicted in Figure 2. In conclusion, a detailed analysis of the recent prevalence of AD and other dementia plus burden from all over India is majorly required.[15]

Distribution of AD in India

In the scenario of India, it is reported that the age-adjusted prevalence of AD in peopled from Southern India (Kerala) was found to be 1.91% whereas dementia including an AD was found to be 4.86%. It is an important element of cognition in aged (older) adults in the Trivandrum (COAT) study.[16] By 2050, this could two-fold from 7.6 million (2030) to 14.3 million.[9] By 2026, more than 5,00,000 AD patients will be housed by Maharashtra and Uttar Pradesh alone[17] Likewise, dementia care facilities are expected to grow to 0.5% of GDP.[9] On behalf of India, five of every 100 elderly people are at a higher chance of developing the disease and many more of them have dementia. Since 1992, ARDSI is working to guarantee support and treatment for all people who have dementia and other dementia-related issues. Recently, ARDSI is established in 24 cities in India and

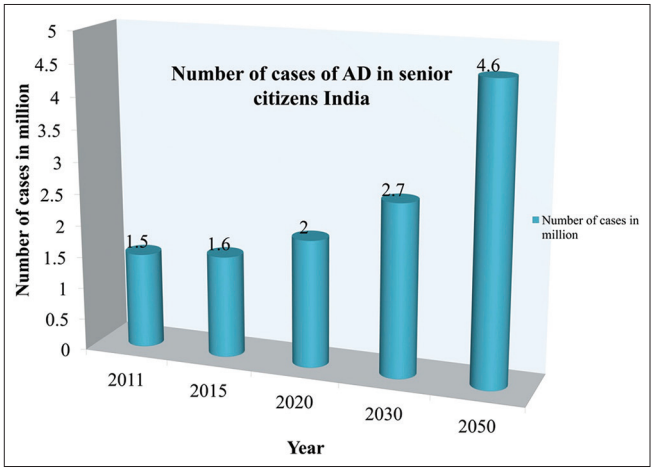


Figure 1: Number of cases of AD in senior citizens across India (from 2011 to 2050)

offering specialist services to dementia patients.[18] Plenty of studies are available about the awareness of AD and types of dementia in India. Yet there is a lack of essential details about AD and dementia in the common peoples of India. As per published literature, in India, 92% of students did not know of a condition called Dementia. Only, 3.8% of students had general disease knowledge and 3.8% of students had a detailed understanding than their basic knowledge.[7] As we know, the elderly population of India has risen from 70 million to 104 million between 2001 and 2011 (Census estimates).[10] Moreover, about 17% of the world's population are living in India only, which just occupies 2.4% of the world's land region. In this line, the progressive demographic transition is resulting in a transformation in the population's age structure, with people aged 60-years-old or older reflecting 7.5% of its 1 billion-plus population.[19] In 1998, in Ballabgarh (Northern India), Chandra *et al*. performed the study to assess the prevalence of AD and other dementias of rural Hindi elderly peoples. Notably, they have reported that the overall AD prevalence rate 0.62% (95% CI, 0.43–0.88) for a population aged 55 years. Furthermore, it was 1.07% (95% CI, 0.72–1.53) for a population with aged +65 years. It is worth revealing that, the incidence rate of AD and other dementias in this population was low. Principally, it has been increased with age and was not linked with gender or literacy. Possible explications include low average life expectancy, short disease survival, and low age-specific incidence. Likely due to variations in the underlying risk and defensive factors distribution relative to higher-prevalence populations.[20] Shaji *et al*. performed the door-to-door survey (city of Kochin). They have investigated the prevalence of AD and other types of dementia in an urban population in Kerala. For this study, they were selected the residents aged ≥65 years by cluster sampling. It has been reported that AD was the most commonly observed (54%) dementia than vascular dementia (39%) in Kerala peoples. In addition, they claimed that the family history of the patient was the main risk factor for AD.[21] In India, especially in rural and underdeveloped regions, the general knowledge of AD is relatively poor and even lower. There is an immediate need to raise the consciousness of dementia and initial AD symptoms.[10] The literature survey disclosed that the AD prevalence rate is higher in west India as compared to the other regions of India. Figure 3 depicted the prevalence of AD and dementia in Southern India.[22]

In 2012, Mathuranath *et al.* reported that the overall incidence rates of AD as well as age-specific incidence rates of AD in a southern region of India (Kerala). In brief, among the 1066 qualified participants who were cognitively stable at baseline, over a follow-up span of 8.1 years, only 104 participants developed dementia (98 with AD). Therefore, the prevalence of AD per 1000 persons was found to be 11.67 (95% CI: 10.9–12.4), which for ≥55 years aged person. Whereas, for ≥65 years aged person, it was 15.54 (95% CI: 14.6–16.5). Overall, it concludes that the AD incidence rate is increased with the proportion of age in the southern region of India. Moreover, incidence rates tend to be much higher than those reported in rural northern India, comparable to those reported in China, and slightly lower than those are reported in the western world.[16]

Management and Economic Effect of AD in India

Family members and doctors are better positioned to identify these early signs of AD. Thus, it is promising that a public awareness campaign is directing at them to have the most impact.[10,23] Unfortunately, research on dementia and associated diseases remains low down, even in the world of healthcare.[2] Much of our current dementia incidence information and projections are coming from limited geographic case studies.[10] It has been mentioned that there are significant variations among states in the incidence of dementia induced by lifestyle and food patterns. It can show a similar incidence to many other non-communicable diseases.[10,21,24] The published data suggested that there is a need to consider genetic factors such as APOE-e4 for genetic screening. In this condition, appropriate finance for such genetic investigation is highly demanded. Principally, Indian scientists are seeking clinical trials that are unique to India. It will help to avoid the dependency on those performed in developed nations. Because it has been mentioned that the risk factors of AD are relying on genetic, lifestyle, environmental, etc. that can be different for each nation.[10] In this regard, better awareness of ad risks will lead to early healthcare and

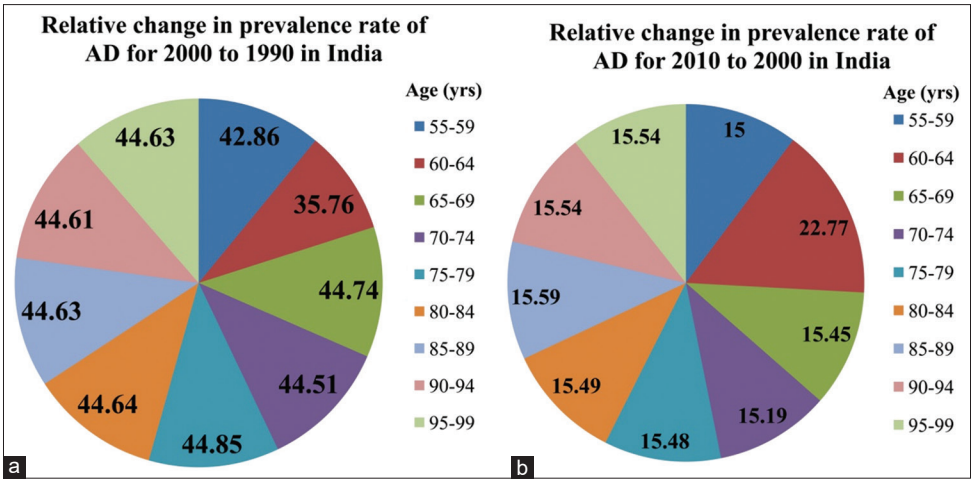


Figure 2: (a and b) The relative change in the prevalence rate of AD in India

early detection of dementia by the physician.[2] Notably, the condition of AD imposes economic burdens on the family. In brief, family members are already giving the majority of treatments to the AD patients that also increase the economic burden.[23,24] The ARDSI 2010 reported that a triple rise in the forecasting of the overall dementia social expense in India. It would be INR 147 billion in the next few decades (2030). In addition to the direct health-care expenses (e.g., drugs and doctors), much of these economic costs are derived from the fact that family members lose their income and earnings by absent employment or retirement from labor.[10,13] It has been revealed that just about 1.2% of India's GDP is spending on public health to date. This is considerably lower than that of many other developing countries. Just a small portion of this allocation is marked for mental wellbeing and even a smaller part for elderly diseases. From 2012 (when the Bharat and Rao analysis was performed) to July 2019, the Consumer Price Index was rose by 1.442. On this basis, the cost of caring for a person with dementia is estimated at Rs 29272-95208 per household in rural areas per annum whereas in urban areas Rs 65755-291933 per annum per household.[25] In conclusion, the current AD management scenario in India, along with the recent economic statistics have been defined in this review. Overall, AD is a major health issue in India. In the upcoming days, it will be the leading cause of health issues. Therefore, AD needed enormous concentration from researchers to design a suitable and effective substitute for AD and other related dementias.

RISK FACTORS FOR AD

In India, there is a prerequisite to authenticating the AD risk factors with the respective region of India. Out of several risk factors, some of the most extremely important risk factors have been briefly described in this subsection. At present, active research is ongoing in India to the confirmation of precise causes and risk factors of AD.[8] Notably, it has been reported that AD develops in a person aged over 65 years.[2]

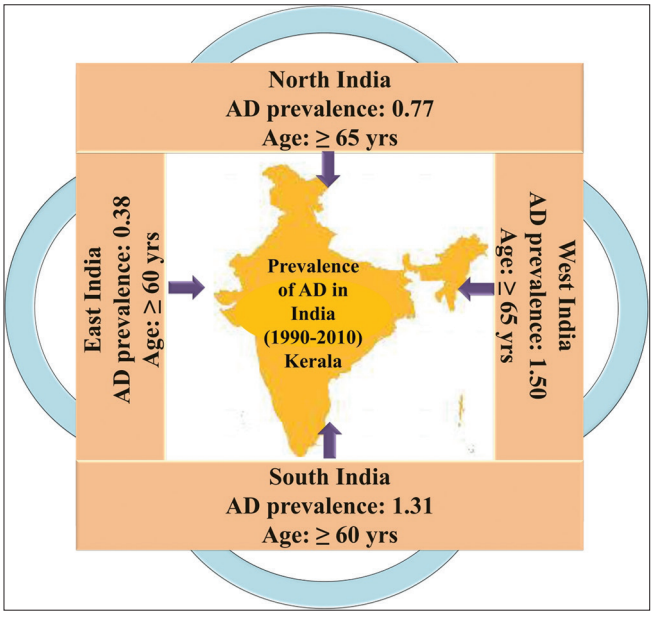


Figure 3: The prevalence distribution of AD and dementia from India

In conclusion, along with the age factor, the likelihood of AD development is getting increased. In addition, AD can be present in both genders (male and female). A rare genetic form of AD is termed early-onset AD that can be developed between the age of 30–60 years.[8] Alzheimer's Association (alz.org) noticed that person could not suffer from AD even there is no control of all the risk factors. On contrary, certain individuals can develop AD due to the risk factors. In this context, the researcher has given us a greater understanding of the underlying causes that place someone at a higher risk.[26] As per Alzheimer's association India, diverse risk factors are playing a crucial part in the development of AD in patient. Nevertheless, the appropriate information related abovementioned factors and AD is not mentioned properly. As per Alzheimer's association India, progress in age is the greatest risk factor for developing AD. Particularly, at least 65 years of age of the person is a large proportion of people with AD. While a younger person can affects much less commonly. It has been reported that up to 5% of younger people are affected by AD. Unfortunately, AD in younger's is sometimes misdiagnosed.[25] Researchers have been found multiple gene variants that increase the risk of AD.[2] Especially, the presence of the APOE-e4 gene is the most common risk gene for the development of AD. Interestingly, deterministic genes vary from risk genes, because they ensure that someone develops a disease. It has been revealed that AD is the only cause of the inheritance of a deterministic gene. The AD caused by the deterministic gene is exceptional and is likely to happen in <1% of AD. Published research studies have shown that the health of the brain is directly linked to the wellbeing of the heart and blood vessels. In general, the brain receives the oxygen and nutrients from the blood, which are required for the proper functioning of the brain. As we know, the heart helps to pumps blood into the brain.[25] Thus, cardiovascular factors, for example, smoke, diabetes, obesity, and high cholesterol and blood pressure in the middle of life, can be associated with an elevated risk of AD and other dementias. After a mild to extreme traumatic brain injury, the risk of AD and other dementias is increased. Especially, head hits or skull fractures is causing amnesia or consciousness failure for more than 30 min. It has been reported that the motorcar crash accounts 50% of traumatic brain injuries that can also the risk factor for the development of AD. Persons with repeated brain trauma especially athletes and fighters are also at greater possibility for dementia and thought disorders.[27] Studies related to fewer formal education years are also increasing the risks of AD and other dementias. There are no obvious explanations for this above-mentioned relation. Some scientists suggested more years of formal schooling that helps create a link between neurons, which encourages the brain to use different ways of communication between neurons, and possibly, it leads the AD and other dementias.[26] The signs of MCI include a shift in thought but are not as serious as those induced by AD or other progressive dementia. In addition, MCI condition cannot interfere with daily life. Particularly MCI with memory difficulties increases the possibility of development of AD and other diseases in a patient. However, MCI can never advance that could be reversed or stays stable in some situations.[26] As per records, depression is on the stage as a primary role in AD development that shows the significantly high influence on elder persons. Interestingly, published literature reported that the depressive

indication occurs into the initial step in the onset of dementia. Based on this, it can be considered as a prime causing factor in AD. In this line, plentiful research works have been published to sustain this statement. Among this development, there is no confirmative relationship to conclude the pathophysiological relationship among depression and AD development risk.[25] Another risk factor for AD development is alcohol consumption. In the support of alcohol consumption, the World Health Organization claimed that the consumption of pure alcohol (more than 60 g for men and more than 40 g for females) per day can boost the risk of dementia or it can develop cognitive impairment. It has been divulged that the alcohol is the fifth causing factor that increases the risk of AD and other related disabilities followed by patient death. In this line, "Dementia in India 2020" report mentioned that the 57% occurrence of early-onset dementia is related to heavy and chronic drinking in peoples with age <65 years. Notably, the high intake of alcohol increases brain shrinkage as well as atrophy. After this, it resulted in very similar neurodegenerative changes such as AD or aging. Notwithstanding this, the alcohol influence might be reversible. In addition, uptake of mild/moderate alcohol use resulted in a less incidence of dementia.[25] As per formerly published studies, smoking is vigorously involved in brain-associated abnormalities that include neurobiological and neurocognitive abnormalities. In 2020, "Dementia in India" reports mentioned that sustained smokers could suffer AD and other types of dementia as compared to the other individuals.[28]

TREATMENT FOR AD IN INDIA

As per the existing literature, an early diagnosis is an excellent preference for AD management.[2] In that, the early diagnosis of AD will maximize the value of the therapies available and also encourage the patient to engage in decisions about livelihoods, healthcare, financial, and legal issues.[8] As we know, there is currently no cure available for AD, but existing medications can relieve the associated symptoms.[2] These medicines can enhance cognitive capacity and can lead to a delay in brain dysfunction. In general, medical therapy includes drugs that can influence and maintain the normal chemical levels engaged in signal transmission between brain cells. Herein, successful medical therapies are available for behavioral disorders such as depression, anxiety, and violence. Interestingly, these are the major health concerns that are more popular in individuals with AD. Particularly for caregivers, these health issues are becoming very frightening.[8] Herein, the absence of established experimental models impedes the preclinical assessment of anti-AD agents. The transgenic animal models do not appropriately describe the pathology and clinical development of AD. Furthermore, due to population heterogeneity, insufficient memory test sensibilities, and low resolution of brain imaging procedures, the clinical design of anti-AD is getting more complexed and demanding. Since 2003, no new molecules or disease-modifying treatment approved for AD management. Notably, the failure rate in clinical trials is highest for anti-AD candidates (about 99.6%). Therefore, new strategies for improving the AD prognosis and developing accurate, translationally relevant, experimental models of AD pathogenesis are explored in the last decades.[5] As per literature, the onset of AD can be prevented through education, maintain an active lifestyle including physical and mental, healthy eating habits. Besides, regular health checks to maintain normal blood pressure, blood sugar, cholesterol, and body weight, etc., can help to avoid progress in AD. Moreover, no smoking and no excessive alcohol intake can also help to control the overall onset of AD.[8] Professional practitioners claimed that AD can be controlled up to certain limits by maintaining a brain-healthy lifestyle, which includes regular exercise, a healthy diet, mental stimulation, quality sleep, stress management, active social life, etc.[8] No medications are currently offered to delay or avoid AD-induced brain damage. On contrary, a variety of drugs can eventually contribute to enhance dementia symptoms in certain people by increasing brain neurotransmitters. Researchers are demanding to look for ways of treating AD and other incremental dementias. Plenty of treatments and anti-drugs for the prevention of AD brain cell death are currently in progress. Furthermore, support services can enhance the quality of life of dementia/AD patient and their caregivers or family members by incorporating non-pharmacological behavioral treatments. These support services include education regarding AD, behavioral interventions, participation in activities, treatment of co-existing medical conditions, coordination of care among health-care professionals, and building a care team for support.[26]

DIAGNOSIS OF AD IN INDIA

The abundant literature reported that the early detection of AD is extremely important to reduce the severity of AD followed by increase the quality of lifestyle. Therefore, there is a tremendous demand for AD diagnostic techniques, which can provide the non-invasive, accurate, and fast diagnosis of AD.[2] Unfortunately, AD diagnosis is not an easy examination. Notably, diagnosis is called for an extensive medical review including a neurological examination, brain imaging, blood tests for confirmation of the probable cause of symptoms, family medical history, and cognitive tests for evaluation of memory and thinking. Herein, a straightforward examination of AD is not available; therefore, it is difficult to conclude whether anyone has AD. In most cases, doctors can usually decide if someone has dementia, but it can be harder to conclude what form of dementia is involved in the respective patient. It has been reported that misdiagnosis is more prevalent for younger-onset AD. Principally, it is crucial to achieving a correct diagnosis early in the disease phase because it enables a greater probability of taking advantage of available treatments that can enhance the quality of life and the chance to access support services. Moreover, it can offer opportunities to deal with clinical trials and studies. Interestingly, early diagnosis can offer the chance to voice wishes about future care and lifestyle choices.[26] In this subsection, different currently engaged techniques for AD diagnosis in India have been discussed.

In India, Kokilaben Dhirubhai Ambani Hospital and medical research institute containing the center of neuroscience[8] reported the two-step for AD diagnosis. In the case of Step I, the cognitive assessment to detect the types of memory and cognitive impairment is carried out. Whereas, the different tests to find the exact cause of memory loss are performed using different techniques such as 3 Tesla MRI, positron

emission tomography (PET) scan, an electroencephalogram (EEG) in the case of Step II.[8] Table 1 enlisted the different commonly used AD diagnosis techniques with their major demerits.[2] Herein, we have illustrated numerous diagnostic methods commonly employed in AD.

Cognitive Assessment

The brain is highly structured and each part promotes an ability to recall, prepare, procedure, feelings, to identify the face, and others.[2] Cognitive evaluation requires responding to questions and resolving puzzles. It helps to decide the brain regions or networks function inadequately. Hence, the cognitive examination helps to diagnose the type of dementia and to evaluate its seriousness and its response to judge therapy.[8] Herein, the lack of accuracy of this method is now subjected to the laboratory diagnosis of AD.

Laboratory Diagnosis Techniques

In India, there is a primary necessitate for specialized testing such as metabolic markers detection, level of vitamins assessment, endocrine evaluation, and cerebrospinal fluid (CSF) testing.[8] Interestingly, a PET scan is a recent move forward in the diagnosis of AD and another type of dementia. Herein, by employing advanced imaging techniques, a PET scan measures the events of different brain regions.[8] In the last few years, the application of PET in the detection of AD biomarkers has been raised. It may because of precise detection and non-invasive nature. In addition to that, the different PET radio ligands namely, [18F]-Flutemetamol, [11C]-PiB (gold standard), ([18F]-Florbetaben) have been utilized during AD diagnosis due to their high affinity for AD biomarkers. Mainly, the application of these abovementioned radio ligands can provide the *in vivo* quantification of AD biomarkers.[29] Another diagnosis method is known as an EEG which works based on a recording of brain rhythm by positioning electrodes (or stickers such as in an electrocardiogram) on the scalp. Fascinatingly, it helps to define the AD stage as well as aid to remove additional dementias.[8] From its inception, commonly used MRI is utilized to capture brain images, and based on this, we expert can diagnosis AD. In MRI, a contrast material or agent such as Gadolinium contrast media (chemical element) is preferred to enhance the detection of AD.[30] Advanced MRI protocols including morphometric MRI, volume MRI, Diffusion Tensor Imaging, Functional MRI, and MRI angiography can also aid in elucidating brain networks, early diagnosis, and disease monitoring. In conclusion, these mentioned methods also contribute to the detection of other conditions of AD.[8] Unfortunately, some of the available methods are getting unsuccessful to accomplish high selectivity and sensitivity for the detection of AD. Therefore, this leads to significant diagnostic delays. Fascinatingly, recently reported most therapeutic choices are advantageous to overcome AD and it is possible through the multidisciplinary way.[31] Despite plenty of merits, currently engaged AD diagnosis techniques are suffering from some major disadvantages. Table 1 enlisted the different important diagnosis techniques along with their demerits.[2]

Table 1: Commonly used AD diagnosis techniques and their demerits

S. No.	AD diagnosis technique	Demerits
1.	MRI	1. It is a very costly process.
		2. It provides low scanning speed.
		3. Motion artifacts
		4. It's an insensitive calcification method.
2.	PET	1. It is an expensive endeavor.
		2. It demonstrates an inadequate spatial resolution.
3.	Enzyme-linked immunosorbent assay	1. It is a time digesting and ineffective framework.
		2. It furnishes less sensitivity at a low level of biomarkers.
		3. There is a major chance of false positives.
4.	Western blot assay	1. It suffers the stability issue.
		2. Discrepancy can affect the actual system in every sequence.
5.	xMAP	1. It curtails use based on the cost factor.
		2. It delivers low to medium-resolution responses.

AD BIOMARKERS

For the past few years, researchers are actively working on AD and related health issues. Despite notable development in science and technology, the precise diagnosis and appropriate treatment for AD are a major barrier. As per literature, laboratory diagnosis techniques are suffering from certain limitations such as sensitivity, tedious process, selectivity, cost, and speed. For the last couple of two decades, the research era has been shifted into the *in vitro* diagnosis based on selective biomarkers. In this vein, several biomarkers have been reported on the account of AD. Recently published pieces of literature enlisted the all-possible biomarkers of AD that are playing a central role in advanced diagnosis techniques. Interestingly, it includes CSF-containing biomarkers, genetic biomarker, and imaging biomarker. In addition, other body fluids such as blood, saliva, and plasma containing AD biomarkers. Figure 4 demonstrates the classification of AD biomarkers.[2] Nowadays, the effective diagnosis of neurological disorders through CSF biomarkers has been established, as they are reliable to brain neurochemical behavior. It should also be noted that CSF biomarkers demonstrated greater identification correctness and strong reliability from 85% to 90%. As per the literature, AD biomarkers are particularly relevant in the CSF because CSF directly interacts with the brain. Besides, the CSF composition is represented small and robust changes in the biochemistry of the brain. Notably, CSF of AD patients contains several important biomarkers including total Tau protein (T-Tau, 6-isoforms), $A\beta_{1-42/1-40}$, and Phosphorylated Tau proteins (example: p-Tau-181).[2] In the Indian population, regional variations are related to the incidence of the APOE4 allele, which is a well-known AD risk factor. It has been revealed that the Northern and Central Indian populations contain a lower APOE4 allele incidence than in the United States. Moreover, the population's education levels of India are significantly linked to incident dementia or AD.[16] A plethora of research has reported that earlier diagnosis of AD can

influence the invasion of CSF. Therefore, the research team sought to predict neurodegenerative illnesses/disorders based on variations in patients' blood or cell levels. Notably, the AD can identify with minutely invasive and sometimes even non-invasive biomarkers of body fluids, namely, blood (serum, plasma). Interestingly, several latest studies shown that the prevalence of AD is associated with level of creatine, serine, phospholipids, glutamate, 5-hydroxycytosine, myoinositol, blood dehydroepiandrostone, etc. Moreover, potential AD biomarkers in blood, that is, neuronally mediated $A\beta_{1-42}$ exosomes, neurogranin, phosphorylated tau protein (p-Tau), have been discovered in current knowledge to estimate the threat of AD. In conclusion, analysis of AD biomarkers in different body fluids can facilitate the monitoring of AD development and able to sort out AD and other related health issues.[2]

IN VITRO DIAGNOSIS OF AD BIOMARKERS

In this section, we have highlighted the meticulous finding of research and advanced development projects conducted by Indian researchers to detect AD. Recently, National Institute on Aging and Alzheimer's Association (NIA-AA) has introduced a biological concept for the use of AD diagnosis. At present, the differential diagnoses of AD are costly or invasive that require cost-efficient and non-invasive alternatives. As we know, peripheral tissue such as skin fibroblast, lymphocytes can be used as a source of molecular markers of AD. In this context, Krishna and colleagues divulged that the plasma containing exosomal protein levels is alerted in AD patients, which may due to the cellular changes in AD. The exosomal concentration of lysosome-associated membrane protein 2 (LAMP-2), cluster of differentiation 81, and Aβ-42 confirmed by ELISA. The concentrations of an exosomal marker in AD patients (n = 14, age = 52–80 years) were observed significantly higher as compared (n = 14, age = 50–82 years) to the normal range in South India. More specifically, the exosomal concentration of LAMP-2, Aβ-42 in AD patients was found to be 307.4 pg/mL and 55.71 pg/mL, whereas control samples showed 141.8 pg/mL and 38.86 pg/mL of LAMP-2 and Aβ-42, respectively.[32] In 2021, Rani *et al.* demonstrated the nanoparticle tracking analysis (NTA) novel method for the diagnosis of AD. In brief, this technique helps to correlate the saliva containing exosome concentration with respective of the progress in the AD. In this study, the saliva samples of AD patients have been collected from patients (n = 12) and the control group (n = 12). Afterward. the exosomes from the saliva have been separated by the esterification process. As a result, the concentration of Aβ oligomer/fibril abundance, phospho-tau, and Aβ protein abundance was found to be significantly higher in the case of a patient with AD and cognitively impaired patients as compared to the control group. Therefore, NTA could be used as an alternative technique for early detection of AD as compared to the previously engaged techniques. In conclusion, it will open a new era for cost-effective diagnosis than the traditional techniques.[33] It has been reported that the sialic acid (SA) level in serum is getting increased due to the presence of more concentration of sialylated acute-phase glycoproteins. As a result, SA shows antioxidant property (scavenging of H_2O_2). Notably, the reduction in SA was found in the AD patient's blood that may due to the prevention of Aβ toxicity. Herein, the level of SA was found to be high in the case of the AD group (n = 60, AUC = 0.7133) as compared to the control group (n = 60, AUC = 0.5972). Overall, the authors claimed that the SA and other related markers including lipid peroxidation and protein carbonyl can be used as a substitute biomarker for prognosis and diagnosis of AD.[34] Huded *et al.* reported the AD diagnosis criteria based on CSF-containing biomarkers, namely, Aβ-42, t-tau, and p-tau181 in the Southern India population. Briefly, the CSF samples of 20 patients have been tested for AD biomarker detection using sandwich ELISA

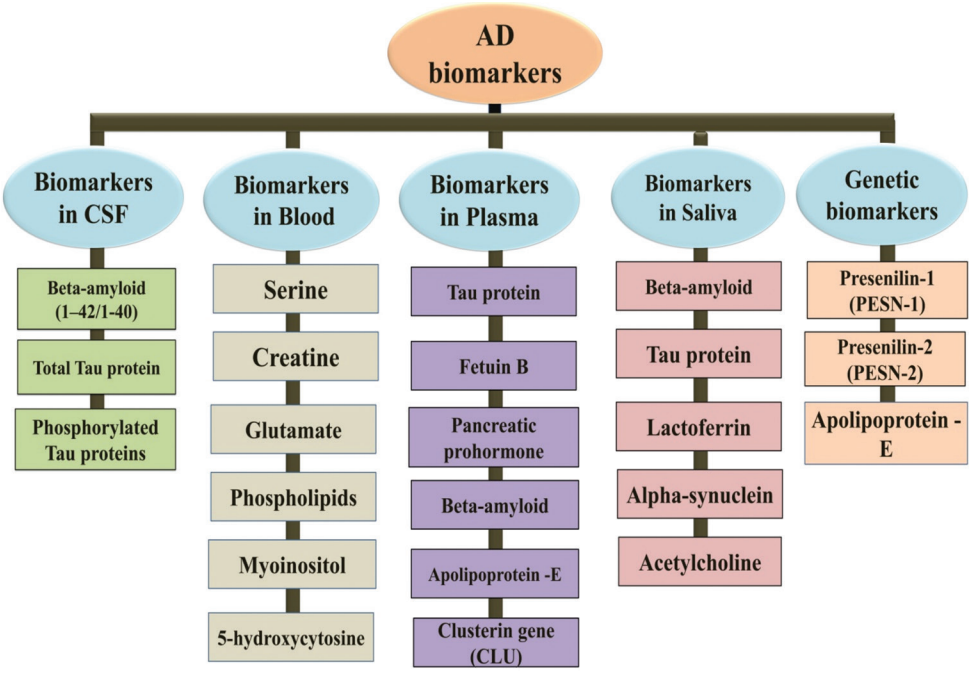


Figure 4: Classifications of AD biomarkers

assay. Based on the clinical dementia rating (CDR) scale, the patient group confirmed mild (CDR = 1) and moderate to severe (CDR > 1) AD dementia. Herein, a patient (CDR > 1) biomarkers concentration in CSF was found to be 49.9 pg/mL and 33.94 pg/mL for t-tau, and p-tau181, respectively. On the other hand, the CDR = 1 group shows the 39.45 pg/mL of t-tau and 13.06 pg/mL of p-tau181 concentration in CSF. The major limitation of this AD diagnosis study is the small sample size and there is no control. Besides this lacuna, the present study offers more reliability in the detection of AD biomarkers. In the future, it would be an exceptional alternative to validate the diagnosis standard of NIA-AA.[35] Low copper and decreased copper-related cytochrome oxidase activity in the AD brain have been correlated with decreasing cognition and elevated Tau in CSF. In comparison, similarly compelling evidence shows that higher serum copper levels can associate with unacceptable cognitive impairment in patients suffering from AD. Unfortunately, there is still a shortage of clarity in current research both in India and globally on this topic. In this context, Shere *et al*. proposed that the patient with AD contains a low level of copper. Herein, 44 subjects (68.32 years) have been selected based on the ICD-10 (ICD-10 is the 10th revision of the International Statistical Classification of Diseases and Related Health Problems, ICD) AD criteria. On the other hand, the healthy subjects (n = 52, 65.75 years) have been selected for the control group. Finally, the serum-containing copper has been determined using the colorimetric detection method. Notably, the total copper content in serum was found to be low in AD (102.2 µg/dL) patients as compared to the control groups (115.9 µg/dL). Moreover, the free copper concentration was found to be 34.1 µg/dL in AD patients whereas 46.1 µg/dL in healthy controls. Overall, the finding of these investigations provides the evidence of the low level of copper in AD patient as compared to the healthy participants. In the future, there is a huge need to conduct a clinical trial for more reliability of copper concentration with AD patients.[36] Literature survey divulged that the serum prolidase activity is a novel biomarker for bipolar issues. In 2020, Krishna *et al*. reported the determination of prolidase enzyme activity into the AD participant (n = 49) body fluids (plasma) with a comparison of healthy control (n = 22). In this study, the prolidase enzyme activity has been measured using a colorimetric assay wherein the AD group shows higher prolidase activity in AD groups (5.62 U/mL) as compared to the healthy control (4.45 U/mL). Despite these findings, there is still a need to evaluate the prolidase level in the initial AD stage. The exact molecular mechanism involved in the prolidase-mediated alteration in the patient with AD needs to be clear. In conclusion, it could be open a new door for the diagnosis of AD using prolidase level as a biomarker.[37] As per published literature, forkhead box O (FOXO) transcription factors are a novel marker for different neurodegenerative diseases. Their broad appearance in the nervous system indicates that particular FOXO can be crucial to targeted cellular plus biological functions, and it can apply to the diagnosis of AD. Notably, FOXO3A is intensely exhibited in the brain. Particularly, it is present in neurodegeneration-sensitive areas of AD but the involvement of FOXOs in AD is unsure. In this shade of light, Pradhan *et al.* performed the identification of FOXO3A in AD patient (n = 21) serum using surface Plasmon resonance (SPR). In this, the serum concentration of the biomarker has been evaluated using respective antibody binding. This SPR assay provides a lower detection limit (1.42 ng/µL) than the MCI (1.61 ng/µL) for FOXO3A in serum. Furthermore, the serum Tau and p-Tau protein concentration were measured using SPR that shows the higher concentration of Tau in the AD patient group (47.55 ng/µL) as compared to the MCI group (43.63 ng/µL). On the other hand, the p-Tau protein concentration was found to be high in the AD patient group (0.176 ng/µL) as compared to the normally aging group (0.15 ng/µL). Therefore, it can be stated that FOXO3A serum might be a readily available new biomarker for slightly earlier recognition of AD. In addition, it can be effective therapeutic involvement to protect further deterrence in AD. Overall, this study supported the invention of a new serum biomarker for the diagnosis of AD.[38] In 2014, Sathish *et al*. revealed the high sensitivity and selective detection of β-amyloid fibrils using a novel probe that relied on rhenium (I) based alkoxy bridged binuclear complex. Herein, rhenium (I) complexes containing naphthalene moiety offer the π-π stacking interaction that resulted in the binding between probe and β-amyloid fibrils that confirmed by the fluorescence spectral method. Herein, the strong binding among complexes resulted in strong fluorescence that also shows the high selectivity towards the target marker. In conclusion, atomic force microscopy can confirmed the construction of fibrils in the range of 30 nm to 40 nm.[39] Due to the requirements of highly precise *in vitro* diagnosis, many research groups are working on the biosensing of AD biomarkers. With the addition of new approaches to diagnosis, different types of biomarkers have been reported. In 2018, Srivastava *et al.* constructed the curcumin-based lanthanum carbonate nanospheres, that is, REM-100 for precise detection of Aβ aggregates. First, surfactant-based lanthanum carbonate nanospheres have been synthesized. Notably, the rapid binding of different proteins (e.g., Casein and Lysozyme) and Aβ aggregates, Aβ monomer, bovine serum albumin, on complex of curcumin-REM100 has examined. Herein, curcumin-REM100 fluorescent probe offers low detection limit of 0.2 µM. Concisely, the experiment is based on label-off method, which offers the different meticulous merits such as highly effectivity, demands at least sample pre-processing, and is suitable for a range of uses.[40]

SUMMARY AND FUTURE PERSPECTIVES

For the past two decades, more than 4 million peoples in India have suffered from dementia whereas it is expected to have about 7.5 million dementia and AD burden by the end of 2030. Unfortunately, there is no proper cure for AD, and current existing symptomatic treatments are effective at an early stage of AD. Therefore, the early diagnosis of AD followed by effective treatments is an upcoming need of the Indian population for successful dealing with the rising burden of AD. In this line, there is a need to spread adequate knowledge of the cause and deaths of AD among Indian peoples. Due to the significant rise in prevalence of AD in the Indian population, several research groups are focusing on ultramodern methods that can be more constructive in the early detection of AD. At present, apart from cognitive assessment, the other laboratory techniques PET, Tesla MRI, EEG, etc., have been suggested for AD diagnosis. Nowadays, In India, different medical and technical research institutions are working together to establish and implement

AQ6 **Table 2:** Summary of an Indian research group working on the *in vitro* diagnosis of AD biomarkers

S. No.	AD Biomarkers	Body fluids	Biomarkers Concentration	Diagnosis technique	Region	AD patient age (Years)	Ref.
1.	LAMP-2	Exosomes	307.4 pg/mL	ELISA	South India	50–80	[32]
	Aβ-42	Exosomes	55.71 pg/mL				
2.	Exosome concentration	Saliva	1.905e+011	NTA	North-central India	72.40	[33]
3.	Sialic acid	Serum	0.7133 (AUC)	ELISA	North India	74.13	[34]
4.	t-Tau	CSF	49.9 pg/mL	ELISA	South India	64.4	[35]
	p-tau181	CSF	33.94 pg/mL				
5.	Copper	Serum	102.2 µg/dL	Colorimetric assay	South India	68.32	[36]
6.	Prolidase activity	Serum	5.62 U/mL	Colorimetric assay	South India	66.43	[37]
7.	FOXO3A	Serum	1.42 ng/µL	SPR biosensor	North-central India	60-76	[38]
8.	β-amyloid fibrils	---	30 nm- 40 nm	AFM	---	---	[39]
9.	Aβ aggregates	---	0.2 µM	Multimode microtiter plate reader	---	---	[40]

customized technologies to meet the needs of a large and varied population. In this vein, in past few years, different diagnostic techniques have been introduced into the arena of diagnosis that shows the exceptional aptitude to distinguish AD from dementias. Regrettably, the traditional methods of AD diagnosis are suffering from many disadvantages including costly, time-intensive, tedious, radiation exposure, less sensitivity, etc. In addition, it requires specialists for handling purposes. Recently documented different AD biomarkers have been detected easily using modern biosensor-based *in vitro* diagnosis techniques. In the future, it needs to be integrated into AD diagnostic practices due to its tremendous potential in biosensing. Although numerous studies on AD have been released that provide noticeable merits, namely, non-invasive, simplistic method, rapid detection, cost-effective techniques that are used for processing, analyzing, and interpreting AD test results without an experienced professional. Still, these techniques are not available for AD diagnosis in India. Therefore, advanced commercialized methods need to be promoted by clinical diagnostic integration practitioners for AD diagnosis. In the future, the selection of appropriate prediction algorithms can be a potential alternative to confirm the actual stage of the AD patient. Definitely, it will facilitate the early treatment of AD management.[41] As we know, India's cultural and socio-economic norms are much stronger than western societies but the burden of treatment for AD and other types of dementia in Indian remains unknown. Indian women are taking the responsibility of AD patient care without formal care services. Therefore, the cure rate of an AD patient is low whereas family members are also suffering mental stress. Hence, there is a demand for reliable institutional services for AD patients including short-term care and long-term care. In addition, the formation of AD care centers can shift the responsibility of family members to skilled professionals and it creates employment. It would entail considerable upfront investment in the curriculum of medical staff to understand and satisfy the requirements of patients with AD dementia. Recently, under the Ministry of Health and Family Welfare, the National Health Care program for elderly people is intended to fill some deficiencies in India's geriatric environment by establishing regional geriatric care facilities. Nevertheless, there is no emphasis on dementia and AD. Cursory qualification courses in AD dementia care are gradually gaining huge consideration from different organizations mainly ARDSI. It is worth revealing that the ARDSI is the first Afro-Asian organization that actively working in the field of AD dementia and other types of dementia since 1992. Mainly, ARDSI is holding 14 chapters throughout India. Besides, some others such as the Helpage India, Nightingales Medical Trust, Dignity Foundation, Dementia Society of Goa, etc. are offering such care services in conjunction with ARDSI or on their own. Notably, most of them are in the South Indian States. They are providing daycare services for AD patients.[13] Despite this, the reports on AD in India are not available for further research on Indian populations in different geographical regions. In addition, only a few new diagnosis techniques such as SPR, fluorescent biosensor have been reported that offer good sensitivity. In the future, there is a need to develop a high sensitivity and selective biosensor for diagnosis of AD biomarker by non-invasive methods. Overall, Indian medical departments must develop a public health strategy focusing on degenerative diseases and invest appropriately in healthcare training.

CONCLUSION

Early diagnosis is a critical step towards reducing the death rate in AD cases. From the past two decades, the overall AD burden of India is rising rapidly. In India, the commonly used traditional test and histopathological examinations are suffering from minimal precision in AD diagnosis. In this context, various study groups are energetically working on novel strategies that can assist in the early recognition of AD biomarkers followed by diagnosis. Consequently, regardless of indisputable advancement in science and technology, several optical techniques are used for AD diagnosis practices. At present, histopathological examinations, biosensors-based biomarker detection, optical image, etc., are utilized for the diagnosis of AD in India. As we know, point-of-care (POC) diagnostic technologies for the diagnosis of several life-threatening diseases have gained tremendous attention. Because the possible changes in patients' survival rate and their quality of life can be managed using POC techniques. Nevertheless,

POC is not feasible in resourced environments and often causes patient distress. In this context, various research groups from India are vigorously working on the latest POC technologies. In that, biomarkers and other diagnostic element detection using POC devices are evolving as an early-stage diagnosis method for non- or minimally invasive AD diagnosis. Principally, several optical technologies have been preferred as an adjuvant in detections of changes in the brain. Nevertheless, it is relatively complicated to diagnose AD at an early stage and its effective treatment in India. In addition, there is a vast communication space between the health system and the rural society of India. At present, various government/private hospitals and research organizations/institutions are working on *in vitro* diagnosis of AD at an early stage in India. On this account, several scientists from India are also reporting the various advanced techniques for the minimally invasive or non-invasive diagnosis of AD. In the future, there is a need for sufficient funding and policies for research from the Indian government and private finding organizations especially for those research groups that are working on AD management in India.

CONFLICT OF INTEREST

None to declare.

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REFERENCES

1. Dubey SK, Lakshmi KK, Krishna KV, Agrawal M, Singhvi G, Saha RN, *et al.* Insulin mediated novel therapies for the treatment of Alzheimer's disease. Life Sci 2020;249:117540.
2. Nangare S, Patil P. Nanoarchitectured bioconjugates and bioreceptors mediated surface plasmon resonance biosensor for *in vitro* diagnosis of Alzheimer's disease: Development and future prospect. Crit Rev Anal Chem 2021;2021:1-31.
3. Janghel R, Rathore Y. Deep convolution neural network based system for early diagnosis of alzheimer's disease. IRBM 2020;42: 258-67. doi.org/10.1016/j.irbm.2020.06.006.
4. Gopalan D, Pandey A, Udupa N, Mutalik S. Receptor specific, stimuli responsive and subcellular targeted approaches for effective therapy of Alzheimer: Role of surface engineered nanocarriers. J Control Release 2020;319:183-200.
5. Bhatt S, Puli L, Patil CR. Role of reactive oxygen species in the progression of Alzheimer's disease. Drug Discov Today 2021;26:794-803.
6. Available from: https://www.nhp.gov.in/disease/neurological/alzheimer-s-disease.2015 [Last accessed on 2021 Feb 21].
7. Shanmugam A, Gopal S. Alzheimers awareness among high school students in India. Am J Geriatr Psychiatry 2020;28:S70-2.
8. Kokilaben Dhirubhai Ambani Hospital and Medical Research Institute; 2020. Available from: https://wwwkokilabenhospitalcom/contactshtml [Last accessed on 2021 Feb 21].
9. Dementia India Strategy Report; 2018. Available from: https://ardsi.org/publication/ [Last accessed on 2021 Apr 06].
10. Brahma D. The Persistence of Memory: The Burden of Alzheimer's Disease in India. 2019. Available from: https://www.brookings.edu.
11. Nair T. Challenges of hypertension and dementia in the Indian subcontinent: A review. J Hum Hypertens 2019;33:568-74.
12. Rogan S, Lippa CF. Alzheimer's disease and other dementias: A review. Am J Alzheimers Dis Other Demen 2002;17:11-7.
13. The Dementia India Report 2010; 2010. Available form: https://www.mhinnovation.net/sites/default/files/downloads/innovation/reports/Dementia-India-Report.pdf [Last accessed on 2021 Mar 08].
14. Department SR. Number of Cases of Alzheimer's Disease in Senior Citizens India 2011-2050; 2020. Available from: https://www.statista.com/statistics/944170/india-number-of-cases-of-alzheimer-s-disease-in-senior-citizens/#statisticContainer [Last accessed on 2021 Mar 08].
15. Toravi J, Vartak A, Yadav V, Mengade M. Assisting technologies for Alzheimer's patients and caretakers. J Emerg Technol Innov Res 2019;6:199-201.
16. Mathuranath PS, George A, Ranjith N, Justus S, Kumar MS, Menon R, *et al.* Incidence of Alzheimer's disease in India: A 10 years follow-up study. Neurol India 2012;60:625-30.
17. Hindustan Times. India Has Second Most Number of Individuals Suffering From Dementia. Pune News; 2017. Available from: https://www.hindustantimes.com/pune-news/india-has-second-most-number-of-individuals-suffering-from-dementia/story-FpMAyRUxeq2yVuVngHtpSM.html [Last accessed on 2021 Mar 08].
18. (ARDSI) AsRDSoI; 2020. Available from: https://ardsi.org/[Last accessed on 2021 Mar 08].
19. Pattanayak RD, Jena R, Tripathi M, Khandelwal SK. Assessment of burden in caregivers of Alzheimer's disease from India. Asian J Psychiatr 2010;3:112-6.
20. Chandra V, Ganguli M, Pandav R, Johnston J, Belle S, DeKosky ST. Prevalence of Alzheimer's disease and other dementias in rural India: The Indo-US study. Neurology 1998;51:1000-8.
21. Shaji S, Bose S, Verghese A. Prevalence of dementia in an urban population in Kerala, India. Br J Psychiatry 2005;186:136-40.
22. Tushar Kanti Ghara KR. Alzheimer's disease - An analytical view. IOSR J Nurs Health Sci 2017;6:31-5.
23. Kumar CS, George S, Kallivayalil RA. Towards a dementia-friendly India. Indian J Psychol Med 2019;41:476-81.
24. Brijnath B, Manderson L. Appropriation and dementia in India. Cult Med Psychiatry 2011;35:501-18.
25. Dementia in India 2020; 2020. Available from: https://dementiacarenotes.in/dcnfiles/Dementia-in-India-2020.pdf [Last accessed on 2021 Apr 06].
26. Alzheimer's and Dementia in India; 2020. Available from: https://www.alz.org/in/dementia-alzheimers-en.asp [Last accessed on 2021 Apr 06].
27. Sivanandam TM, Thakur MK. Traumatic brain injury: A risk factor for Alzheimer's disease. Neurosci Biobehav Rev 2012;36:1376-81.
28. Ragubathy PK, Adikane H. Prevalence of risk factors for dementia in elderly population in a tribal area of central india – A community-based cross-sectional study. J Med Sci Health 2019;5:19-30.
29. Selvan ST, Ravichandar R, Ghosh KK, Mohan A, Mahalakshmi P, Gulyás B, *et al*. Coordination chemistry of ligands: Insights into the design of amyloid beta/tau-PET imaging probes and nanoparticles-based therapies for Alzheimer's disease. Coord Chem Rev 2020;430:213659.
30. Soundarya S, Sruthi M, Bama SS, Kiruthika S, Dhiyaneswaran J. Early Detection of Alzheimer Disease Using Gadolinium Material. Materials Today: Proceedings; 2020.
31. Chandra SR. Alzheimer's disease: An alternative approach. Indian J Med Res 2017;145:723.
32. Krishna G, Kn A, Kumar RS, Sagar BC, Philip M, Dahale AB, *et al*. Higher levels of lysosomal associated membrane protein-2 (LAMP-2) in plasma exosomes from Alzheimer's disease: An exploratory study from South India. Asian J Psychiatr 2020;48:101898.
33. Rani K, Rastogi S, Vishwakarma P, Bharti PS, Sharma V, Renu K,

et al. A novel approach to correlate the salivary exosomes and their protein cargo in the progression of cognitive impairment into Alzheimer's disease. J Neurosci Methods 2021;347:108980.
34. Yadav J, Verma AK, Garg RK, Ahmad K, Shiuli, Mahdi AA, *et al.* Sialic acid associated with oxidative stress and total antioxidant capacity (TAC) expression level as a predictive indicator in moderate to severe Alzheimer's disease. Exp Gerontol 2020;141:111092.
35. Huded CB, Bharath S, Chandra SR, Sivakumar PT, Varghese M, Subramanian S. Supportive CSF biomarker evidence to enhance the National Institute on Aging-Alzheimer's Association criteria for diagnosis of Alzheimer's type dementia – A study from Southern India. Asian J Psychiatr 2015;13:44-7.
36. Shere S, Subramanian S, Bharath S, Purushottam M. Lower levels of serum copper in patients with Alzheimer's dementia: A controlled study from India. Asian J Psychiatr 2018;32:73-4.
37. Krishna G, Sivakumar PT, Dahale AB, Issac TG, Mukku SSR, M LP, *et al.* Increased prolidase activity in Alzheimer's dementia: A case-control study. Asian J Psychiatr 2020;53:102242.
38. Pradhan R, Yadav SK, Prem NN, Bhagel V, Pathak M, Shekhar S, *et al.* Serum FOXO3A: A ray of hope for early diagnosis of Alzheimer's disease. Mech Ageing Dev 2020;190:111290.
39. Sathish V, Babu E, Ramdass A, Lu ZZ, Velayudham M, Thanasekaran P, *et al*. Alkoxy bridged binuclear rhenium (I) complexes as a potential sensor for β-amyloid aggregation. Talanta 2014;130:274-9.
40. Srivastava AK, Dev A, Choudhury SR, Karmakar S. New insight into curcumin tethered lanthanum carbonate nanospheres and protein corona conferring fluorescence enhancement based sensitive detection of Amyloid-β aggregates. Sens Actuators B Chem 2018;262:687-95.
41. Kishore P, Kumari CU, Kumar M, Pavani T. Detection and Analysis of Alzheimer's Disease Using Various Machine Learning Algorithms. Materials Today: Proceedings; 2020.

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