SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

THIRUVANANTHAPURAM, KERALA

LIPID PROFILE IN PATIENTS WITH PARKINSON'S DISEASE - A CROSS-SECTIONAL COMPARATOR GROUP STUDY

Thesis submitted in partial fulfilment of the rules and regulations for PDF Movement Disorders course of

Sree Chitra Tirunal Institute for Medical Sciences and Technology

By

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PDF Movement Disorders

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Department of Neurology

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2020

DECLARATION

I, Dr. Saraf Udit Umesh , hereby declare that this project was undertaken by me under the supervision of the faculty, Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology.

Thiruvananthapuram Dr.Saraf Udit Umesh
Date: 30-12-2020

Forwarded :

The candidate, Dr Saraf Udit Umesh, has completed the project under my guidance.

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Date:30/12/2020

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Thiruvananthapuram
Date: 30-12-2020

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INTRODUCTION

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease presenting with motor and non-motor symptoms, with pathological hallmarks of α-synuclein immunopositive Lewy neurites and Lewy bodies in the dopaminergic system, predominantly substantia nigra. Updated discoveries have highlighted that four main processes – oxidative stress response, endosomal-lysosomal functioning, endoplasmic reticulum stress response, and immune response activation— interact with each other and regulate dopaminergic neuron function and death in PD[1]. Interestingly, lipids and lipoproteins are functionally involved in and influenced by all these processes and affect dopaminergic neuron-specific signaling cascades[1, 2]. The loss-of-function mutation identified in the *LPA* gene provided the first genetic link that directly connects cholesterol metabolism and neuronal cell death in PD.

Several population-based studies point towards the role and influence of cholesterol metabolism in PD[2-5]. The serum levels of total cholesterol, low-density lipoprotein – cholesterol (LDL-cholesterol), very low-density lipoprotein-cholesterol (VLDL-cholesterol) and triglycerides have been found to be significantly lower in PD patients as compared to controls[4]. This low level of cholesterol has been linked with a decrease in cholesterol biosynthesis in fibroblasts from PD patients[6]. Although most studies have shown association between lower cholesterol level and PD, a meta-analysis failed to establish an association between serum cholesterol and PD risk, suggesting that direct cholesterol intake through food may not play a major role in PD aetiology[2]. In contrast to population-based studies, a high-level of brain cholesterol seems to aggravate the PD phenotype in an animal model system. For example, a high cholesterol diet has been shown to raise brain cholesterol level and exacerbate 1-

methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) induced neurodegeneration in PD[7]. Similarly, a cholesterol overload induced in SH-SY5Y cells showed a decrease in dopaminergic neuronal survivability via increased depolarization of mitochondrial membrane potential[8]. The relationship between neuronal cell death and cholesterol is probably mediated via α-synuclein. High cholesterol is suggested to increase *α*-synuclein levels via proteasomal inhibition in human dopaminergic neurons, which further suggests that dysfunction of cholesterol metabolism increases the *α*-synuclein aggregation in PD[9]. PD landscape showed the involvement of lipid/lipoprotein in PD pathogenesis, and an ongoing lipidomics study using the tg-snca rat model also corroborated the genetic findings, thus suggesting that lipid pathway is important in understanding PD mechanism.

With this background, we planned our study to compare the lipid profile parameters, including lipoprotein A, in patients with Parkinson's disease with that of normal healthy controls in an Indian population.

REVIEW OF LITERATURE

1. Parkinson's disease (PD)

PD is the second most common neurodegenerative disease, second only to Alzheimer's disease, affecting 1% of the population above 60 years and up to 4% of individuals in the highest age groups.[10] Its current diagnosis is based on a clinical examination and presence of bradykinesia, rigidity, and/or rest tremor.[11] PD patients also suffer from non-motor symptoms like depression, hyposmia, sleep disorders, cognitive impairment, and hallucinations.[12] These symptoms are associated with a progressive loss of dopaminergic (DA) neurons from the nigrostriatal pathway, formation of Lewy bodies (LBs), and microgliosis[13]. In familial PD, which constitutes 5–10% of all cases, these abnormalities may be caused by a mutation in one of the thus far known 19 familial genes, including *SNCA, LRRK2, PRKN, PINK1* and *DJ-1*, among others[14]. The remaining 90–95% of PD cases are of sporadic nature, with both genetic and environmental contributing risk factors.

2. Pathophysiology of PD

The main pathological features of PD are the loss of dopaminergic neurons with subsequent depigmentation of the substantia nigra pars compacta (SNc) and the presence of LBs. LBs are intraneuronal, round, eosinophilic inclusions with a hyaline core and a pale peripheral halo that are composed of more than 90 proteins[15]; their main components are a α-synuclein (αS) and ubiquitin[16]. It has been estimated that at the time of the diagnosis, up to 60-65% of dopaminergic neurons have already been lost[17, 18].

SNCA, the gene encoding for αS, was the first gene linked to PD[19]. Pathogenic mutations confer to αS a greater tendency to misfold and aggregate than the wild-type; or affect the quantity of αS (either through duplications or triplications, either altering its expression or its clearance), and alter its post-transcriptional modifications, and/or its interaction with other cellular organelles and transport systems. Current evidence has highlighted the role of αS in activating immunological response, and it has been demonstrated that activated microglial cells directly engulf αS in an attempt to clear it[20]. Notably, upregulation of the expression of αS has also been found in patients with idiopathic PD[21].

αS has the propensity to misfold, become insoluble and form β-sheet-rich amyloid aggregates that accumulate and form intracellular inclusions. The intermediates in this aggregation process are the toxic oligomeric and proto-fibrillar forms that impair mitochondrial[22], proteasomal and lysosomal[23] function, damage biological membranes[24] and cytoskeleton[25], alter synaptic function[26] and cause neuronal degeneration.

3. Role of αS in the pathogenesis of PD

Oligomeric αS was first observed in in-vitro studies on the aggregation of recombinantly produced αS[27]. These αS aggregates may be harmful to dopaminergic neurons in the SN, especially when αS is present in the form of toxic oligomers. αS oligomers were reported to be present in postmortem brain of patients with PD[28], cell line cultures[29] and neurons[30].

Aggregation of αS tends to spread through neurons in a prion-like manner. This propagation occurs most likely by exosomes,[31] and this mechanism of transmission probably underlies the progression of pathological alterations[32]. Some data suggest that αS aggregation may begin in the autonomic plexi of the gut and spread rostrally[33] and this could be influenced by the gut microbiome[34]. A sequential model of the deposition of αS and formation of Lewy bodies, initiating in the dorsal motor nucleus of the glossopharyngeal and vagal nerves and anterior olfactory nucleus, with gradual spread to the brain stem, and finally to the neocortex, has been proposed[35].

αS can be secreted from cells into the extracellular space and is found in human body fluids, including the cerebrospinal fluid (CSF) and blood.[36] This has led to considerable interest in αS as a potential biomarker for PD and other synucleinopathies.[37]

4. Interaction of α-synuclein with other molecules

During biogenesis, proteins form multiple interactions with other proteins for proper folding, modification, transport, regulation of activity, recycling, and degradation. Majority of these interactions occur co-translationally when proteins are being synthesized on the ribosome. Alteration of such interactions can interfere with protein biogenesis and lead to multiple human disorders. αS protein-protein interactions can occur on a co-translational and a post-translational level. Starting at the early stage of translation, the nascent chain is already exposed to a variety of interacting partners, which includes chaperones/chaperonins, translocating and targeting factors, modifying enzymes, and others.[38]

5. Role of lipids in Neurological disorders

A sufficient availability of cholesterol is necessary for normal neuronal function and morphology, and both a lack and surplus of cholesterol can cause impairment[39, 40]. Cholesterol in neurons can be synthesized by neurons themselves[41] and can also be taken up from other cells within the CNS, namely, from oligodendrocytes[42]. Study by Ko et al.[39] shows that cholesterol demand and the optimal cholesterol concentration for neurite outgrowth depend completely on the neuronal type and that the mechanism underlying the effect of cholesterol on neuronal maturation involves the attainment of the optimal cholesterol concentration. Consensus is that cholesterol in the brain is insulated from changes in circulating cholesterol.[43] Although the mature brain contains 25% of total body cholesterol, essentially all brain cholesterol is synthesized de novo in astrocytes via the mevalonate pathway, and is then transported to neurons via endocytosis and interaction with the LDL receptor and apolipoprotein E. But, nearly all 24S-hydroxycholesterol present in human circulation is of cerebral origin, and its level may be used as a surrogate marker for brain cholesterol homeostasis.

Evidence of a possible link between cholesterol and neurodegeneration has accumulated in the last few years. Some of the earliest observations of this link were the recognition of ε4 isotype of apoE as an important risk factor for late-onset Alzheimer disease[44] and that hypercholesterolemia was associated with increased brain Aβ immunoreactivity in rabbits.[45]

A molecular landscape of Parkinson's disease has been proposed, guided by the most significantly enriched genetic network and extensive literature searches.[1] Lipid and lipoprotein signalling represents the ''common denominator'' that functionally

integrates, regulates and is regulated by the landscape processes - increased oxidative stress response, dysregulation of endosomal-lysosomal functioning, endoplasmic reticulum stress response and exaggerated immune response. Individually or in combination, deficits or impairments in any of these processes can contribute to the degeneration and ultimately death of DA neurons.

6. Animal and Cellular Studies on Cholesterol in Parkinson's disease

The association of cholesterol metabolism with PD in human studies is debatable, and the effect of cholesterol on the phenotype of PD in model systems is also conflicting.

A protective role of higher plasma cholesterol levels on PD risk is biologically possible. The proposed potential mechanisms focus mainly on cholesterol biosynthesis and expenditure. Individuals with PD may have on average lower cholesterol biosynthesis but a higher cholesterol expenditure, compared with others.[6] For instance, a cholesterol precursor, lanosterol, was found to be significantly reduced in the nigrostriatal region of a PD mouse model, indicative of altered lanosterol metabolism during PD pathogenesis. Exogenous addition of lanosterol was found to resurrect dopaminergic neurons from death.[46] Cholesterol is one of the essential components of cell membrane and synaptic vesicle.[47] Neurons are unable to produce sufficient cholesterol to support synaptogenesis and are therefore dependent on cholesterol through other sources.[48] Because there is increased need of cholesterol metabolism during neuron repair and remodelling, it is possible that higher levels of cholesterol facilitate neuron repair and thereby reduce the risk of PD. Increased cholesterol reduces cell death[49] and modulates presynaptic DA phenotype by

increasing TH and VMAT2 expression in SH-SY5Y cells[50], a human neuroblastoma cell line, and enhancing ligand binding of DAT and VMAT2 in the brains from rats and monkeys[51].

However, the high cholesterol level incorporated into differentiated SH-SY5Y cells worsens dopaminergic neuronal survivability through increased depolarization of mitochondrial membrane potential[8]. Increased αS aggregation is seen when the neuronal cultures are grown in cholesterol-rich medium[49]. High cholesterol diet has been shown to increase brain cholesterol level and exacerbate 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced reduction of striatal dopamine and dopaminergic neurons in the substantia nigra with motor manifestations in mice[7]. Hypercholesterolemia seems to cause DA neuronal loss and oxidative stress in the SN and the striatum, leading to motor impairment[7].

These findings suggest that the effect of cholesterol levels on PD may be dose dependent. Cholesterol also modulates the development of PD through other pathways. For example, iron deposit and accumulation have been implicated in the pathology and pathogenesis of PD and substantia nigra might be the primary region for iron deposit.[52] It should be noted that SH-SY5Y cell studies in which cholesterol levels are modulated might give ambiguous results due to its effect on cell differentiation.[53]

7. Interaction of αS with lipids

αS is concentrated in presynaptic terminals, where it has been proposed to be involved in synaptic vesicle docking, fusion, clustering, and homeostasis[54]. As these functions occur on the lipid membrane surface, the ability for αS to interact with lipids is essential[55]. αS–lipid interactions are modulated by lipid composition, membrane fluidity, and curvature. N-terminal and C-terminal regions of αS can bind to fatty acids.[56] αS has a strong propensity to bind to lipid membranes, especially the regions enriched in cholesterol. It can interact with the cholesterol component of lipoproteins via its cholesterol binding domain[57]. α-Synuclein shares marked structural similarities with ApoE[58] and contains two cholesterol-binding domains. α-Synuclein has been reported to form structures that are similar to nascent lipoproteins, which are the premature forms of the larger spherical lipoproteins (i.e., HDL)[59], and interact with lipoproteins in human plasma[60]. Emamzadeh et al.[60] found that αS appears to interact either directly or indirectly with apoA1, apoE, and apoJ. All these apolipoproteins are found in the HDL sub-fraction of lipoproteins. It is hypothesized that HDL, containing apoA1, may be involved in the transport of αS out of the brain. The intermediate fraction between HDL and LDL is referred to as lipoprotein (a) (Lp(a)) and is a risk factor for cardiovascular disease. Presence of αS in the intermediate fractions between HDL and LDL is suggested to be due to an interaction between αS and the high amount of cholesterol contained in the Lp(a) fractions. Large amount of ApoJ has been detected in the Lp(a) fractions, suggesting a possible role for apoJ in the trafficking of αS through the BBB. ApoJ is mainly produced by astrocytes and along with apoE is more abundant in the brain than any other apoprotein, and both

interact with LRP. Extracellular αS may function similarly to apolipoproteins, and extracellular αS may promote cholesterol transfer.

αS has role in brain cholesterol homeostasis[61], together with these lipid-binding properties[59]. Cholesterol biosynthesis is reported to be decreased in fibroblasts from patients with PD owing to reduced β-Hydroxy β-methylglutaryl-CoA (HMGCoA) reductase activity[6]. As cholesterols are critical for membrane biogenesis, decreased cholesterol biosynthesis has the potential for profound toxic synergy with αS-induced vesicle trafficking defects.[62] Upon binding, αS can influence lipid packing within the bilayer, induce clustering of vesicles, and generate formation of curved lipid structures, such as tubules and nanodiscs[63].

αS -cholesterol interaction seems to be associated with αS accumulation[49, 64] and aggregation[65] and is a determining factor in αS ability to form pores[66]. Accordingly, reducing cholesterol levels leads to decreased αS accumulation and damage in the synapse[67]. Hence, high levels of cholesterol aggravate αS associated pathology. Furthermore, αS potentiates cholesterol efflux[68], antagonizes cholesterol in lipid rafts[69] and enhances production of oxidative cholesterol metabolites[70]. Finally, A53T-αS -overexpressing mice have increased levels of serum cholesterol[71] , while WT- αS -overexpressing mice have upregulation of genes involved in cholesterol biosynthesis in DA neurons from the SN[72], indicating a tight reciprocal relationship between α-synuclein and cholesterol metabolism.

Cholesterol mediates the interaction of oligomeric α-synuclein with the cell membrane, leading to membrane disruption and cell death[73]. Isopentenyl diphosphate isomerase, a cholesterol-synthesizing enzyme, is localized in Lewy bodies. This

suggests that cholesterol metabolites may play a role in the aggregation of α-synuclein, enhancing Lewy body formation[74].

Increased levels of oxidized cholesterol metabolites in Lewy body disease brains accelerate αS fibrillization[70]. α-Synuclein aggregation was found to increase at low concentrations of ApoE and decrease at high concentrations of ApoE[75]. 27-HC increases αS protein levels through proteasomal inhibition in human dopaminergic neurons[9], suggesting that dysfunctions of cholesterol metabolism may also induce the aggregation of α-synuclein, thus causing PD. These studies corroborate that α-synuclein may influence cholesterol metabolism and cholesterol may also contribute to the aggregation of α-synuclein.

8. Genetic evidence for the role of lipids in PD

Many genetic factors associated with familial PD are reported to be involved in cholesterol metabolism. Increased cholesterol levels have been observed in the brain of α-synuclein transgenic mice[76]. Brain cholesterol, cholesteryl ester, and triacylglycerol mass have also been reported to increase by 1.1-, 1.6-, and 1.4-fold, respectively, in *SNCA* knock out (KO) mice[61]. A E3 ubiquitin ligase, parkin, of which genetic mutations were considered a common cause of early onset PD[77], is also associated with cholesterol metabolism. It has been reported that the membrane fluidity is decreased and the total cellular cholesterol level is increased in parkin deficient mouse embryonic fibroblast (MEF) cells, thus dysregulating lipid rafts-dependent endocytosis[78]. Parkin has been reported to be a lipid-responsive regulator of fat uptake, and the increase in serum cholesterol level by high fat diet is less

pronounced in parkin KO mice[79]. DJ-1, a multifunctional protein and a causative gene product associated with autosomal recessive familial PD[80], has been reported to be associated with cholesterol metabolism. DJ-1 deficiency in astrocytes causes increase in membrane fluidity, decrease in cellular cholesterol level, and decrease in lipid rafts-dependent endocytosis[81, 82]. In addition, cholesterol supplementation rescues the synaptic endocytic defects observed in DJ-1-deficient neurons[83]. Reduced expression of LDLR mRNA and protein has been observed in DJ-1-knockdown cells and DJ-1 KO mice[84]. Elevated plasma cholesterol level in leucine-rich repeat kinase 2 (LRRK2) KO rats has also been reported[85]. Additionally, parkin, PINK1, DJ-1, LRRK2, and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) have been elucidated to be associated with lipid rafts in in-vitro models[81, 86-89]. The molecular pathways of neurodegeneration triggered may be shared by several genetic forms of PD, and may play a common role in its pathogenesis. As each mutation of genetic factors alters cholesterol metabolism, alterations in cholesterol metabolism can contribute to the pathogenesis of PD.

Mutations in the gene encoding the lipid-producing enzyme glucocerebrosidase (GBA) are associated with familial PD[90]. GWAS have identified several proteins that help regulate lipid metabolism, including lipid droplet biology, to be associated with PD. This has been confirmed by postmortem brain analyses. Multiple single-nucleotide polymorphisms (SNPs) located in other genes involved in lipid metabolism have also been linked to sporadic PD. ASAH1[91] and SMPD1[92] participate in ceramide metabolism, similar to GBA, and have been implicated as Parkinson's disease susceptibility genes. In addition, a diacylglycerol kinase, DGKQ, which controls diglyceride and phosphatidic acid content, emerged from several GWAS as a PD risk

factor.[93, 94] FA elongase 7, recently identified as a significant PD risk gene, is a determinant of fatty acyl side-chain length, and membrane composition and fluidity.[95] A phospholipase, PLA2G6, has been proposed to affect risk for PD and other brain diseases with brain iron accumulation[96]. Seipin, an integral membrane protein localized at endoplasmic reticulum (ER)/LD contact sites and involved in LD biogenesis and maintenance,[97] may be differentially expressed in the brains of PD vs. control subjects.[98] Knockdown of seipin has been found to rescue αS toxicity in the neurons. These findings suggest that phospholipid group and side-chain nature play a critical role in PD, likely through αS interaction alterations. LRP1 seems to be involved in α-synuclein efflux to the periphery in mice[99]. Variants in LRP10, a low-density lipoprotein receptor protein, is also reported be linked to PD dementia, DLB, and Lewy pathology,[100] but this remains controversial[101]. *SREBF1* encodes SREBP-1 (sterol regulatory element-binding protein 1), a transcriptional activator required for lipid homeostasis, which regulates cholesterol synthesis and its cellular uptake from plasma LDL, has been identified as a PD risk factor.[102] In addition to certain studies on statin use, this finding suggests that sterol pathways in PD pathogenesis should be considered significant.

9. Clinical evidence for the role of lipids in Parkinson's disease

Low plasma levels of ApoA-I, a major component of HDL, are found in PD[103] and associated with age at onset and motor severity in early PD patients[103, 104]. Lower apoA-I levels was also associated with greater putaminal DAT deficit evaluated by DAT dopamine transporter imaging in PD and in subjects at risk to develop PD.[104]

Additionally, low level of ApoA-I has been found in PD especially in the prodromal stage of the disease, supporting the role of plasma ApoA-I as biomarker for PD risk.[105] ApoE and LRP1 immunoreactivity are increased in melanized neurons of the substantia nigra in PD patients[106].

The analysis of the DATATOP trial showed that higher total serum cholesterol may be associated with a slower clinical progression of PD[107]. Epidemiologic studies,[108-111] including four prospective analyses,[112-115] have found that higher cholesterol levels are associated with a lower risk of Parkinson's disease (PD), while lower plasma cholesterol has been associated with PD.[116-121] Higher plasma cholesterol levels have also been linked to slower clinical progression of PD[107]. However, other studies, including a meta-analysis[122], have found no association between plasma cholesterol levels and PD[121, 123] or PD risk [124, 125]. Even higher plasma cholesterol levels in PD patients compared to controls[126, 127] have been reported.

HDL cholesterol

Lower plasma HDL have been associated with earlier PD onset[103] and higher PD risk[128, 129], and HDL levels are positively correlated with disease duration[130]. Plasma levels of HDL-cholesterol are lower[116, 131] or not different[119, 120, 127] in PD patients compared to controls.

LDL cholesterol

High LDL-cholesterol levels in plasma are protective for PD and associated with preserved executive and fine motor functions in PD[4, 123, 127, 132] and later age of PD diagnosis[133], while lower LDL-cholesterol levels are associated with higher PD risk[108, 114, 118-120, 134]. Also, PD patients seem to have higher levels of oxidized LDL

compared to controls[135], which is able to enter neuronal cells and elicit neurotoxicity[136]. Higher LDL-cholesterol in PD also has been linked to slower loss of motor and executive function[123] These data suggest that higher LDL-cholesterol status may be favorable for people with, or at risk for, PD. However, conflicting results have also been reported.[126, 137]

Triglycerides

A meta-analysis has revealed higher triglyceride levels to be statistically useful as protective factor for the onset of PD[138].

Lipoprotein(a)

Lipoprotein(a) is a low-density lipoprotein (LDL) particle with an added apolipoprotein(a) (apo[a]) attached to the apolipoprotein(b) (apo[b]) component of the LDL particle via a disulfide bridge. The structure of Lp(a) is highly heterogeneous secondary to many different apo(a) isoforms within the population. An individual's Lp(a) level is 80-90% genetically determined[139, 140] in an autosomal codominant inheritance pattern with full expression by 1-2 years of age and adult-like levels achieved by approximately 5 years of age. Apart from acute inflammatory states, the Lp(a) level remains stable through an individual's lifetime regardless of lifestyle. [141] The Lp(a) concentration was statistically lower in PD patients compared with controls in a Chinese population.[142]

10. Role of statins in Parkinson's disease

In a prospective study including 38,192 healthy men and 90,874 healthy women, it was found that the regular use of statins was associated with a modest reduction in PD

risk.[143] Risk of PD was lower in statin users as compared to non-users in diabetic patients.[144] Another study[145] demonstrated that statin use was associated with lower PD risk, after adjusting for smoking, caffeine, history of heart disease and hypercholesterolemia. The protective effect of statins was demonstrated in younger cohort (<60 years).[145, 146] As statins are known to lower cholesterol levels, this somewhat contradicts the reports from several case control studies which identified higher levels of cholesterol as potentially protective. One explanation for this could be that the beneficial anti-inflammatory or anti-oxidant effects of statins could compensate for the decreased cholesterol levels. These beneficial effects may be offset by their effect on lowering Coenzyme Q10 and urate levels.[147] Meanwhile, one study found that the use of statins was associated with increased risk of PD.[148] Studies that did not adjust for cholesterol suggested a protective effect of statins on the risk of PD, and the protective effect was not observed in studies that performed adjustment for cholesterol.[149]

AIM AND OBJECTIVES

Aim of the Study

1) To compare the lipid profile parameters in patients with Parkinson's disease with that of normal healthy subjects.

MATERIALS AND METHODS

Materials and Methods

Subjects:

Patients with diagnosis of Parkinson's Disease were recruited from Movement Disorder Clinic. Age and gender-matched controls were recruited from the friends of patients / visitors to the hospital and from the community. All patients were recruited after informed consent from patient/caregiver.

Sample size:

Based on the number of patients with Parkinson's disease attending Movement Disorders clinic over duration of 6 months, 150 patients and 150 controls were planned to be recruited.

Inclusion criteria:

1. Patients with diagnosis of Parkinson's Disease as per UKPDS Brain Bank Criteria[150]

Exclusion criteria:

1. Patients on treatment with statins or other lipid lowering drugs

Methodology:

Consecutive patients attending to Movement Disorders services at SCTIMST were taken up in the study after applying the selection criteria. Information regarding clinical, demographic & risk factors were collected. Healthy volunteers were selected as controls from among the friends and visitors of patients, and from the community.

Plasma/serum were taken from the PD cases and age and gender-matched controls to measure lipid profile and Lp(a). Fasting (9-12 hours) blood samples were collected for lipid profile and Lp(a) measurement. Blood was collected in tubes containing sodium citrate as anticoagulant (for plasma) or serum separator tube or serum gel tubes (for serum). Lipid profile (total cholesterol, high-density [HDL] and low-density lipoprotein [LDL] cholesterol, triglycerides) was measured in the Central Clinical Laboratory, SCTIMST using Siemens Dimension RxL Max autoanalyzer. The serum/plasma level of Lp(a) was measured using the Lipoprotein Human ELISA kit (Abcam) based on the manufacturer's recommendations.

Statistical methods:

Data is presented as mean ± SEM and for statistical comparison of two groups, unpaired, two-tailed student's t-test was done and for the comparison of three or more groups, ANOVA followed by Fisher's post-test were done. Differences were considered as significant when $p \leq 0.05$.

The data was analyzed using SPSS version 25 software (SPSS Inc, Illinois, Chicago). Univariate analysis was undertaken to examine relationship of various factors. Crude odds ratio with 95% confidence interval is reported. Chi square test/ Fisher's exact test was applied to evaluate statistical significance. Multivariate analysis/ logistic regression has been used to evaluate the independent and joint effect of the variable of interest on the outcome.

Ethical considerations

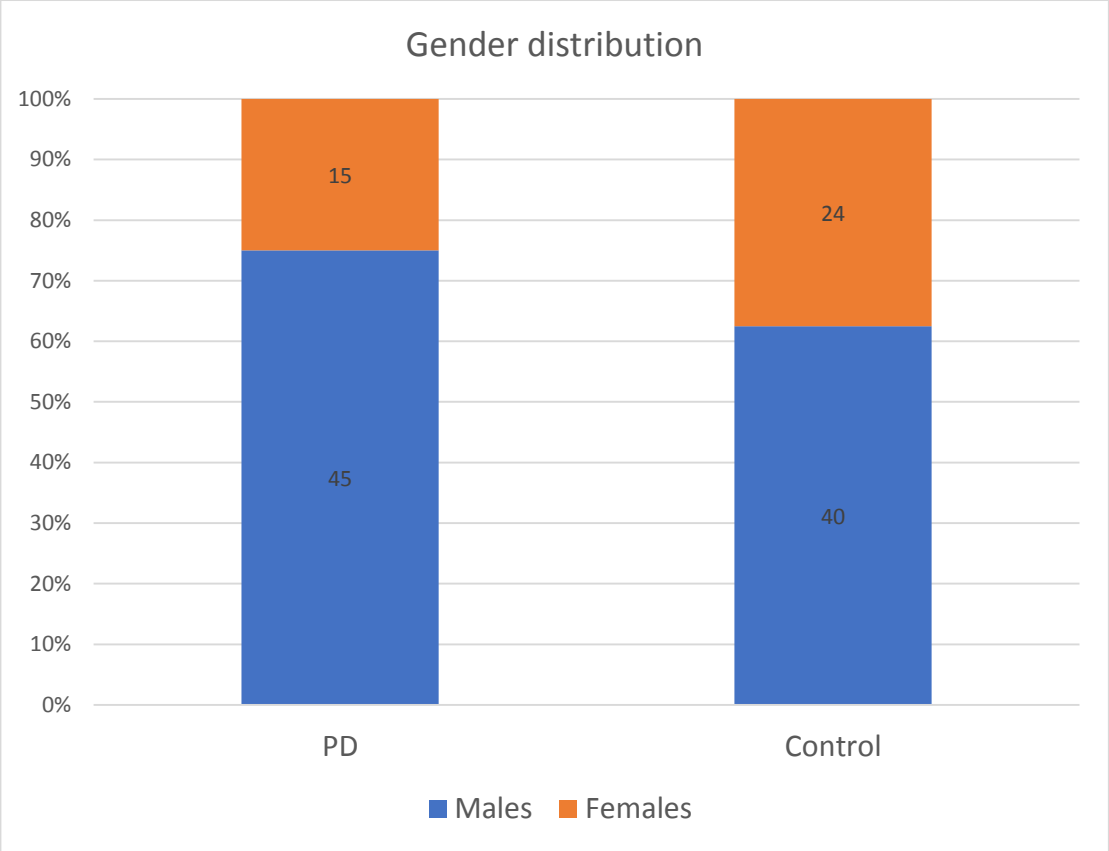
This study has the approval of the Institutional Ethics Committee and informed consent was obtained from the patient or caregiver.

RESULTS

Results

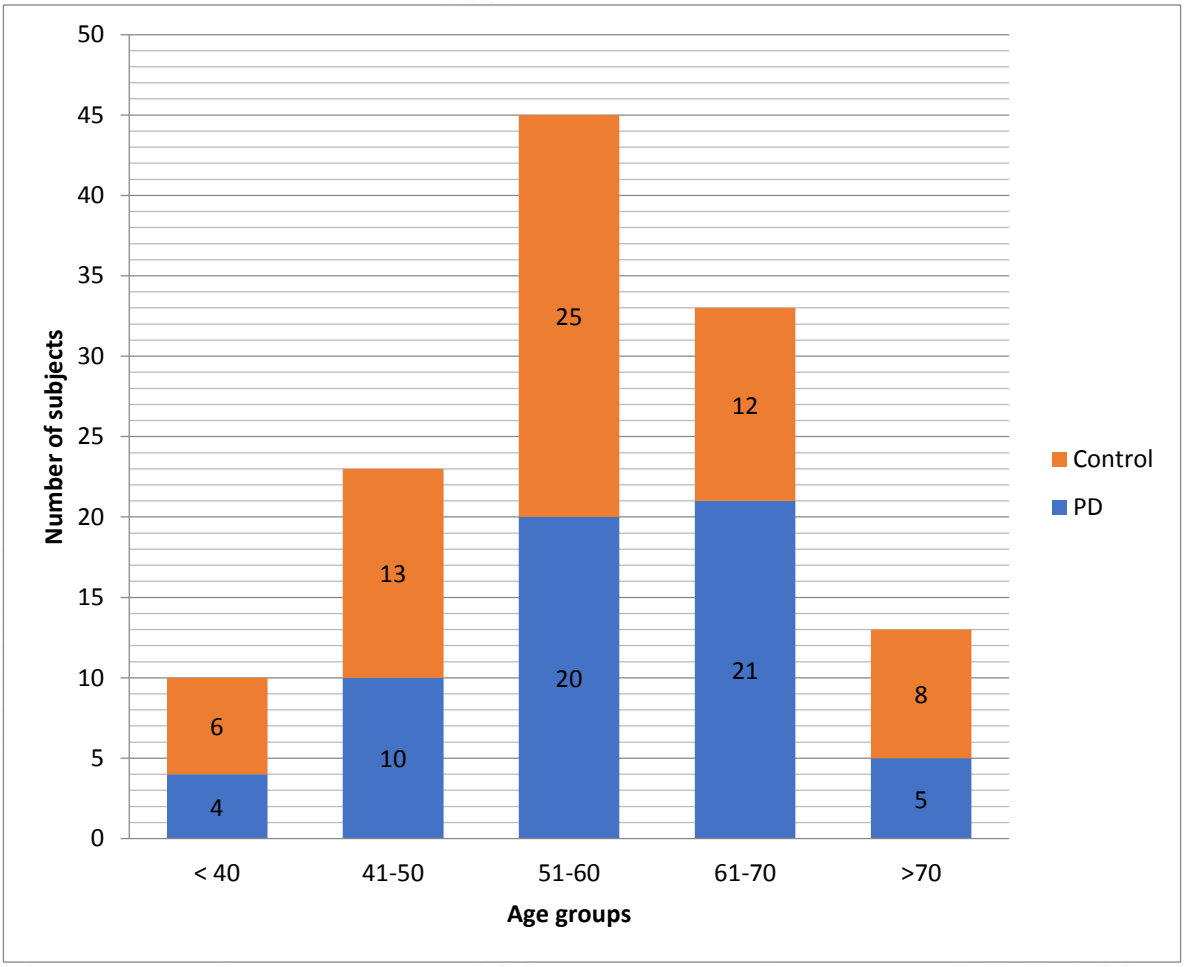
60 patients (45 males, 15 females) and 64 controls (40 males, 24 females) were included in the study. The planned sample size of 150 patients and 150 controls could not be reached due to Covid-19 restrictions.

Figure 1: Gender distribution of study subjects



Overall, 85 (68.55%) were males.

Figure 2: Age distribution of subjects



Mean age of the complete cohort was 56.52 years (SD=11.3)

Overall, maximum subjects were in the age group of 51-60 years. However, maximum PD patients belonged to the age group of 61-70 years.

Mean duration of illness in PD patients was 10.56 years (SD = 6.94).

Mean Levodopa equivalent daily dose (LEDD) was 639.77 mg (SD = 305.36).

Median worst H & Y score was 4.

We assessed impact of age on lipid profile parameters. We found significant negative correlation between age and serum triglyceride levels (Pearson's correlation coefficient=-0.234, p=0.009). There was no significant correlation of age with other lipid parameters.

Figure 3: Correlation of age with lipid profile

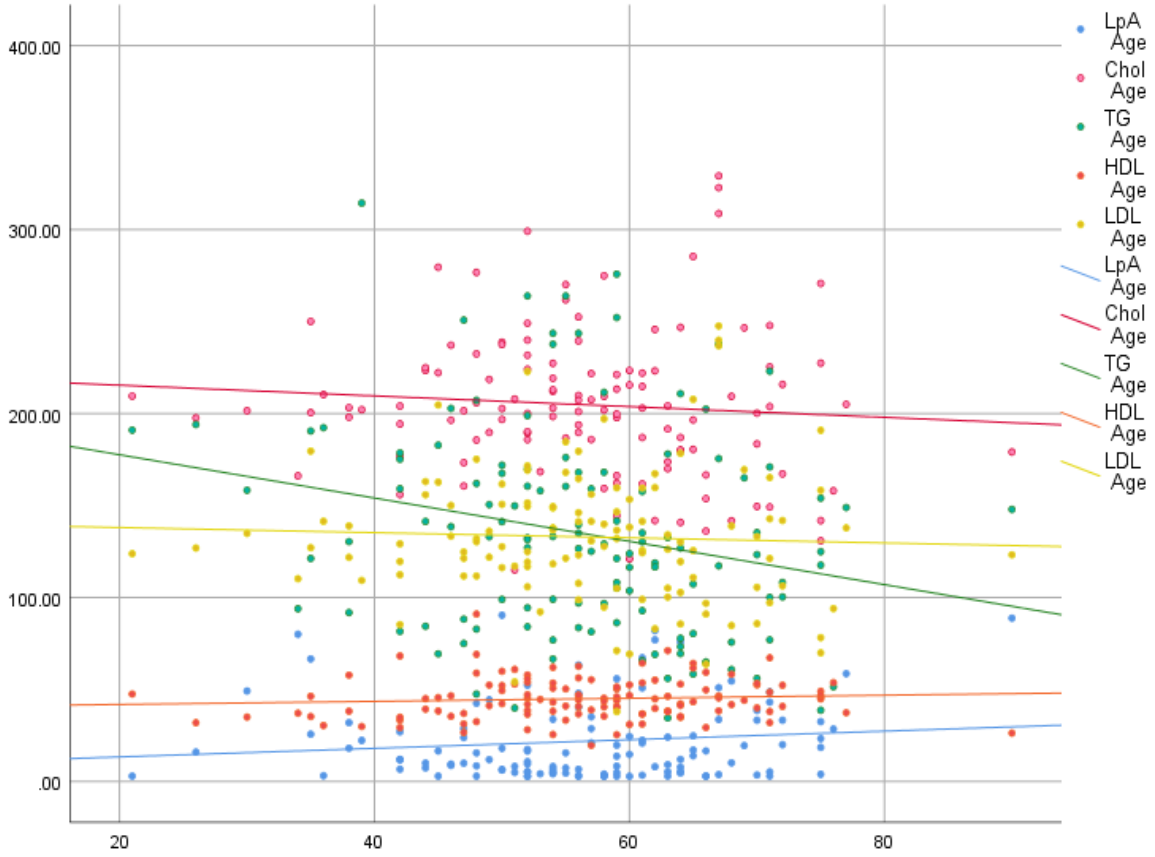


Table 1: Comparison between male and female subjects

Sex	Male (n=85)	Female (n=39)	P value
Age, Mean (SD)	55.92 (12.08)	57.82 (9.37)	0.342
Cholesterol, Mean (SD)	199.2 (36.98)	217.41 (42.8)	0.025
TG, Mean (SD)	139.47 (58.59)	124.04 (52.3)	0.146
HDL, Mean (SD)	42.37(9.98)	50.45(11.72)	< 0.001
LDL, Mean (SD)	128.93 (34.11)	142.09 (39.3)	0.076
Lp(a), Mean (SD)	21.88 (22.18)	21.8 (20.66)	0.985

Females had significantly higher serum cholesterol levels as compared to males, with difference in HDL cholesterol being the most significant. This difference was also noted individually in both PD and control group. Serum triglyceride levels were found to be lower in females, but this did not reach statistical significance.

Table 2: Comparison between cases and controls

Variable	Cases (n=60)	Controls (n=64)	P value
Age, Mean (SD)	57.85 (10.23)	55.27 (12.16)	0.204
Male gender, n(%)	45 (75%)	40 (62.5%)	0.176
Hypertension, n(%)	20 (33.3%)	13 (20.32%)	0.109
Diabetes mellitus, n(%)	11 (18.3%)	6 (9.38%)	0.193
Cholesterol, Mean (SD)	203.28 (41.64)	206.47 (37.95)	0.657
HDL, Mean (SD)	45.00 (11.99)	44.83 (10.42)	0.935
LDL, Mean (SD)	134.47 (36.74)	131.76 (35.89)	0.678
Triglycerides, Mean (SD)	118.86 (55.46)	149.38 (54.67)	0.003
Lp(a), Mean (SD)	19.87 (17.86)	23.68 (24.62)	0.332

PD patients and controls did not differ significantly in age and gender distribution. Prevalence of hypertension and diabetes mellitus was similar in the 2 groups.

Serum triglycerides levels were significantly lower in PD patients, as compared to controls. Serum total cholesterol, HDL cholesterol, LDL cholesterol and Lp(a) levels did not differ significantly between patients and controls.

We examined the differences in lipid profile between the DBS group and medically managed groups to find out whether neurostimulation and subsequent medication adjustments have any impact on lipid profiles.

Table 3: Comparison between PD patients on medical management alone vs PD patients post DBS

	Post DBS (13)	Medical management alone (47)	P value
Age, Mean (SD)	56.85 (9.83)	58.13 (10.43)	0.686
Duration of illness, Mean (SD)	16.3 (5.99)	8.97 (6.36)	0.001
LEDD, Mean (SD)	626.92 (319.74)	643.32 (304.73)	0.871
Cholesterol, Mean (SD)	192.98 (46.66)	206.13 (40.22)	0.368
TG, Mean (SD)	119.72 (75.74)	118.63 (49.49)	0.961
HDL, Mean (SD)	42.92 (11.24)	45.57(12.25)	0.469
LDL, Mean (SD)	126.11 (39.92)	136.79 (35.92)	0.395
Lp(a), Mean (SD)	24.28 (20.75)	18.63 (17.0)	0.381

Lipid profile in PD patients who underwent DBS did not differ significantly from PD patients who were under medical management alone.

The duration of disease of post-DBS patients at the time of the study was significantly more compared to medically managed patients. However, LEDD did not differ between the 2 groups.

We examined whether lipid profile parameters showed any difference between PD and control group, after adjusting for demographic variables like age and gender, and risk factors like Diabetes mellitus and hypertension.

Table 4: Linear regression for association of Lp(a) levels with demographic factors

	Estimate	Standard Error	P
Intercept	3.161	12.837	0.806
Age	0.289	0.182	0.115
Female	-1.723	4.333	0.692
HTN	-1.452	4.687	0.757
DM	-6.949	5.752	0.229
Control	3.919	4.029	0.333

There was no difference in LPA levels between PD and control group after adjusting for age, gender, hypertension and diabetes mellitus.

Table 5: Linear regression for association of total cholesterol levels with demographic factors

	Estimate	Standard Error	P
Intercept	201.476	23.205	<0.001
Age	-0.286	0.329	0.386
Female	17.975	7.767	0.022
HTN	-3.619	8.459	0.670
DM	-9.608	10.393	0.357
Control	-1.135	7.234	0.876

There was no difference in total cholesterol levels between PD and control group after adjusting for age, gender, diabetes and hypertension.

Females had significantly higher total cholesterol levels after adjusting for other variables.

Table 6: Linear regression for association of serum triglyceride levels with demographic factors

	Estimate	Standard Error	P
Intercept	165.280	31.770	<0.001
Age	-1.127	0.450	0.014
Female	-15.761	10.634	0.141
HTN	13.327	11.581	0.252
DM	10.006	14.229	0.483
Control	32.202	9.904	0.001

Triglyceride levels were significantly higher in controls as compared to PD patients, after adjusting for age, gender, diabetes and hypertension. Age was another predictor for triglyceride levels, and showed a negative correlation.

Table 7: Linear regression for association of serum HDL cholesterol levels with demographic factors

	Estimate	Standard Error	P
Intercept	32.622	6.293	<0.001
Age	0.083	0.089	0.356
Female	7.758	2.106	<0.001
HTN	-3.036	2.294	0.188
DM	0.829	2.818	0.769
Control	-1.243	1.962	0.528

HDL cholesterol levels did not differ significantly between PD and control groups, after adjusting for age, gender, hypertension and diabetes mellitus.

HDL cholesterol levels remained significantly higher in females, even after accounting for other variables.

Table 8: Linear regression for association of serum LDL cholesterol levels with demographic factors

	Estimate	Standard Error	P
Intercept	135.827	21.314	<0.001
Age	-0.145	0.302	0.633
Female	13.317	7.134	0.064
HTN	-3.221	7.770	0.679
DM	-12.416	9.546	0.196
Control	-6.289	6.645	0.346

LDL cholesterol levels did not differ between PD patients and controls, after adjusting for age, gender, hypertension and diabetes mellitus.

DISCUSSION

Discussion

124 subjects (60 PD, 64 controls) were recruited for the study. We intended to include 150 PD patients and 150 controls, but could not recruit due to reduced patient inflow because of Covid-19 related lockdown. The control group was age and sex-matched to patients, as planned. Greater prevalence of PD in males is known[151], and was seen in our study as well. This explains the skewed sex ratio in our cohort.

Overall, females had higher total cholesterol and HDL cholesterol levels, as compared to males. However, triglyceride levels did not differ between males and females. This finding is similar to earlier studies.[152] The most significant gender difference in HDL levels is expected, as shown in multiple studies[153, 154] and demonstrated by different cut-off values for abnormal levels of HDL[155]. In contrast to HDL, LDL-cholesterol levels do not differ between male and female PD patients[130, 156].

Triglyceride levels were found to be significantly lower in patients as compared to controls. The significance persisted even after adjusting for age, sex, presence of hypertension and diabetes mellitus. This finding is consistent with earlier studies. A meta-analysis has revealed higher triglyceride levels to be statistically useful as protective factor for the onset of PD[138]. Triglycerides are protective against αS cytotoxicity and associated ER trafficking defects.[157] Another hypothesis for low triglyceride levels in PD patients has been suggested. Hasuike et al[158] showed a high prevalence of small intestinal bacterial overgrowth (SIBO) in PD patients, which was associated with low triglyceride levels. It was proposed that there is dominance of unconjugated bile acids and inhibition of bile acid synthesis due to bacterial

overgrowth. Lipid absorption is decreased with decreased bile acid, and serum TG levels may become low.

Serum total cholesterol levels, as well as HDL and LDL cholesterol levels, did not differ between patients and controls in our study. Lower plasma cholesterol has been associated with PD,[116-121] and higher plasma cholesterol levels have been linked to reduced PD risk[4, 109, 110, 112, 113, 148] and slower clinical progression of PD[107]. However, others, including a meta-analysis[122], have found no association between plasma cholesterol levels and PD[121, 123] or PD risk [124, 125]. Even higher plasma cholesterol levels in PD patients compared to controls[126, 127] have been reported. The differential outcome of the studies could be attributed to demographic factors such as age and gender, among others. Lower plasma cholesterol levels have been reported in PD male patients of more than 55 years compared to controls[111], a high total cholesterol baseline has been associated with increased risk of PD in subjects of 25–54 years (but not in those above 55) [159], and female PD patients seem to have higher cholesterol levels compared to male PD patients.[156]

LDL cholesterol levels have been found to be negatively correlated with the risk of PD in few studies, but final verdict could not be reached due to marked heterogeneity of the studies.[138] Two meta-analyses have found no association between LDL cholesterol and risk of PD.[160, 161]

Plasma levels of HDL-cholesterol have been found to be lower[116, 131] or not different[119, 120, 127] in PD patients compared to controls in previous studies. This controversial relationship is complex, as both sex[156] and *APOE* polymorphisms[126]

seem to affect HDL-cholesterol levels in PD patients. No definite significant association between HDL levels and risk of PD has been found till date.

Our study did not find a significant difference in Lp(a) levels between PD patients and controls. Any influence of other demographic factors also could not be demonstrated. Only one other study has assessed Lp(a) levels in PD patients, and found statistically lower concentrations in PD patients compared with controls.[142] However, an individual's Lp(a) level is 80-90% genetically determined[139, 140] in an autosomal codominant inheritance pattern with full expression by 1-2 years of age and adult-like levels are achieved by approximately 5 years of age. Outside of acute inflammatory states, the Lp(a) level remains stable through an individual's lifetime regardless of lifestyle.[141] This was demonstrated by wide variability in Lp(a) levels in both patients and controls, and lack of correlation with other factors.

Parkinson's disease is considered a catabolic state. Although PD patients do not exhibit reduced energy intake, they typically lose significant weight and present with lower BMI than their age-matched controls. Whether weight loss is etiologically linked to PD, or is a result of the disease processes itself still remains obscure.[162] Patients who undergo Subthalamic nucleus Deep Brain stimulation (STN-DBS) gain weight post-operatively. A recent study found changes in lipid profile post DBS – LDL levels increased early post-surgery, while changes in HDL and triglyceride concentrations were more gradual.[163] Our study did not find significant difference in lipid profile of patients who had undergone DBS.

Strengths of the study:

1. This study assessed lipid profile in statin free patients and compared with age-and-sex matched controls, resulting in a comparable population.

Limitations of the study

1. The major limitation of the study was the small sample size.
2. This was a cross-sectional study.

CONCLUSIONS

Conclusions and Summary

1. We intended to compare lipid profile between PD patients and age and sex matched controls. Serum triglyceride levels were lower in PD patients as compared to controls.
2. Serum triglyceride levels remained significantly lower in PD patients, even after adjusting for age, sex, presence of diabetes mellitus and hypertension.
3. Other lipid profile parameters, including total cholesterol, HDL cholesterol, LDL cholesterol and Lipoprotein A levels, did not differ between PD patients and controls.

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APPENDIX

a. Abbreviations

αS: α- synuclein

DA: Dopaminergic

DAT: Dopamine transporter

DBS: Deep brain stimulation

GWAS: Genome wide association studies

HDL: High density lipoprotein

LB: Lewy bodies

LDL: Low density lipoprotein

Lp(a): Lipoprotein A

PD: Parkinson's disease

SN: Substantia nigra

STN: Subthalamic nucleus

VLDL: Very low density lipoproteins

VMAT2: Vesicular monoamine transporter-2