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Reviewl Article

Inflammatory Biomarkers for Parkinson's Disease

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Abstract: Parkinson disease (PD) is caused by degeneration of dopaminergic neurons. Clinically it is characterized by bradykinesia, rigidity and resting tremors. The central hallmark of the PD is accumulation of alpha synuclein (α-SN). Aetiology of the PD includes exposure to the environmental toxins, low serum urate, genes and sporadic forms of the disease. Till date PD is a clinical diagnosis with almost 80% of dopaminergic neurons disintegrated when motor signs begin to appear. Therefore, there is urgent need for the definite biomarkers that indicate death of the dopaminergic neurons and provide accuracy in diagnosis. The cerebrospinal fluid (CSF) and imaging biomarkers are already available with varying and inconsistent results. Genetic biomarkers have also significantly contributed in revealing the physiological and pathological process underlying the PD. Most important of them is the leucine rich repeat kinase (LRRK2) which has role in both sporadic and familial forms of the PD. Inflammation appears also to be an important player in PD pathogenesis. The crosstalk between immune system of the CNS and peripheral immune system results in generation of cytokines and chemokines that are useful inflammatory biomarkers. Importantly LRRK2 expressed in different immune cells of the innate immunity which further strengths the role of inflammation in the PD pathogenesis. Hence inflammatory biomarkers could aid in early diagnosis of the PD, helps in identification of new pathways and novel therapeutic targets. Key words Parkinson disease (PD), leucine rich repeat kinase(LRRK2), MRI, innate immunity.

Introduction

PD is a neurodegenerative disorder, where the accumulation of α-SN aggregates is a central hallmark of the disease pathogenesis. However, the precise pathophysiological processes remain largely unidentified yet.[1] It is one of the most prevalent neurodegenerative disorders worldwide, affecting about 2%-3% of community older than age 65.[1] Until now 20 genes have been associated to the familial forms of the PD, whereas more than 20 genetic loci linked to increased susceptibility for the development of the PD were identified from genome-wide association studies (GWAS). Some of these genes seem to be particular relevant for the disease, including for example α-SN, glucocerebrosidase (GBA), parkin (PARK2), Pten-induced kinase 1 (PINK1), microtubule-associated protein tau (MAPT) and LRRK2.[2] The onset of PD can be characterised as juvenile (age < 21 yr), early onset (2150 yr) and late onset (generally >60 yr). In addition mutation carriers are clinically indistinguishable from idiopathic PD.[3] The clinical identification of PD is mainly based on the motor symptoms, which normally appears when 60-70% of the dopaminergic neurons are already deteriorated in the substantia nigra.[4] This depletion translates into the PD distinct motor signs, such as bradykinesia, rigidity and tremor.[5] Clinically PD is detected according to the UK Brain Bank Society Criteria based on motor signs and its upgrading by dopaminergic medication.[6] Besides disabling motor symptoms, PD development also comprise of noteworthy non-motor symptoms (NMS), such as depression, anxiety, fatigue, and cognitive decline.[7,8] The neuropathological alterations linked to these NM seems to result from α-synuclein containing Lewy bodies and Lewy neurites in the peripheral autonomic nervous arrangement and from the neuronal loss in the dorsal motor nucleus of the vagal nerve, the olfactory bulb, and the lower brainstem nuclei that control random eye movement (REM) sleep atonia.[4] The NMS anomalies of the PD includes dysautonomia, hyposmia, and REM sleep behaviour disorders (iRBD), that have been linked to the PD in people and cohort studies. Inflammation appears also to be an important player in PD pathogenesis. Immune alterations have been reported in the peripheral immune system, with altered cytokine levels and monocyte and lymphocyte subsets. Old age is the main risk for development of the PD. Similar to other immune cells, microglia exhibit age-dependent alteration. Studies from human and animal models suggest that inflammation-derived oxidative strain and cytokine-dependent toxicity add to nigrostriatal

path deterioration Furthermore, degeneration of dopaminergic neurons that releases of reactive oxygen species (ROS), chronic activation of microglia, and mitochondrial dysfunction all seems to contribute towards the neuroinflammation observed in the PD patients. [9] The inflammation statistics so far has largely focus on the risk of developing the PD. However, the significant issue is whether the immune reaction manipulates the pace of the PD development after its clinical identification. This issue is of special significance since the treatment should start with immunomodulatory drugs in the early phase of the PD. However, prior to this, proper immune-related biomarkers needs to be identified to ease the prospective tests of immuno-modulatory therapies to hinder disease progression in the PD patients. Inflammation biomarkers of the PD have achieved attention as probable, early markers of neurodegenerative disease course and may have a prognostic value. So far, the detection of biomarkers for the PD has focused exclusively on neuronal proteins (e.g., α-SN, tau, and β-amyloid). This is because these proteins are acknowledged to play a role in the primary pathophysiology of other neurodegenerative diseases including the PD.[9] Collectively, the data suggest that immune activation happened in the PD and play a vital role in its development in the central nervous systems (CNS) as well as the peripheral nervous system (PNS). Here we discuss recent advances in novel inflammatory significant biomarkers and how the cross-talk between the immune system of the brain and peripheral immune system give rise to the inflammatory biomarkers. Importantly cells of the immune system are potential biomarkers involved in the progression of the PD and will be discussed in detail here. Finally, the perspectives linked with inflammation biomarkers and therapeutic significance related to the PD will be concluded.

I. PD & biomarkers

a.CSF and blood based biomarkers in PD:

Biomarkers in body fluids and tissues may provide an effective route to detect proteins and other molecules correlated with the early diagnosis and progression of PD.[18] As multiple disease processes might coexist in PD, combination of different biomarkers that reflect each contributing pathogenic mechanism is likely to be the most appropriate approach. For example, tau and α-SN pathology coexist in PD, as well as in AD and dementia with Lewy bodies (DLB).[19] In the cerebrospinal fluid (CSF), α-SN and related molecules emerge as the most promising biomarkers,[19] although several others were also investigated such as urate or Aβ42 among others. [20] Data showed decreased tau and α-SN in the PD but inconsistency was found regarding other biomarkers i.e Aβ42, DJ-1, 8-OHdG and urate in the PD and related disorders. Nevertheless, these CSF biomarkers provide good indication of cellular turn over regarding pathways in the aging brain and subsequently the disease prognosis.

b.Genetic Biomarkers of PD:

Increasing evidence suggests that both genetic and environmental factors contribute to the aetiology of the PD. This means if a person suffered PD due to duplications, triplications or missense mutations in a gene even in these patients, age-related physiological alteration or environmental exposures contribute to the disease progression.[21] Several loci termed as PARK accounting for PD were identified in the last years. Almost half of the genes identify for PD affects the patients in a dominant manner and rest half exerts their expression in a recessive pattern.[21] The clinical symptoms varies with respect to genetic mutation in the PD. Some of the patients show more of the autonomic and resting tremors features than the others and vice versa however the decline in quality of life remains the same with the passage of time.[22] This suggests that pathogeneses varies with mutation in genes and not only disintegration of dopaminergic neurons but involvement of some other parts of the CNS occurred in the PD. The effect of the genetic alteration in the PD patients with regard to the symptoms could help in the clinical diagnosis in the future. However, the genetic testing for the PD currently used as a research tool. Some of the important genes with their inheritance and clinical features are summarized in **Table-1.**

II. Imaging Biomarkers of PD

It is well established that PD results from disintegration of dopaminergic neurons in the CNS[23] Collectively dopaminergic, serotonergic and cholinergic neurons contribute to the overlapping clinical features and presentation observed in the PD and parkinsonism. Thus, it is imperative to scan the vital structures of the brain supplied by these neurons to check their integrity in the aging brain. Substantia nigra pars compacta is especially important with regards to the dopaminergic neurons. Caudate, putamen, and Raphe nuclei are vital with regard to serotonergic neurons in patients with tremor-

Table-1: The genes with their inheritance pattern, clinical features and pathogenesis Adapted and modified.[22] Abbreviations: AD: autosomal dominant, AR: autosomal recessive.

Gene	Age of onset with inferitance	Features	Pathogenesis
SNCA	Early onset with AD	Classical PD to a more atypical	Diffuse lewy body (DLB)
PARKIN	Very early onset with AR	Classical PD	Inactivation of its E3 ligase function
PINK1	AR	Atypical features	Typical LB pathology
DJ-1	Very early onset with AR	EOPD	Increase oxidative stress and mitochondrial damage
LRRK2	AD	Classical PD	Diverse pathology comprising Lbs pathology and tauopathy with neurofibrillary tangles
Vacoular protien sorting associated protien 35 (Vps-35)	Old onset with AD	Classical PD	Disturs endosomal lysomomal trafficking

Table-2: Table described different imaging modalities with their functions specifically in the PD and related parkinsonism. (modified from literature).[25]

Imaging Modalities	Assessment in the PD and parkinsonism
SPECT	DAT-SPECT is particularly useful for the differential diagnosis between PD and nondegenerative parkinsonism, such as drug-induced parkinsonism, essential tremor (ET), dystonic tremor, or psychogenic parkinsonism
PET	PET measures the integrity of dopaminergic, serotonergic and cholinergic neurons in the PD and parkinsonism. It also assess the role of glutamatergic transmission, Cannabinoid type 1 receptors, opioid receptors A, adenosine receptors and Sigma 1 receptors.
Volumetric MRI	Gray matter (GM) changes have been assessed with voxel-based morphometry and cortical thickness analyses in PD.
T2 and T2*, and susceptibility -weighted imaging (SWI)	Iron accumulation in the brain can be detected in brain nuclei using sequences sensitive to local magnetic field in homogeneities.
Diffusion-weighted imaging and diffusion tensor imaging (DTI)	Useful to detect changes in white matter (WM) integrity in the PD and related disorders.
Functional MRI (rs-fMRI)	Measure the blood oxygenation level-dependent signal when subjects are positioned in the scanner in an awake-state without performing any particular task.
Task-Related Functional MRI	Investigate brain activity in patients with PD in order to elucidate pathophysiological mechanisms underlying PD symptoms and complications.

dominant PD. Putamen, the insular cortex, and the supplementary motor area and lower in the caudate nucleus, the orbitofrontal cortex, and the middle temporal gyrus are important in relation to the cholinergic neurons.[24] Imaging could provide information in the PD patients regarding anatomy and functional status of the whole brain and its important structures. PET radioligand scan applied can check the integrity of the projecting neurons in these brain regions. Proton magnetic resonance spectroscopy (1H-MRS) show gross physical changes (structural MRI) and metabolic changes at synaptic levels.[24] Studies have shown mixed and inconsistencies in results when comparing PD and healthy controls and between PD and parkinsonism.[25] Nevertheless, imaging provides good picture of the anatomical, metabolic and integral changes to study the disease details thus enhancing our diagnostic and treatment precision. **Table-2** highlights some of the functional performance of different imaging modalities in the PD patients.

II. Importance of LRRK2 gene in PD Neuroinflammation:

There are many genes involved in aetiology of the PD but the LRRK2 is only gene discovered so far with familial and sporadic form of the PD. The clinically indistinguishable symptoms of the LRRK2 from the idiopathic form of the disease points towards the same pathogenic process. LRRK2 has established role in inducing mitochondrial functioning, inflammatory responses, apoptosis, deregulating the immune system and endocytosis.[22] The role of the LRRK2 in immune system has been established too. It is well documented that LRRK2 expressed on the immune cells. The highest expression found in the macrophages followed by the B-cells and the dendritic cells.[28] The macrophages and DCs are the antigen presenting cells (APCs) of the innate immune system that are professional in presenting cells to the adaptive immune system. The function of microglia in the CNS is the same as that is performed by the macrophages in the peripheral immune system. Presentation of the processed antigen by the

macrophages activates the T-lymphocytes of the adaptive immunity that release cytokines to activate B-cells to generate immunoglobulin against the antigen. Importantly the antibodies produce by the B-cells can crossed the BBB and exert their influence via Fc receptors present in the microglia.[29] Figure 2 describes the axis through which B-cells antibodies activates the inflammatory process in the CNS. However the influence of the LRRK2 mutation in the B-cells antibodies differentiation process is still unclear.

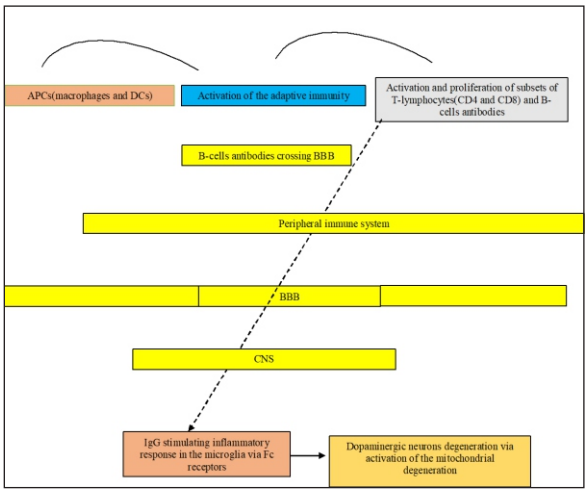

Fig-1: Schematic diagram showing the crossing of activated antibodies(IgG) that has specification for the Fc receptors in the microglia.

II. Biomarkers of immune dysregulation:

Studies showed a role of innate and adaptive immune system dysregulation in the development of the PD and its progression. The macrophages, neutrophils, natural killer cells, T-cells and B-cells have documented role at the cellular level. [30] T lymphocytes have a vital role in igniting and worsening of the PD. Importantly the T-cells occupies different dopaminergic receptors on their surface. With the introduction of most commonly used drug levodopa in the PD, a change happened in these receptors. With this alteration the a-synuclein presented to the T-cells with the help of MHC processed in a different way via dopaminergic receptors than it was done previously leading to abnormality in the pathways. Indeed, a-synuclein is a pathological hallmark of the PD and its proper disposition is vvital to halt the neuronal disintegration in the PD. Figure 3 depicts the simple pathway of discarding of a-synuclein via T-cells and the potential biomarkers originating in doing so. An important aspect in diagnosing the PD is its early detection with non-motor symptoms(NMS) as they precede the development of motor signs many years. Different cytokines have been found in the PD with non motor features. C-reactive protein (CRP), interleukin-6, tumor necrosis factor-alpha, eotaxin, interferon gamma-induced protein-10, monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein 1-β are in higher amount in the CSF of the PD patients. These cytokines are related with the NMS of depression, anxiety, fatigue, and cognition. [29]

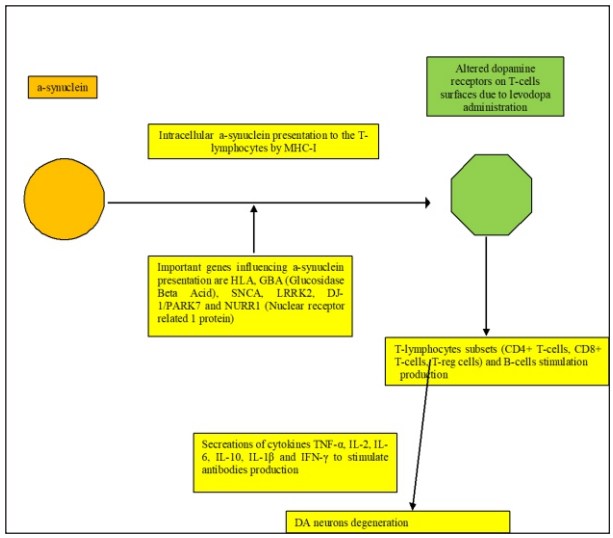

Fig-2: Immune system dysregulation generates specific biomarkers in the form of T and B lymphocytes and their subsets. The cytokines and important genes influencing the pathways provides special biomarkers for the PD.

II. Human leukocyte antigen(HLA) association to neuroinflammation in the PD:

The overexpression of PD linked genes disturb the countenance of pro-inflammatory cytokines. Besides this there are immunological genes that have a role in inflammatory process of the PD. The HLA complex is a gene complex encoding the MHC proteins which are accountable for the regulation of the immune system in humans. Association between PD and the HLA region was found on chromosome 6p21.3. It is particularly strong for sporadic and late-onset PD and men. Studies has identified four loci, including the HLA region, that contain a secondary independent risk variant for PD. This exerts an effect independently of the primary risk allele. The antigen-presenting cells, including microglia in the brain interact with T-cell receptors protein chains encoded by the closely linked HLA-DRA and HLA-DRB.

III. Conclusion and future directions:

To date PD is a clinical diagnosis and there is an urgent need to develop biomarkers that shows death of dopaminergic neurons well before the symptoms appears. Aging is the physiological process and biggest risk factor for the PD. The discovery of LRRK2 as a neurodegenerative process. Moreover whether inflammation in the CNS accelerate the neurodegeneration after its clinical identification needs investigation. This query is important in order to elucidate the role of anti-inflammatory and immunomodulatory therapy to slow the neurodegeneration in the PD.

Culprit gene for idiopathic and sporadic forms of the PD opens the gate for better understanding of the PD. In addition the LRRK2 expression in the immune cells (mononuclear, dendritic, T and B lymphocytes) further enhances the role of inflammation in the PD. The periphery inflammation is ill-studied in the PD and parkinsonism especially in the early prodromal phase when the NMS dominates. This is vital since most of the movement controlling neurons have been damaged when the motor signs starting to appear in the PD. However, efforts with large cohorts required to detect the inflammatory cytokines and chemokines at NMS stage of the PD. Since current drugs recommended for the PD depends on the disease features but levodopa remains the first choice of the treatment among clinicians. The studies of different genes and their influence on the inflammatory cells especially B cells provides new pharmacological targets and subsequently delaying the neurodegenerative process. Moreover whether inflammation in the CNS accelerate the neurodegeneration after its clinical identification needs investigation. This query is important in order to elucidate the role of anti-inflammatory and immunomodulatory therapy to slow the neuro-degeneration in the PD.

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