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Review article

Early Diagnosis and Biomarkers in Parkinsonism

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ABSTRACT:

Parkinsonism disease (PD) is a progressive neurological disorder. It is age-related disorder and affects more than 6 million population every year. It is associated with neuronaldegenerationinsubstantianigraandtolesserextent,intheGlobuspallidus,putamenandc audatenucleus.Thedegeneration of the neurons of the substantia nigra that send their axons to the corpus striatum results in reduction in the release of neurotransmitter dopamine within corpus striatum. This leads to hypersensitivity of the dopamine receptors in the post synaptic neurons in the striatum. Individuals have characteristics signs and symptoms like Tremors, Bradykinesia, Postural instabilities, Rigidity. Neither loss of sense nor muscle power is usually seen in such cases. Deep tendon, Superficial and Abdominal reflexes are well retained on evaluation. Together with aging, genetics, environment and the role of biological sex as important factor in development of PD has been widely discussed in the past decade. Investigations in PD remains a challenge, recent studies included 7 Tesla MRI,PET and SPECT have proved to be confirmatory in diagnosis of PD. Understanding the biomarkers involved in PD may help in early detection of the disease and this will definitely helpful in the timely management of disease.

Keywords: Parkinsonism, Degeneration, Tremors, Bradykinesia, Rigidity.

1. INTRODUCTION

Parkinsonism is second most common neurodegenerative progressive disorder with wide umbrella of symptoms and wider disparity in both the gender from early features to diagnosis treatment and response to treatment. Parkinsonism shows a broad spectrum of changes in both the gender [1]. PD is age related disorder affecting almost 5% above the age group of 85 years and 3% over the age 65years. It is found twice common in men than women [2]. PD is a movement disorder but its management and more than that diagnosis remains a big challenge with no definite bio marker available in it till date [1]. The diagnosis is made on the basis of clinical symptoms and is difficult at times due to the wide umbrella of motor and non-motor symptoms. Reduction in the release of neurotransmitter dopamine within corpus striatum leads to hypersensitivity of the dopamine receptors in the post synaptic neurons in the striatum.Together with ageing, genetics, environment and immune status, the role of biological axis also an important factor in development of PD has been widely discussed in the pastdecade. There is clear demarcation in gender related differences in epidemiology and clinical features of the disease[3]. PD affects men double the women still females have higher mortality rate and faster progression of disease leading to speedy functional immobility and is ability among women compared to men [2,3,4]. In India, there is not much uniform data on PD and its prevalence. It was reported14.1 per100000 amongst population of 63,644 in NORTHERN INDIA, prevalence was found low in SOUTH-INDIA and it was reported16.1 per 100000 from state of Bengal and states of seven sisters that's EASTERN INDIA [5].

Parkinson's is now classified into many classes:

1. According to age group: Juvenile parkinsonism (JPD) before the age of 21years,young onset parkinsonism (YOPD) age range of 21- 40 years, Early onset parkinsonism (EOPD)features appear at age of 40-65,Primary and Secondary parkinsonism[6].
2. According to the cause of onset: Atherosclerotic PD seen in geriatric population with hypertension, Idiopathic PD, Iatrogenic PD (as aside effect of antipsychotic drugs)[7].
3. According to Clinical classification: Tremor Dominant (It has absence of motor symptoms and slow rate of progression and reduced functional disability.) Non-Tremor Dominant (It includes Gait disorders, Posturalin stabilities and Akinetic rigid syndrome [8].
4. Patient history: An evaluation of the results of assessment data must be taken into accountforthestageandchronicityofthedisease.Duringthe earlystages, physical impairments and physical performance is not much affected and is quite stable [9].

2. INCIDENCE &PATHOPHYSIOLOGY

Parkinson's disease is the second most common neurodegenerative disease with a global prevalence of more than 6 million individuals. Disease has increased 2.5 times in incidence in the last decade taking PD at first number in neurological disorders [1, 2]. Loss of cells from areas of brain such as substantia nigra, brain stem from distal to proximal and then moving towards neo cortex [3].

INVESTIGATIONS

Biomarkers are the characteristics feature or molecules that can be considered for the identification, monitoring and prediction of the progression of the ailment. PD involves neuropathological changes that leads to depletion of dopaminergic neuron formation of intraneuronal protein inclusion i.e. Lewy bodies, primarily made from α-synuclien. Many study cleared that the PD not only involve movement disorder but also include complex non motor symptoms too. Therefore, a specific biomarker cannot be considered as "gold standard" biomarkers. **Figure 1** represented the various biomarker of PD. Mainly used biomarkers of PD can be categories in 4 groups. These includes clinical, biochemical, genetics, and imaging. First, Cranialnerve, olfactory nerve, sniffing stick test evaluation helps in diagnosis and differentiation of PD from other PD syndromes and is found to be positive in 90% of individuals with PD. Clinical features and detailed history account for 80% of diagnostic accuracy in PD at the initial visit following development of early PD symptoms in an individual[10, 11]. Biochemical estimation of the body fluids such as blood, saliva, cerebrospinal fluid and biopsies of brain further considered for diagnosis and determining the stage of the PD. Presence of a-Syn can be counted as a prominent characteristic in PD.

The genetic biomarker are well understood by studying the etiology of Parkinsonism. Studies showed a complex relationship between the environmental and genetic factors in PD etiology. Slight genetic mutation leads to classical parkinsonism which combine a-Syn, Parkin, DJ-1, PTEN-induced kinase 1, Leucine repeat kinase 2 in about 3% PD cases.

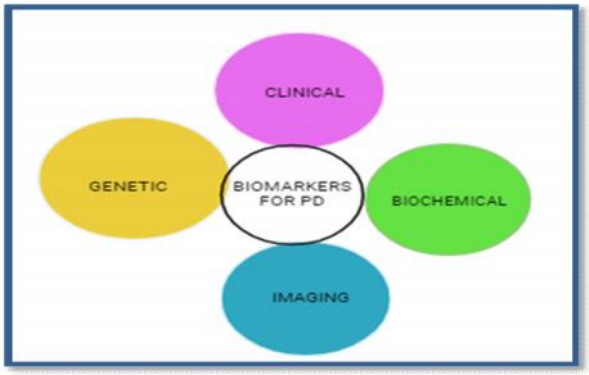

Fig 1: Category of biomarkers used for Parkinson's Diseases (PD) investigations

3. LEWY BODIES AND ALPHA SYNUCLEIN

Lewy bodies discovered by Friedrich Lewy are proteins seen in neurons [12]. Strong evidence proves the presence of alpha-synuclein in clusters of larger cell bodies known as Lewy bodies and Lewy neuritis typical feature of PD and its cell-to-cell transmission and template of synuclein also describes the progression and severity of disease[4]. Alpha-synuclein species are sensitive for fluid and tissue in PD biomarkers. Studies via biopsies strongly recommend immuno histochemical assessment for presence of phosphorylated and aggregated alpha-synuclein in the enteric and autonomic nervous system in saliva /skin confirms the presence of PD in an individual. Alpha synuclein is a reliable biomarker present in Plasma [13]. Factors such as environmental (various pollutants: water, chemical), behavioral (caffeine, smoking), trauma (head injury), metabolic disorders and others also trigger pathological changes[14,15].Role of biological ex hormone plays a role in females in reducing the prevalence of PD among females [6]. Alpha synuclein is one protein in brain that is responsible for stability of neuronal membranes, transportation via membrane and pre-synaptic signaling and Lewy bodies were seen with abnormal deposition of alpha synuclein[16].The production of neurotoxic effects in brain confirms the presence of alpha synuclein modification precisely of phosphorylation at 129-ser [17].Lewy bodies are seen in DLB along with PD[18].Dopaminergic neuronal cell death is caused by lewy bodies in PD. Markedly raised levels of alpha synuclein deposits are seen in PD especially with mutation in GABA gene. Many researchers on alpha synuclein proved markedly reduced levels of alpha synuclein in CSF in PD compared to control group [19]. In this decade SAA (seed amplification assays) have developed over CSF alpha synuclein. SAA is used for identification of alpha synuclein in lewy body with specificity and sensitivity over 95% in PD patients from control group [20]. In recent advancements alpha synuclein in blood has correlation with or within erythrocyte depicts PD presence and its progressive stage [21]. About 70% of reliability and confirmation is found in alpha synuclein erythrocyte aggregation in a logistic model that used PS 129 [22].Extra cellular vesicle loaded with alpha synuclein are important peripheral biomarkers in PD as neuron derived vesicles had increased levels of alpha synuclein in blood in PD patients[23]. Similar studies proved in differentiating PD from DLB and PSP [24]. More recently, alpha synuclein in blood can be detected by seeding assay [25]. Alpha synuclein is a trouble shooter protein for PD encoded by SNCA gene (synuclein alpha gene) though not much is known about it but its presence is proven harmful for the recovery of PD.

Alpha synuclein causes cell organelles damage, its metabolism damage, causes inflammation in central nervous system and brain and disrupts progression of nerve impulses and synapse formation [26].

3.1. MUTATIONS GENE

Dopamine presence in CNS is dependent on two catabolic enzymes: catechol-o-methy ltransferase (COMT)and monoamineoxidase-B(MAO-B)there encoding genes are present on chromosome X and 22[27].There has been strong evidence of genetic contribution in PD[28][26].Patients with mutations in the genes *GBA*, *SNCA*, or *VPS13C* can present with typical Parkinson's disease, but more commonly develop progressive cognitive impairment consistent with Lewy body dementia [9,29].The neuroprotective effect of urate in men, especially in terms of cognitive functions, seems to be related to its ability to influence resting-state networks (RSN), which reflect the spontaneous neural activities and provide indirect information regarding brain functional status[30]. Interestingly, a recent study focused on the potential of urate plasma levels in subjects carrying mutations in the gene encoding leucine-rich repeat kinase 2 (*LRRK2*) [27][25], the most common genetic cause of PD[31]. Genetic technology has driven great improvements in sequencing and genotypeing and their successful implications in this disorder. Genomics efforts have revealed that PD is highly complex and both simple common and rare variants contribute to the disease pathogenesis [27,31,32]. More than 90 genetic risk mutations are therefor common form of this disorder. Mutations in 20 genes are recognized to cause hereditary PD [33, 34].Study of family tree helps gain more insight into gene defect which further lay foundation for betterpredictive testing and family counselling. More clinical trials are hitting the bull's eye to precise genetic forms of neurodegenerative changes in PD. Advancements in technology and genetic studies are greatly significant in PD. There are either autosomal dominant or autosomal recessive genes responsible for causing PD [35].

PARK2 is one autosomal recessive gene mutation responsible for PD [36]. Atypical PD is also result of mitochondrial dysfunction due to mutation of PARK2 & PARK6, PARK7 and DJ-1[37]. PD has always found a close association with Alzheimer's disease in imaging, biomarker or gene mutation. MAPT is one such gene that codes for tau proteins also been for PD along with Alzheimer's[38]. Gene mutation is commonest to be seen with neurodegenerative disease and its high probability is found in PD with family history positive 115. LRRK2 is a protein responsible for vesicular trafficking[39]. LRRK2 gene along with chains of proteins Rab is linked to protein Kinase and its activity with PD[40].

3.2. FECAL

Recent research has suggested, and hypothesis proves that pathological process of PD initiates from gut to later reach the brain [7]. Before the classical motor symptoms, improper functioning of brain-gut micro bio-organisms may find the positive correlation non-motor symptoms that can be found before motor dysfunction features appear [8, 41].Recent study by Finnish has shown that variations in micro bio-organisms composition especially in abundance of ENTERO bacteria has direct and association with severity of postural instabilities and gait disturbances in PD patients[9]. FECAL biomarkers in PD cases contained lesser number of short chain fattyacids (SCFA) including butyrate, that produce bacteria that could exert anti-inflammatory properties [7,8,9].

4. CLINCAL FEATURES

Bradykinesia Is A Characteristic of Basal Ganglia Disorders, and it encompasses difficulties with planning, initiation and execution of movement and with performing sequential and simultaneous tasks[42]. It is dependent on the emotional state of patient and this phenomenon is called as kinesia paradoxa and is also one of the early symptoms to occur in PD. It is a cardinal feature and best appears with degree of dopamine deficiency [43].PD has decreased reaction time and slowness of movement [44]. Dysarthria is monotonic and hypophonic. Reduced eye blinking and reduced arm swing while walking.

Motorsymptoms: Tremors, Bradykinesia, Rigidity, Postural instabilities, Micrography. Gait changes include decreased arm swing, shuffling gait, difficulty raising up from chair and turning in bed.

*Non-motorsymptoms:*Cognitive impairment, bradyphrenia, depression, apathy, fatigue, dysautonomia, sleep disorders, dysarthria, sleep disorders, dysphagia, hypomimia and somato sensory disturbances.

Any of the above non-motor symptoms can be precursor for further onset of motor symptoms cardinalinnature over the period of time [34]. Motor symptoms can be observed unilaterally like pain in dominant and should every commonly seen as early symptom or may be bilateral and asymmetry persist throughout course of disease. Pain is also one of the factors commonly prevalent in early sign and symptoms of PD patients [45,46]. Autonomic symptoms in PD increase with age in severity and progression. Painful sensory symptoms areseenin2/3ofpatientsandarethought to be due to abnormal nociceptive processing[47, 48].

4.1. IMAGING

Due to widespread symptoms, it has always been a challenge to diagnose PD especially its association with striatal dopamine deficiency and nigral degeneration. For confirmation and better understanding of PD pathophysiology, functional imaging is the best choice.

4.2. DAT SPECT

DAT (Dopamine transporter) SPECT (Single – Photon emission computed tomography) biomarker markedly used in PD and Dementia with Lewy body disease (DLB).DAT is a protein that is permeable to sodium chloride membrane which is expressed in dopaminergic cells. It reabsorbs dopamine from synaptic cleft. The International Consensus Criteria has mentioned the low uptake of DAT in the basal ganglia per SPECT as a diagnostic feature for PD. It shows significant correlation between PD dopaminergic terminals within striatum and acts as a differentiating factor from other neurodegenerative disorders (also known as 123I- ioflupane single photon emission computed tomography,SPECT)

[49,50].The Food and Drug Administration (FDA) has given approval to DaTscan (ioflupane I 123) injection (also known as phenyltropane) in 2011 as a diagnostic test for Parkinson's Disease. PD diagnosis is confirmed with its related symptoms with DAT SPECT and finding in atypical tremors (unilateral postural, resting/ mixed tremors) with absence of bradykinesia or rigidity.

4.3. FDG–PET

2-Deoxy-floroglucose positron emission tomography (FDG-PET) is explored as a biomarker of progression of PD. Evidence has proved it to be of great help in progressive supra nuclear palsy (PSP), cortico basal degeneration(CBD) and accurately describes the mono aminergic changes in PD [31,49,51].

4.4. F-DOPA PET

Dopamine precursors are measured with 18F-DOPA which is fluoro-3,4-dihydroxyphenylalanine positron emission tomography (18F-DOPA PET). PET measures metabolic activities of tissues and cells of the body. 18F-DOPA had high diagnostic accuracy for PD with a high sensitivity of 95.4% and specificity and predictive value both of 100%. F-DOPA PET gives clear information about decline from caudate nucleus to the putamen observed in IPD patients of PD.Trans cranial sonography is done to confirm different stages of PD [53].

4.5. MRI: Basic MRI is not of much significance in differential detailed diagnosis of Parkinsonism, but 7 TESLA MRIgives detailed information and is of great significance in PD, it helps in early diagnosis, gives clear values of dopamine levels and also changes in brain structures such as basal ganglia [54].The areas of neuro degeneration atrophy in Brain is gyrus region of temporal, inferior, middle, parietal and occipital lobes and hippocampus, basal ganglia and amygdala. Research has always supported MRI in investigating and differentiating PD from DLB. In a systemic review study reveals atrophy in PD with PDD or PD with cognitive impairment. Recent approaches with 7 TESLA MRI compared to clinical history and features mark distinguishing in its diagnosis [55].

4.6. NMI: Neuro melanin imaging (NMI) is a boom to technology as it differentiates PD from other neurological progressive disorders with high reliability and sensitivity in more than 80% of population size. Its results are positive in interaction of pharma properties with neuromelanin. NMI has visual assessment of dorsal nigral hypersensitivity which is a distinct feature of PD. Neuromelanin is seen in substantia nigra and is possible biomarker for PD and DLB. Dopamine metabolism releases neuromelan in and is a polymer dark in color.

4.7. MICRO RNA (miRNA): miRNA is a form of RNA involved in genetics which can be isolated from CSF where it has shown discriminating results in PD and normal individuals thus making it easily accessible and minimally invasive bio marker in diagnosis and even in determining progression of PD. miRNA CELLS in brain had specific changes in cellular process of cells in PD individuals[30,56,57].Micro RNA's (Mi RNA's) and other small noncoding RNA are vast majorly explored areas in PD serum biomarker in Plasma exosomes. PD Rating Scale (Unified) confirmed to be reduced with reduced MiRNA's in blood related to alpha synuclein [38].

4.8. CSF: CSF examination is widely used in investigation of medical, neurological, autoimmune and inflammatory disorder[58].As with medications in PD there is exchange via blood brain barrier so CSF examination is of high validity and reliability in confirmation of PD and its prognosis [59].

4.9. GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP*)*

Among many types of neurons in CNS one is astrocytes containing GFAP (glial fibillary acidic protein) in the form of filament in the astrocytes cells. Astrocytes are majorly seen in Brain and injury to it releases increased levels of GFAP in CSF and serum confirming some insult to Brain. Thus, elevated levels of GFAP and its breakdown products are suggestive of progressive neurological disorders like PD, Alzheimer's, Dementia [60]. PD progression and degeneration is precisely tracked with GFAP[61]. GFAP levels are inversely proportional to cognitive function in PD when documented with MMMSE [62].Wang's group in recent study with raised levels of GFAP plasma predicted PDD conversion in PD patients over 3 years follow up with mild cognitive dysfunction[63].Another study of both GFAP and NfL together distinguished patients of PD from REM sleep disorders[64].

4.10. Beta-Amyloid 1-42, Tau, P-Tau

Track of evaluation of movement, motor and cognitive impairments can be correlated with reduced levels of bio markers beta-amyloid 1–42, tau, and phosphorylated-tau (p-tau) in patients with PD. Higher levels are seen in PD Dementia compared to PD with cognitive impairments[65,66].

4.11. Neuro Filament Light Chain (NfL)

Similar to GFAP, is NfL its raised levels are seen in CNS of patients with some neurological insult. Researchers have confirmed higher levels of NfL in PD subtypes, PD Plus more than in PD Studies have confirmed higher values of NfL in PD blood samples irrespective of motor symptoms. [67]. Early PD development was seen twice elevated levels of NfL with demographic levels details predicted PD development 5 years before the clinical diagnosis [68]. NfL levels are of great importance in confirming PD, its types and other movement disorders.

To drive any conclusion on the clinical application NfL levels, Sun's group has compared NfL level in various age match groups with PD and healthy control. Study included 146 PD patients, 82 essential tremor and 40 healthy individuals as control group. A test has been created by considering the cut-off value and it was 77% sensitive and 86% specific against essential tremor patients as well as comparable against control group [69]. This study reported NfL level as a biomarker in PD but of limited clinical use due to inability to distinguish tremor patient and PD [70].

Another group of researchers, created assays using plasma NfL that could distinguish between PD patients and patients with MSA and PSP each, along with Parkinson's plus syndromes, with a sensitivity and specificity of about 90 %, which was comparable to results derived from CSF NfL. This study included 153 patients with PD, 80 patients with MSA, 58 patients with PSP and 64 patients with DLB [71].

4.12. Amyloid and TAU

Though with fewer research, still serum biomarker amyloid and tau are important biomarker of PD [72]. Study by Kapogiann is group forward that p-tau levels are significantly high in PD patients [73]. Glial and immune cells are released as a result of neuron inflammation in PD and neuro inflammation results from PD pathogenesis [74]. Many studies have been done on PD with inflammatory bio markers like CRP, cytokines and other interleukins in serum. Systemic study and meta-analysis result proved increased cytokine levels (IL-6, TNF- gamma and IL-beta) in serum of PD [75].

5. CONCLUSION

Parkinson's disease remains a great challenge for medical professionals with its profound symptoms and implications for patients and families. Despite recent advances in its diagnostics, simplification of biomarkers with high reliability is still under process of research. Increasing work on PD also lay down the foundation of greater knowledge of PD among men and women in detail explaining from initial to progression, response to pharma and other therapies differ greatly among both gender and various age groups. The International Parkinson and Movement Disorder Society has proposed for a research diagnosis of prodromal Parkinson's disease. These practices provide an evidence-based frame work to work statistically for future Parkinson's disease at an individual level based on a large set of well featured markers of PD. Finally, there is a need to better define Parkinson's disease subtypes, which not only have different clinical presentation and prognosis, but also differ in underlying disease mechanisms, calling for personalized treatment approaches.

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