ALTERATION LEVELS OF MONOAMINE NEUROTRANSMITTER RELATED WITH NON-MOTOR SYMPTOMS AND THEIR FLUCTUATION PATTERNS DURING ON/OFF PERIODS IN PARKINSON'S PATIENTS

Miss Patsorn Wichit

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การเปลี่ยนแปลงระดับสารสื่อประสาทกลุ่มโมโนเอมีนเชื่อมโยงกับอาการนอกเหนือจากการเคลื่อนไหวและรูปแบบการผันแปรในช่วงที่ยาออกฤทธิ์และไม่ออกฤทธิ์ในผู้ป่วยพาร์กินสัน

น.ส.ภัสสร วิชิต

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By	Miss Patsorn Wichit
Field of Study	Medical Sciences
Thesis Advisor	Associate Professor SAKNAN BONGSEBANDHU-PHUBHAKDI, Ph.D.
Thesis Co Advisor	Professor ROONGROJ BHIDAYASIRI, M.D., FRCP, FRCPI
	Onanong Phokaewvarangkul, M.D., Ph.D.

Accepted by the FACULTY OF MEDICINE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

..... Dean of the FACULTY OF MEDICINE
(Professor SUTTIPONG WACHARASINDHU, M.D.)

DISSERTATION COMMITTEE

..... Chairman
(Professor VILAI CHENTANEZ, M.D., Ph.D.)

..... Thesis Advisor
(Associate Professor SAKNAN BONGSEBANDHU-PHUBHAKDI, Ph.D.)

..... Thesis Co-Advisor
(Professor ROONGROJ BHIDAYASIRI, M.D., FRCP, FRCPI)

..... Thesis Co-Advisor
(Onanong Phokaewvarangkul, M.D., Ph.D.)

..... Examiner
(Associate Professor POONLARP CHEEPSUNTHORN, Ph.D.)

..... Examiner
(Sekh Thanprasertsuk, M.D.)

..... External Examiner
(Professor Banthit Chetsawang, Ph.D.)

ภัสสร วิชิต : การเปลี่ยนแปลงระดับสารสื่อประสาทกลุ่มโมโนเอมีนเชื่อมโยงกับอาการนอกเหนือจากการเคลื่อนไหวและรูปแบบการผันแปรในช่วงที่ยาออกฤทธิ์และไม่ออกฤทธิ์ในผู้ป่วยพาร์กินสัน. (ALTERATION LEVELS OF MONOAMINE NEUROTRANSMITTER RELATED WITH NON-MOTOR SYMPTOMS AND THEIR FLUCTUATION PATTERNS DURING ON/OFF PERIODS IN PARKINSON'S PATIENTS) อ.ที่ปรึกษาหลัก : รศ. ดร.ศักนัน พงศ์พันธุ์ผู้ภักดี, อ.ที่ปรึกษาร่วม : ศ. นพ.รุ่งโรจน์ พิทยศิริ,ดร. พญ.อรอนงค์ โพธิ์แก้ววรางกูล

การศึกษาครั้งนี้ประกอบด้วย 2 ส่วน ส่วนที่หนึ่งมีวัตถุประสงค์เพื่อศึกษาการเปลี่ยนแปลงระดับสารสื่อประสาทกลุ่มโมโนเอมีนในเลือดและน้ำลาย รวมถึงเมตาบอไลต์ในปัสสาวะของผู้ป่วยพาร์กินสัน ร่วมกับศึกษาความสัมพันธ์ระหว่างสารโมโนเอมีนในเลือดและอาการวิตกกังวล ซึมเศร้า ปัญหาการนอนหลับ และปัญหาด้านเพศสัมพันธ์ ส่วนที่สองเพื่อศึกษาการผันแปรของสารโมโนเอมีนในเลือดในช่วงที่ยาออกฤทธิ์และไม่ออกฤทธิ์ในผู้ป่วยพาร์กินสันระยะ advance ในส่วนที่ 1 ผู้เข้าร่วมงานวิจัยแบ่งเป็นกลุ่มผู้ป่วยพาร์กินสันและกลุ่มควบคุม กลุ่มละ 40 คน เข้ารับการประเมินอาการนอกเหนือจากการเคลื่อนไหวด้วยแบบคัดกรองปัญหาอาการวิตกกังวลและอาการซึมเศร้า แบบสอบถามความผิดปกติการนอนหลับในผู้ป่วยโรคพาร์กินสันฉบับปรับปรุง แบบประเมินประสบการณ์ทางเพศของอริโซน่า และแบบสอบถามอาการของโรคพาร์กินสันที่นอกเหนือจากการเคลื่อนไหว จากนั้นทำการเก็บตัวอย่างเลือดและน้ำลาย เพื่อตรวจวัดระดับ dopamine (DA) norepinephrine (NE) และ serotonin (5-HT) และเก็บตัวอย่างปัสสาวะเพื่อวัดระดับ 3-Methoxytyramine (3-MET), homovanillic acid (HVA), noremetanephrine (NM), vanilly mandelic acid (VMA) และ 5-hydroxyindoleacetic acid (5-HIAA) ด้วยวิธี High-Performance Liquid Chromatography ร่วมกับ electrochemical detection ส่วนที่ 2 ผู้ป่วยพาร์กินสันระยะ advance จำนวน 10 คน เข้ารับการเก็บตัวอย่างเลือด 3 ครั้ง ได้แก่ ก่อนรับประทานยา (OFF time) หลังรับประทานยา 45 และ 120 นาที (ON time) ผลการศึกษาในส่วนที่ 1 พบว่าในเลือดของผู้ป่วยพาร์กินสันมีปริมาณ DA เพิ่มขึ้นเล็กน้อย ขณะที่ NE เพิ่มสูงขึ้น ส่วน 5-HT ลดลงอย่างมีนัยสำคัญทางสถิติ ระดับของ DA และ NE ในน้ำลายของผู้ป่วยพาร์กินสันเพิ่มสูงขึ้นเล็กน้อย ขณะที่ 5-HT ตรวจไม่พบในน้ำลาย ในปัสสาวะพบว่าผู้ป่วยพาร์กินสันมีระดับของ 3-MET และ HVA เพิ่มสูงขึ้น ขณะที่ NM และ 5-HIAA มีระดับลดลงอย่างมีนัยสำคัญทางสถิติ จากการประเมินอาการนอกเหนือจากการเคลื่อนไหวพบว่าผู้ป่วยพาร์กินสันมีระดับอาการวิตกกังวล ซึมเศร้า ปัญหาการนอนหลับ และปัญหาด้านเพศสัมพันธ์สูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ อย่างไรก็ตามพบว่าไม่มีความสัมพันธ์ระหว่างระดับของโมโนเอมีนในเลือดกับอาการนอกเหนือจากการเคลื่อนไหว ผลการศึกษาส่วนที่ 2 พบว่าระดับ DA, NE และ 5-HT ในเลือดสูงขึ้นหลังได้รับยา 45 นาที และลดต่ำลงหลังรับยา120 นาที ยกเว้น NE ที่ยังเพิ่มสูงขึ้นอย่างต่อเนื่อง อย่างไรก็ตามพบว่าระดับโมโนเอมีนในเลือดทั้ง 3 ช่วงเวลาไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ จากการศึกษาครั้งนี้แสดงให้เห็นการเปลี่ยนแปลงของระดับโมโนเอมีนในของเหลวชนิดต่างๆของร่างกาย ลักษณะการผันแปรในช่วงที่ยาออกฤทธ์และไม่ออกฤทธิ์ รวมถึงปัญหาเกี่ยวกับอาการนอกเหนือจากการเคลื่อนไหว ซึ่งจะเป็นประโยชน์ต่อการตรวจประเมิน คาดการณ์อาการนอกเหนือจากการเคลื่อนไหวที่อาจเกิดตามมา และใช้ความรู้นี้ในการช่วยวางแผนการรักษาที่เหมาะสมให้กับผู้ป่วยพาร์กินสัน

สาขาวิชา	วิทยาศาสตร์การแพทย์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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Patsorn Wichit : ALTERATION LEVELS OF MONOAMINE NEUROTRANSMITTER RELATED WITH NON-MOTOR SYMPTOMS AND THEIR FLUCTUATION PATTERNS DURING ON/OFF PERIODS IN PARKINSON'S PATIENTS. Advisor: Assoc. Prof. SAKNAN BONGSEBANDHU-PHUBHAKDI, Ph.D. Co-advisor: Prof. ROONGROJ BHIDAYASIRI, M.D., FRCP, FRCPI, Onanong Phokaewvarangkul, M.D., Ph.D.

Parkinson's disease (PD) is the second most common neurodegenerative disorder. This study consisted of two parts. The purpose of part I was to investigate the difference in concentrations of DA, NE, 5-HT and their metabolites (3-methoxytyramine (3-MET), homovanillic acid (HVA), normetanephrine (NM), vanillylmandelic acid (VMA) and 5-hydroxyindoleacetic acid (5-HIAA)) in peripheral body fluids between control and PD groups. In addition, the correlation between these neurotransmitter levels and NMSs were evaluated. The purpose of part II was to investigate the fluctuation of plasma DA, NE and 5-HT concentrations during medication ON/OFF periods in advanced-PD patients. In part I, the control and PD patients (n=40/group), aged between 30-80 years were randomly recruited from the King Chulalongkorn Memorial Hospital. All participants were assessed anxiety and depression, sleep problems, and sexual dysfunction by using Hospital Anxiety and Depression Scale (HADS), Modified Parkinson's disease sleep scale (MPDSS), and The Arizona Sexual Experience Scale (ASEX), respectively. Plasma, saliva, and urine were collected for determined the levels of monoamine and their metabolites by using high performance liquid chromatography with electrochemical detector. In part II, the plasma DA, NE and 5-HT levels were determined in advanced-PD patients at 3 points time during medication ON/OFF periods (5 minutes before, 45 and 120 minutes after drug taking). Plasma DA level did not show significant differences between two groups. However, there were significantly higher in plasma NE and lower plasma 5-HT levels in PD patients than control group. Levels of urinary 3-Met and HVA were significantly higher in the PD patients, while urinary NM and 5-HIAA levels were significantly lower in PD than control. Salivary DA and NE tend to increase in PD but do not show significant differences when compared to control. However, salivary 5-HT could not be detected in this study. PD patients were significantly higher in HADS (anxiety and depression) and ASEX score with lower in MPDSS score than in control. For the study of correlation, the results did not show any relationship between monoamines levels and NMSs in PD patients. In part II, we found that plasma DA, NE and 5-HT levels increase after medication taking for 45 minutes in ON comparing to the OFF period. Plasma DA and 5-HT levels tend to be decrease after medication taking for 120 minutes. Meanwhile, NE level in plasma was continuously increased. However, there were no significant difference among 3 time points of these plasma monoamine levels. From these findings, there is dysregulations of monoamines in peripheral body fluids. Additionally, PD patients exhibited anxiety, depression, sleep disturbances and sexual dysfunction. This knowledge could benefit appropriate pharmacological treatment planning in respect of monoamine changes and might also help predict subsequent clinical symptoms.

Field of Study:	Medical Sciences	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....
		Co-advisor's Signature .....
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Patsorn Wichit

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LIST OF ABBREVIATIONS

PD	Parkinson’s disease
NMSs	Non-motor symptoms
DA	Dopamine
5-HT	5-Hydroxytryptamine or Serotonin
NE	Norepinephrine
VMA	Vanillymandelic acid
HVA	Homovanillic acid
5-HIAA	5-hydroxyindoleacetic acid
NM	Normetanephrine
3-MET	3-Methoxytyramine
L-DOPA	Levodopa
SN	Substantia nigra
LC	Locus coeruleus
H&Y	Modified Hoehn and Yahr Staging
HPLC	High-performance liquid chromatography
ECD	Electrochemical detector
EDTA	Ethylenediamine tetraacetic acid
MMSE	Mini – Mental State Examination
ASEX	The Arizona Sexual Experience Scale
MPDSS	The Modified Parkinson's Disease Sleep Scale

NMSQ	Non-motor Symptoms Questionnaire for Parkinson's disease
PDQ-8	The eight items-Parkinson's disease questionnaires
HADS	The Hospital Anxiety and Depression Scale
SD	Sexual dysfunctions
NMF	Non-motor fluctuation
MF	Motor fluctuation
RN	Raphe nuclei
LC	Locus coeruleus
SNpc	Substantia nigra par compacta
GABA	Gamma-amino butyric acid
ACh	Acetylcholine
RLS	Restless leg syndrome
RBD	Rapid eye movement-sleep behavior disorder
VTA	Ventral tegmental area
SNRIs	Serotonin-norepinephrine reuptake inhibitors
HCL	Hydrochloric acid
TH	Tyrosine hydroxylase
AADC	Aromatic amino acid decarboxylase
COMT	Catechol-O-methyltransferase
DHPG	Dihydroxyphenylglycol

CHAPTER I
INTRODUCTION

1. Background and Rationale

Parkinson's disease (PD) is the second most common chronic progressive neurodegenerative disorder. It has 4 distinctive characteristics, including resting tremor, bradykinesia, muscle rigidity, and postural instability (1). The pathophysiology of PD is still unclear. However, a large number of studies demonstrated 2 remarkable pathologic features of PD. The first one is depigmentation of substantia nigra (SN) resulting from deterioration of dopaminergic neurons that produce important neurotransmitter named "dopamine" (2). The second pathologic feature is the widespread of Lewy bodies, which are the accumulation of unfolding proteins causing of neuronal death in many brain areas. PD is classified as movement disorder because motor symptoms are main problems of PD patients in clinic (3). Furthermore, numerous studies revealed that almost PD patients have been exposed to non-motor symptoms (NMSs) since early stage. A variety of NMSs include autonomic dysfunctions, sleep disorder, fatigue, neuropsychiatric problems, cognitive impairment, abnormal sensation and pain. Interestingly, the studies in postmortem and animal model of PD showed the disturbances of multi-neurotransmission systems particularly dopamine (DA), norepinephrine (NE), and serotonin (5-HT) (4, 5). DA is mainly produced in SN and has a variety functions; including motor, emotional, behavioral, cognitive, and motivational control. Likewise, NE is a neurotransmitter releasing from locus coeruleus neuron, which is responsible to autonomic nervous system, memory, learning, attention, behavior, and sleep-wake regulations (6). Another one of altered-neurotransmitter in PD is 5-HT. It is produced in raphe nucleus and plays a role in mood, cognition, and motor function (7). Considering from their functions, deficit of these neurotransmitters may involve to pathogenesis of NMSs in PD. However, the

altered pattern of these neurotransmitters, the concentration changes in body fluids of PD patient and their correlation with non-motor conditions remain unclear.

Managements of PD consist of pharmacological treatment and surgery. Pharmacological treatment is the primary management that is used to control the symptoms by replenishing deficient neurotransmitters. It is generally accepted that levodopa (L-dopa) is the most effective drug of PD (4). Even though this agent has benefit, it is able to contribute adverse effects. The long-term use of L-dopa (more than five years) usually causes both of motor and non-motor fluctuations (5). Motor fluctuation may result from pulsatile stimulated dopaminergic receptor with drug. The occurrence of motor fluctuation also relates to medicinal taking time (6). In addition to motor fluctuation, a large number of PD patients especially in advance stage also experience the fluctuations of NMSs. The most common non-motor fluctuations include autonomic, psychiatric, and sensory symptoms, which are mainly regulated by DA, NE, as well as 5-HT (7). The exact causes of these non-motor fluctuations are still not understood. The pathophysiology of them may involve to the alteration of both dopaminergic and non-dopaminergic neurotransmission during medicinal ON/OFF periods like a motor fluctuation. However, there is no study about the alteration pattern and levels of DA, NE, and 5-HT during ON/OFF periods in advanced-PD patients.

The present study consisted of two parts. Aims of first part were to investigate the alteration of DA, NE, 5-HT and their metabolite levels in peripheral body fluids and evaluated their correlations with NMSs of PD patients. The second part aimed to investigate the fluctuations of plasma DA, NE, and 5-HT levels during medicinal “ON/ OFF” periods of PD patients in advance stage.

2. Research Questions

1) Are there the differences of DA, 5-HT, NE and their metabolites levels in plasma, saliva, and urine between PD patient and control groups?

2) Are there the correlations between levels of plasma DA, 5-HT and NE levels and NMSs (i.e. anxiety, depression, sleep disturbances, and sexual dysfunction) in PD patients?

3) Are there the differences of plasma DA, NE, and 5-HT levels during medication on/off times in advanced PD patients?

3. Objectives

Part I

1) To investigate the differences in levels of DA, NE, and 5-HT and their metabolites in plasma, saliva, and urine between PD patients and control groups

2) To evaluate the correlations between the NMSs (i.e. anxiety, depression, sleep disturbances, and sexual dysfunction) and levels of plasma DA, NE and 5-HT of PD patients

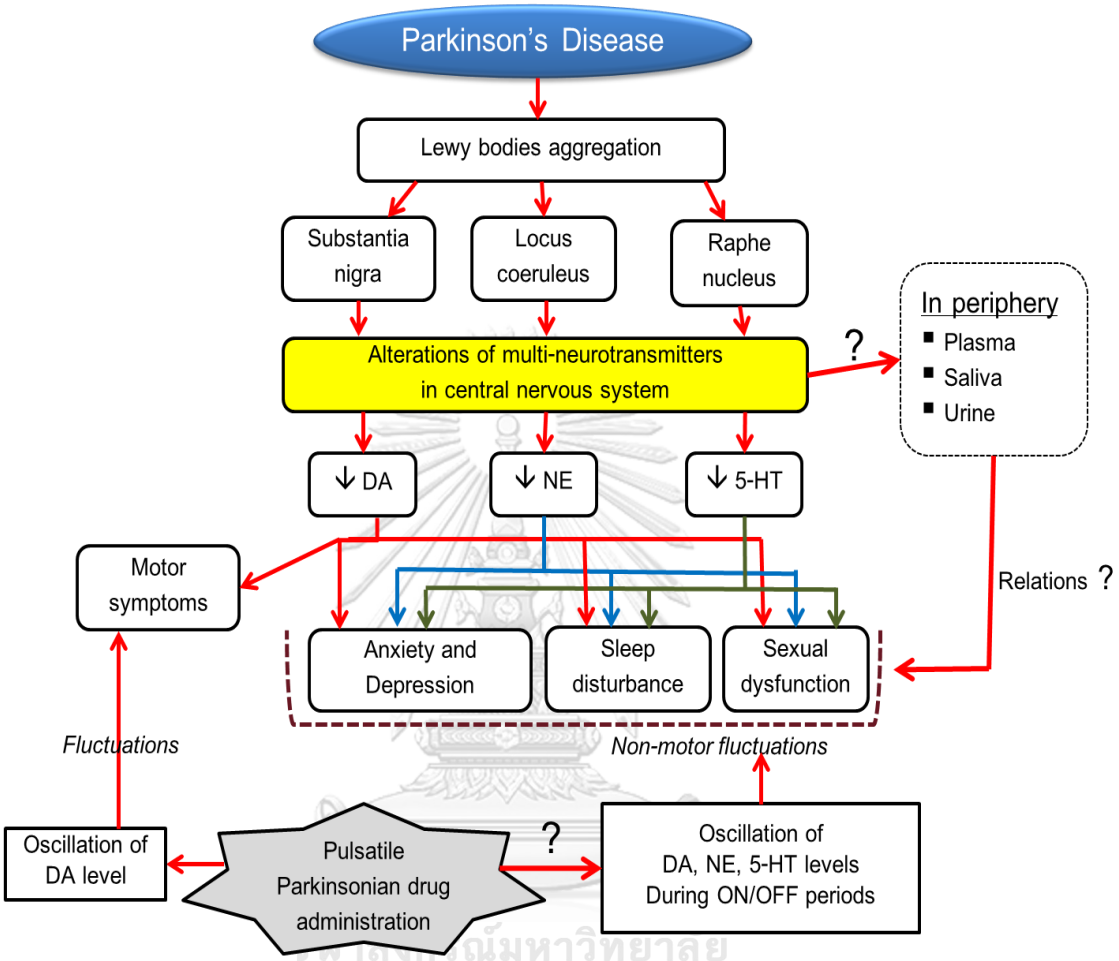
Part II

To investigate the fluctuations of plasma DA, NE and 5-HT levels during medication ON/OFF times in advanced-PD patients

4. Keywords

Parkinson's disease, Non-motor symptoms, Dopamine, Norepinephrine, Serotonin, Monoamine fluctuations

5. Conceptual Framework



CHAPTER II
LITERATURE REVIEW

1. Parkinson's Disease

Parkinson's Disease (PD) is the second most common chronic progressive neurodegenerative disorder in aging population next to Alzheimer's disease. From the survey finding, there were new PD patients 5 to more than 35 per 100,000 of population yearly around the world (8). In Thailand, the National Statistical Office revealed the prevalence of PD tends to increase. The number of inpatients with PD is 13,140 in 2013 and extend to 16,845 in 2016 (9). The incidence of PD is more increased over 60 years old and slightly higher in men than women (10).

PD is neurological condition, which was discovered in 1817 by English physician named "James Parkinson". In the past, this condition was called as "shaking palsy" because the main symptom of patients is tremor at rest. Thereafter physicians defined 4 distinctive characteristics, including resting tremor, bradykinesia, muscle rigidity, and postural instability and renamed this condition as "Parkinson's disease" (1).

PD has also been classified as movement disorder because motor symptoms are main problems and are essential criteria for diagnosis of PD in clinic (3). Resting tremor usually occurs at distal part of limbs in the frequency range 4-6 Hz. The fingers, hands, arms, feet, and legs are common affected parts, respectively. Particularly, tremor of fingers looks like "pill-rolling" action. Rigidity is abnormality of muscle tone, which is characterized by increase resistance to passive movement and stretching. This resistance is a result of increase muscle tone of both flexor and extensor groups. Bradykinesia is slowness of motion; especially on initiation period. In the mid-late stages, PD patients have postural and balance problems which are the result of severe tremor and muscle rigidity (11). Although PD is classified as movement disorder, almost PD patients have been exposed to non-motor symptoms. A variety of NMSs include

autonomic dysfunctions, sleep disorder, fatigue, neuropsychiatric problems, cognitive impairment, abnormal sensation and pain (12, 13).

2. Pathophysiology of Parkinson's Disease

Over the past century, pathophysiology of PD had been investigated but it still unclear. However, a large number of studies revealed 2 remarkable pathologic features of PD, including depigmentation of substantia nigra (SN) and widespread of Lewy pathology in the brain.

Depigmentation of SN results from deterioration of dopaminergic neurons, which contain neuromelanin pigments. These dopaminergic neurons produce an important neurotransmitter named "dopamine". The destruction of dopaminergic neurons in substantia nigra pars compacta (SNpc) relates to marked reduction of DA level and dysfunction of the basal ganglia. A group of nuclei at basal ganglia is responsible for movement initiation and execution.

Motor impairments which are characteristics of PD usually occur when the dopaminergic neurons loss about 80%. These motor impairments consist of rigidity, tremor, and bradykinesia (14). Treatment with dopamine precursor is able to decrease motor symptoms. From these evidences, neurologists have been proposed and concluded that dopamine depletion involve in pathology of PD (2).

Another remarkable feature of PD is the widespread of Lewy pathology in the brain. Lewy pathology is defined as abnormal accumulation of unfolding alpha-synuclein protein, which results in neuronal cells death. The model of Lewy pathology in PD was demonstrated by Braak and was divided into 6 stages (fig. 1). In the first stage of the Lewy body deposition starts in gastric myenteric plexus, motor vagus nerve, and olfactory bulb. In stage 2, Lewy pathology spread to the medulla nuclei; including locus coeruleus (LC) and raphe nuclei (RN). In stage 3, distribution of Lewy pathology reaches to SNpc, the pedunculopontine tegmental nucleus (PPN), and the central subnucleus of amygdala. In stage 4, midline and intralaminar nuclei of the thalamus

present Lewy pathology. In stage 5, Lewy pathology presents extreme accumulation in the area in stages 1-4. In this stage, Lewy pathology also extends into insular and subgenual mesocortex, where regulate autonomic functions. In last stage, Lewy pathology distribute to sensory association areas, the premotor cortex, primary motor, and sensory cortex. This work supports that Lewy pathology involves in pathophysiology of PD (15, 16).

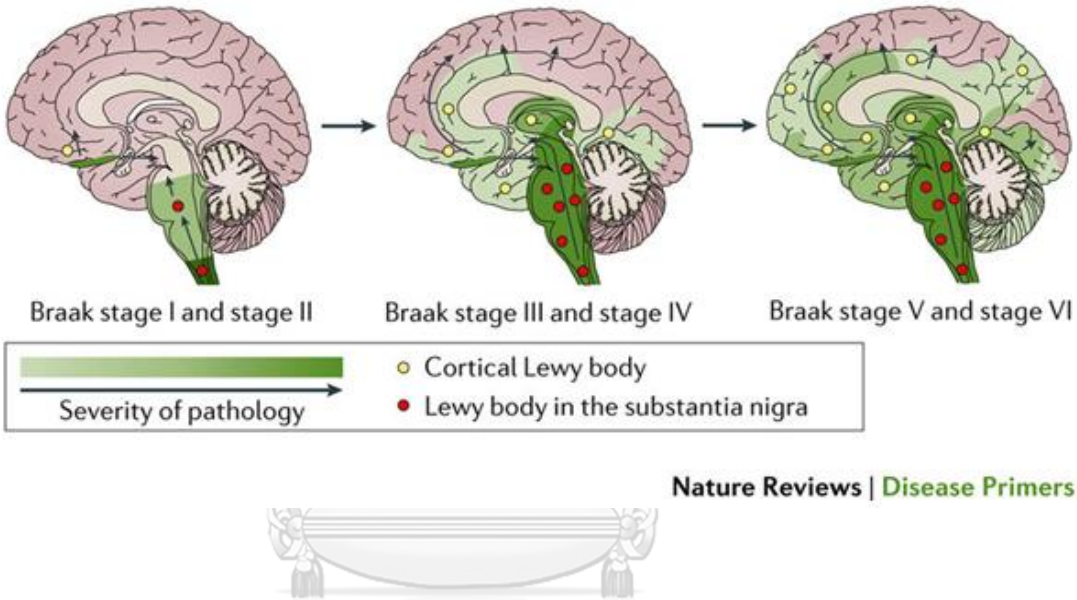


Figure 1 Lewy pathology hypothesis of Braak in 6 stages

(17)

From the hypothesis of Braak, there is several brain regions are implicated in PD. Moreover, many reports found that some of symptoms do not improved after treatment with dopamine precursor, especially NMSs. So, pathology of PD may associate to dysfunction of other neurotransmissions beside dopaminergic system.

3. The Alterations of Multi-Neurotransmissions in Parkinson's Disease

The histopathological, imaging, and postmortem studies reveal that pathology of PD involves not only in dopaminergic system, but also involves a variety of neurotransmitters in the central brain, which are acetylcholine (Ach), glutamate,

gamma-aminobutyric acid (GABA), DA, NE, epinephrine (E), 5-HT (18, 19). In present study, we emphasize the alteration of neurotransmitters in monoamine group, including DA, NE, and 5-HT, which are more related to pathogenesis of NMSs (20).

DA and NE are catecholamine neurotransmitters, which are proteins containing one group of amines. DA is synthesized by using tyrosine as a substrate. Then an enzyme named “tyrosine hydroxylase (TH)” catalyzes tyrosine to levodopa (L-dopa). After that aromatic L-amino acid decarboxylase (AADC) or DOPA- decarboxylase converts L-dopa to DA. Moreover, DA becomes a precursor of NE. The dopamine ß-hydroxylase converts DA to NE (fig. 2). In degradation process, DA is broken down to dihydroxyphenyl acetic acid (DOPAC) and homovanyllic acid (HVA) through the catalytic reaction of monoamine oxidase-A (MAO-A) and catechol-O-methyltransferase (COMT), respectively. In the meantime, 3-Methoxytyramine (3-MET) is formed by direct metabolism of dopamine by COMT. 3-MET is considered as reflection of dopamine release from neurons, which low level of them indicate rapidly DA metabolism. However, HVA is a main metabolite of DA. HVA from bloodstream circulates to kidneys and excretes into urine. Similarly, NE is metabolized into normetanephrine (NM) and vanillylmandelic acid (VMA) by MAO and COMT, respectively. These products are also excreted to urine (fig. 3).

In addition to DA and NE, serotonin (or 5-hydroxytryptamine; 5-HT) is also important monoamine protein. It uses tryptophan as a substrate. Tryptophan is converted to 5-hydroxytryptophan and 5-HT by tryptophan hydroxylase and AADC enzyme, respectively (fig. 2). 5-HT is broken down to 5-hydroxy-indol-acetaldehyde and 5-hydroxy-indol-acetic acid (5-HIAA) by enzyme MAO-B and aldehyde dehydrogenase, which excrete into urine (figure 2.3) (21, 22).

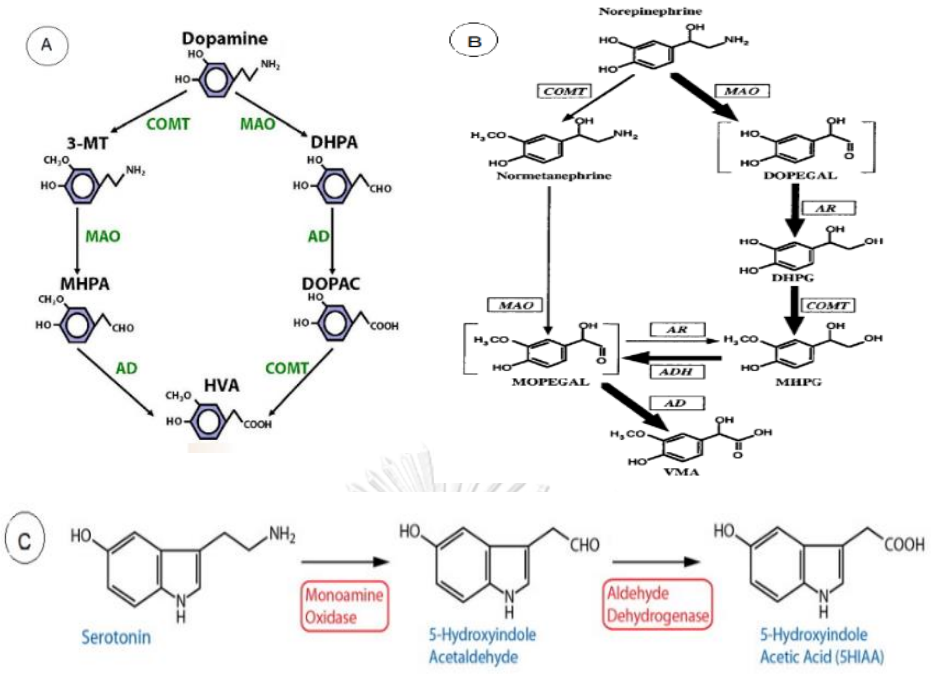


Figure 2 Catecholamine Biosynthesis modified from Hare and Loer, 2004

(23)

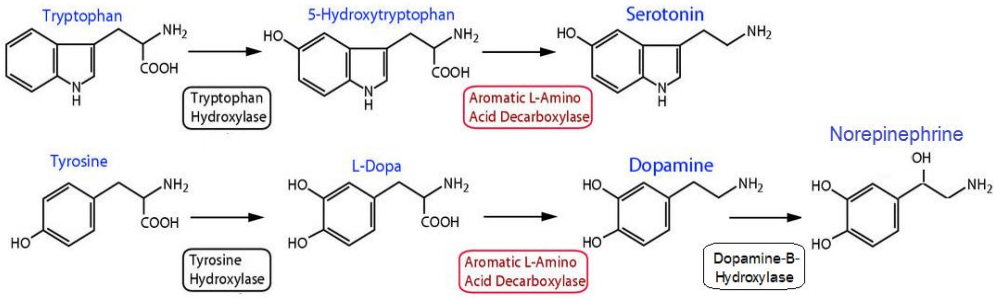


Figure 3 Degradation pathways of DA (A), NE (B), and 5-HT (C)

(24)

Distribution, functions and abnormality related with NMSs of these monoamine neurotransmitters are mentioned below.

- **Dopaminergic system**

Major pathology of PD is the loss of dopaminergic neurons in SNpc and subsequent decreasing of dopamine level in striatum (11). Dopaminergic neurons release catecholamine neurotransmitter named “dopamine, DA” and project their fibers into striatum, limbic, and associative cortical areas (fig. 4). A variety of dopamine functions are including motor, emotional, behavioral, cognitive, and motivational control. Depletion of DA contributes to disruption of cortical-basal ganglia-thalamus-cortical circuit. Inadequate dopamine also leads to increasing GABA and decreasing glutamate. Imbalance of these neurotransmitters results in impairment of motor activity (11). Patients with PD present motor symptoms after losing 50-70% dopaminergic neurons in SN (25). Additionally, DA depletion causes disruption of its interaction with the other neurotransmitters, which may cause non-motor problems (17).

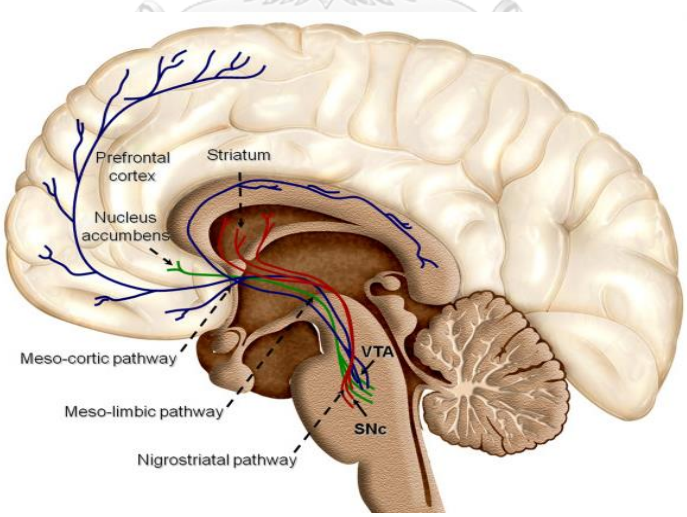


Figure 4 Dopaminergic neurons, their fibers projection, and pathways

(26)

- **Noradrenergic system**

Norepinephrine (NE) is a neurotransmitter releasing from locus coeruleus (LC) neuron in PPV, which is important for regulation of autonomic nervous system. LC neuron fibers project into many brain regions including, cortical, subcortical, brainstem, and spinal cord nuclei. NE from LC plays a role in memory, learning, attention, behavior, and sleep-wake regulations (Fig. 5). In peripheral autonomic nervous system, NE regulates postganglionic sympathetic neuron that innervate to vascular and cardiac systems (27). The work of Braak revealed that Lewy pathology presented in pontine LC before in SN, which mean the NE deficit from adrenergic degeneration occur earlier than DA deficiency. NE deficiency is the main cause of autonomic dysfunction in PD. A study by Iwanaga demonstrated Lewy bodies in sympathetic ganglia (cardiac plexus) in PD patients (28). Furthermore, more than 30% of PD patients have the orthostatic hypotension. In the study of Hurst showed the reduction of cerebrospinal concentrations of DA-beta-hydroxylase (enzyme converse DA to NE) in PD patients (29). As Goldstein reported dihydroxyphenylglycol (DHPG) (the main neuronal metabolite of NE) is lower in PD (30).

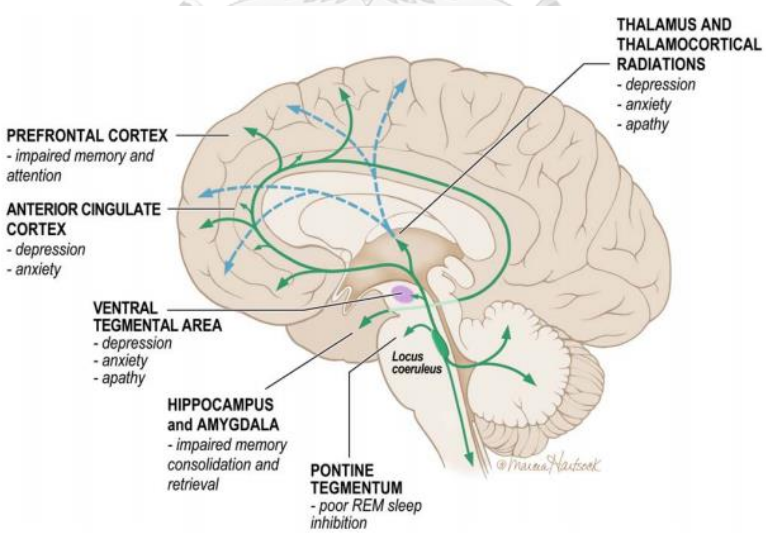


Figure 5 Noradrenergic neurons, their fibers projection and deficit-symptoms

(27)

- **Serotonergic system**

Serotonin or 5-hydroxytryptamine (5-HT) is produced by serotonergic neurons in raphe nucleus. Serotoninergic fibers project into basal ganglia, amygdala, hippocampus, hypothalamus, and cortical regions (fig. 6). 5-HT plays a role in mood, cognition, and motor function. Presence of Lewy pathology in raphe nuclei in Braak stage 2 relates to decrease in 5-HT levels and NMSs occurring in early PD. The NMSs result from reduction of 5-HT are including, apathy, anxiety, and depression (31).

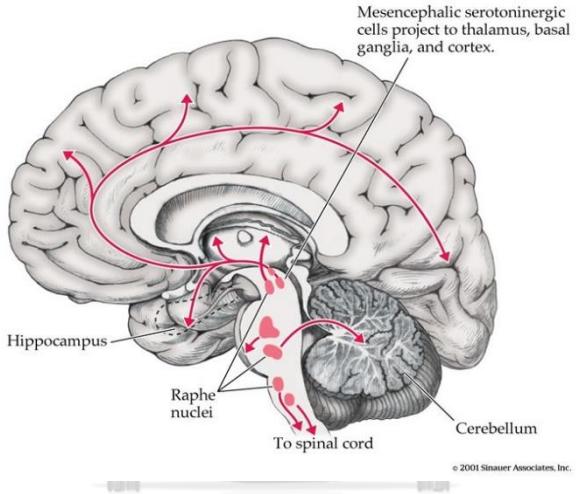


Figure 6 Serotonergic neurons and their fibers projection

At present, increasing evidences support that pathology in widespread brain and fluctuation of many brainstem neurotransmission systems lead to non-motor problems. A large number of non-motor symptoms include olfactory and visual problems, sleep disorders, mood disturbance (anxiety and depression), autonomic dysfunction (orthostatic hypotension, gastrointestinal and sexual dysfunction), neuropathic and central pain (allodynia, hyperalgesia, headache and migraine), and cognitive impairment (13, 32, 33). This study focuses on anxiety, depression, sleep disorder, and sexual dysfunction.

4. Non-motor Symptoms in Parkinson's Disease

- **Anxiety and Depression**

Anxiety disorders refers to feeling excessive fear, worried thoughts and associated with muscle tension, increasing of respiratory rate and avoidance behavior. Anxiety disorder is diagnosed when anxiety condition frequently and high intensity occurs and inappropriate situation. Prefrontal cortex and amygdala are the areas where involve anxiety in human. Amygdala is the stored-emotional memories area that is stimulated from environment. The efferent fibers of amygdala project into the parabrachial nucleus, vagus nerve, nucleus ambiguous, and lateral hypothalamus which result in hyperventilation, parasympathetic, and sympathetic activation, respectively. The prevalence of anxiety in PD patients varies between 20-52%. Anxiety is comorbidity of depression and usually fluctuates during medication on/off phases and motor freezing (34).

Depression occurs in about 30-40% of PD patients and gives more impact to their quality of life. The onset of depression is in the early stage of PD and may manifest before motor impairment (35, 36). Symptoms of depression are slow movement, bradyphrenia, poor concentration, loss of libido, sleep and appetite disturbances (37).

The pathophysiology of depression in PD has been involved in monoamine-deficiency theory that is disturbances of mesocorticolimbic dopaminergic, noradrenergic, and serotoninergic pathways (38, 39). These monoamine neurons project to several areas of the brain, which are responsible for behavioral control including mood, motivation, and fatigue. So, abnormal functions of these neurotransmission systems lead to depression or mood disorders. DA is the important neurotransmitter that regulates mood, motivation, and reward. The imaging and post-mortem studies of PD patients revealed deficiency of DA in mesolimbic system (40). In 2005, Remy revealed the reduction of catecholamine innervation in LC, limbic regions, and ventral striatum in PD patients with depression (41). Depressive patients showed decrease in NE and 5-HT turnover in the brain (42). Moreover, there are the correlation between loss of serotoninergic neurons in raphe nucleus and depression in PD patients (36).

- **Sleep Disturbances**

Sleep disorders are a group of conditions that involve to the change in sleep-wake cycle. This problem occurs about 80-90% in PD patients (43). Common sleep problems in PD include insomnia, excessive daytime sleepiness, sleep fragmentation, restless legs syndrome, rapid eye movement-sleep behavior disorder, and nocturia (32).

Normal sleep architecture consists of two alternative stages including non-rapid eye movement (NREM) and REM stages. Each stage has different in brain activity, movements of eye, and muscle tone. NREM sleep is divided into stages 1, 2, 3, and 4. Sleep cycle start with NREM stage 1, 2, 3, 4, and finally to REM (fig. 7). Time spent in NREM and REM stages are about 80% and 20%, respectively. NREM stage 1 is 2-5% of total cycle. This stage is easily to interruption. Brain activity changes from alpha wave to low-voltage wave. Similarly, NREM stage 2 shows relatively low-voltage and mixed frequency waves. However, NREM stage 2 present K-complex waves and is difficult to awake than stage 1. Stage 3 and 4 of NREM presents high-voltage and slow-wave activity. In contrast, REM stage present burst of rapid eye movements and muscle atonia. During REM stage, there are increasing of brain activity in motor and sensory areas, heart rate, blood pressure, and sympathetic activity. Dreaming is often occurring in this stage. However, acting out or nightmare are prevented during REM from muscle atonia (44).

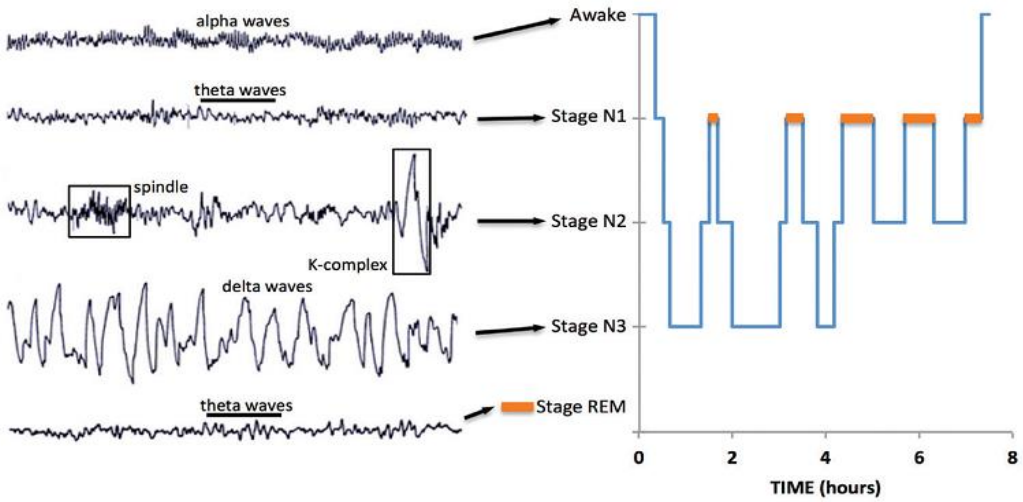


Figure 7 Electroencephalography of sleep/wake stages

Normally, sleep-wake cycle is regulated by two major processes including maintain wakefulness and promote sleep processes. Wakefulness is regulated by ascending arousal system through 2 pathways. First pathway, cholinergic neurons in pedunculo-pontine tegmentum (PPN) of pons send signal to thalamus and cerebral cortex. The second pathway originates by neurons which contain monoamine neurotransmitters in brainstem including LC, raphe nuclei, and ventral periaqueductal gray matter. These neurons send signals (NE, 5-HT, and DA) to activated neurons in hypothalamus to releasing of orexin for maintain arousal state (fig. 8). Sleep is generated in brainstem region. The neurons in preoptic area of hypothalamus are responsible for turn off the arousal system during sleep. In addition, neurons in pons involve to switching of NREM and REM by send output to lower brainstem and spinal cord to inhibit muscle tone (45).

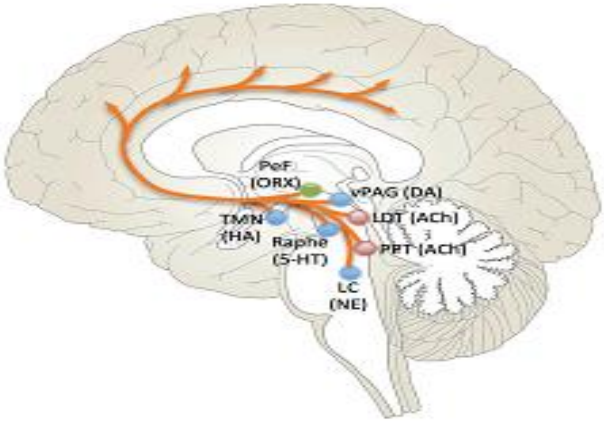


Figure 8 The brain networks that control wakefulness

The causes of sleep disturbance in PD result from destruction of brainstem-controlled sleep-wake cycle from Lewy body accumulation. These destructible regions include raphe nuclei, LC, SN, ventral tegmental area (VTA), neocortex, hippocampus, and PPN. Moreover, they associate to the impairments of sleep architecture (REM and non-REM stages), and the thalamocortical arousal system (orexin, 5-HT, NE, DA, and Ach). In addition to these pathological changes, sleep disturbances also involve nocturnal motor symptoms (such as rigidity, bradykinesia, tremor, dyskinesia, and dystonia), non-motor symptoms (depression and cognitive impairment), and medication use (such as dopaminergic and antipsychotic drugs) (46). Excessive daytime sleepiness (EDS) results from impairments of arousal system, which involves change in 5-HT, NE, DA, Ach, and orexin levels. The study of Fronczek (2007) found the reduction of orexin in hypothalamus of PD patients, which related to severity of disease (47). Moreover, using of dopaminergic drug contributes to sedative effect and decrease daytime alertness (48).

Rapid eye movement-sleep behavior disorder (RBD) is the common sleep problem occurring in early state of PD and precede motor symptoms. It is manifested by lack of muscle atonia during REM state leading to vivid dream and nightmare. According to Braak theory stage 2, Lewy bodies accumulate in brain areas where regulate sleep/ wake cycle and REM/ non-REM states. These areas include PPN, LC, and magnocellular reticular formation, which are involve to disturbances of cholinergic and monoamine neurotransmitters. RBD is more affect in men than women (49).

Restless legs syndrome (RLS) is characterized by dysesthesias in limbs which can be diminished by movement. This symptom is prominent in the evening and at night. The pathophysiology of RLS involves in dysregulation of A11 dopaminergic nucleus and hypothalamus which control sensory suppression through the spinal tract (48).

Nocturia is the condition that patients have excessive and frequent urination during night time. The cause of nocturia in PD is dopaminergic dysfunction. The D1-receptor is under stimulation, whereas D2-receptor is over-stimulation. These conditions lead to hyper-activation of detrusor muscle of urinary bladder (50).

- **Sexual Dysfunction**

Sexual dysfunction (SD) is the one of NMSs, which is usually disregarded. Symptoms of SD include decreased libido, erectile impairment, insufficient lubrication, genital pain during sexual intercourse and difficult to reaching orgasm.

Normally, sexual arousal is controlled by medial preoptic and anterior hypothalamic region that related to limbic-hippocampal pathway. In men, libido (biological need for sexual activity) and erection are controlled by medial preoptic area and paraventricular nucleus. Libido is activated by dopaminergic neurotransmission and testosterone. Testosterone promotes releasing of DA in medial preoptic area to activated sexual behavior (sexual motivation, arousal, and reward). Moreover, neurons in preoptic area and hypothalamus send signal to activated parasympathetic and sympathetic nervous systems to modulated smooth muscle tone and vasoactivity, Sympathetic neurons release NE that responsible for regulate smooth muscle contraction and vasoconstriction that provoke erection in male.

SD problem in PD mainly results from impairments of motor (rigidity and bradykinesia), mood disorder, cognition, and sleep. In addition, it involves to the reducing of monoamine neurotransmitters regulated function of the hypothalamo-hypophyseal-gonadal system (51). DA depletion contributes hyposexual in PD. However, there were reports of hypersexual condition in PD patients who use L-dopa

and apomorphine for a long time (52). NE depletion is also relating to SD. NE is a neurotransmitter for regulated vasoconstriction, so decrease in NE level causes of sexual impairment in male patient. Likewise the study of Goldstein found decreasing of NE correlated to drop in sexual activity (53). In contrast, 5-HT suppresses libido by decreasing sensitivity of brain to testosterone. The antidepressant drugs that use in PD with depression usually implicate SD. These drugs include selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (54).

5. Non-motor Fluctuations in Parkinson's Disease

Almost PD patients who have had long term of levodopa treatment (more than 5 years) will develop motor fluctuations. This condition is the variations in motor symptoms control. Motor fluctuations relate to the alteration of DA concentration in striatum. Another cause of motor fluctuation is pulsatile non-physiological dopaminergic stimulation by levodopa therapy. The fluctuation of DA is depending on level of levodopa in blood circulation (fig. 9). These fluctuations establish ON/ OFF phenomena in PD. The period that has high concentration of DA and is able to control the symptoms called "ON state". On the other hand, OFF state correlates with less DA level causing inability to control the symptoms (55). Moreover, most PD patients usually experience non-motor fluctuations (NMFs), which can easily be neglected. However, the cause and pattern of NMFs remain unclear. There is hypothesized that NMFs may result from oscillating dopamine modulated other neurotransmitters (i.e. 5-HT, NE, and Ach) (6). Some of studies demonstrated that NMFs present along with motor fluctuations. Conversely, many reports found NMFs are not always occur together with motor fluctuations (56).

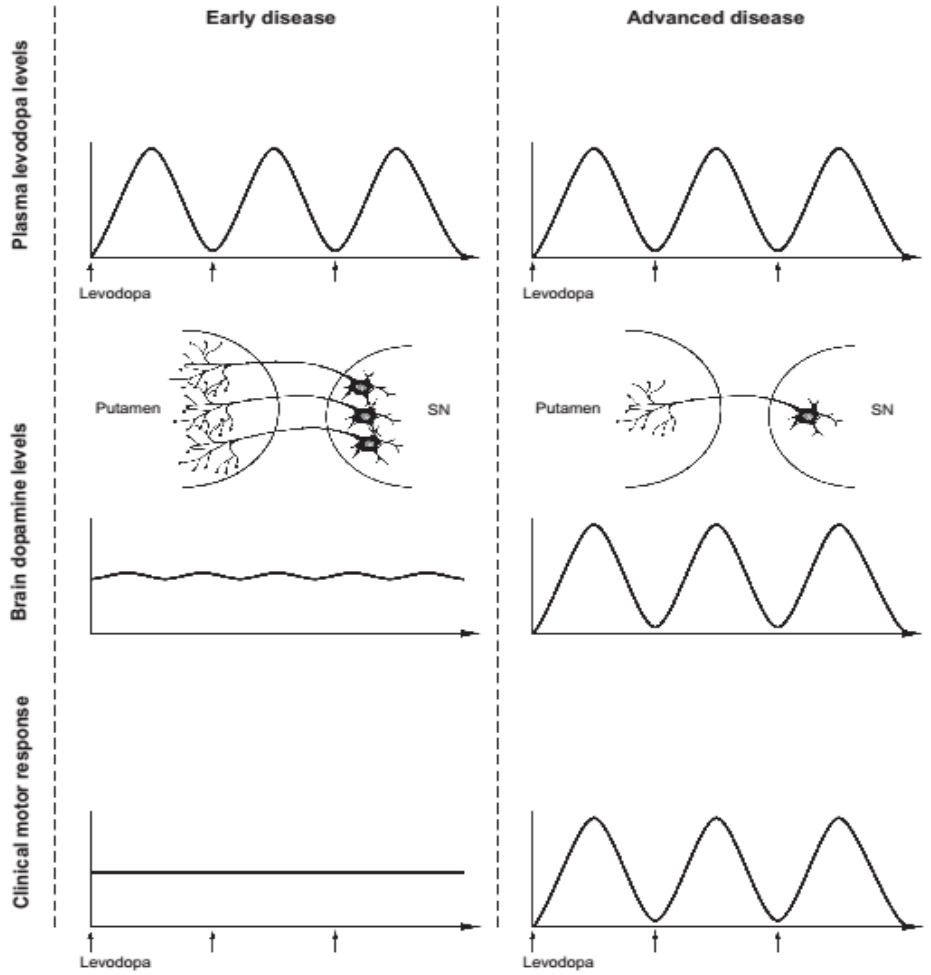


Figure 9 The association of levodopa level, brain dopamine level, and motor fluctuation in early and advanced stages of PD.

(57)

6. Diagnosis for Parkinson's Disease

Nowadays, there is no specific test for diagnosis of PD. Clinicians consider base on patient's medical history and signs from neurological examinations. Occasionally, the brain image technique and a variety of biomarkers are used to confirm it and distinguished from the other diseases.

1) Clinical signs and symptoms

Motor symptoms which are characteristics of PD include resting tremor, rigidity, bradykinesia, and postural instability. These symptoms have asymmetrical onset and effective response to dopaminergic therapy. Masked face, loss of arm swing, micrographic and hypophonia also are the features of PD. In addition to motor, non-motor problems such as abnormal sleep, sensation, mood and behavior, and autonomic system are the common symptoms of PD patients (17).

2) Brain imaging study

Neuroimaging is able to explore the depletion of striatal dopamine and destruction of involved brain areas. Computerized tomography (CT) scan and magnetic resonance neuroimaging (MRI) are used for detecting vascular diseases and changes in basal ganglia structure. Positron emission tomography (PET) and single-photon emission CT (SPECT) are used for identifying the loss of neurons, dysfunctions of presynaptic and postsynaptic terminal in nigrostriatal areas. However, these neuroimaging techniques are more expensive and are not available in many clinical settings.

3) Biochemical markers

There are many researches revealed the neurodegenerative biomarkers correlated with pathogenesis of PD such as tau and alpha-synuclein proteins (3).

7. Management of Parkinson's Disease

1) Dopaminergic medication

Levodopa (L-dopa) is a precursor of DA. It is the most effective and primary medication for improving motor disability. L-dopa has short half-life (approximately 1-3 hours) (58). It is converted by decarboxylase enzyme in peripheral system before entering the brain (fig. 10). To prevent the decreasing of bioavailability in the brain, levodopa is usually given combine with carbidopa or benserazide to inhibit peripheral decarboxylation. However, patients who receive long period of L-dopa therapy (>5 years) often develop motor fluctuations and complications such as levodopa-induced

dyskinesia. These patients have wearing off effect that refers to decreasing of duration of drug benefits in each dose. So, patients usually describe motor fluctuations as ON period (medication is able to control the symptoms) and OFF period (medication is not effective to control the symptoms). Catechol-O-methyltransferase (COMT) and monoamine oxidase type B (MAO) inhibitors are benefits to reduced degradation and increase half-life of levodopa, and decrease OFF period (59). Unlike levodopa, dopamine agonist is an agent that directly activates dopamine receptor (mainly in D2 receptor). It has longer half-life than levodopa and has less chance for generating motor fluctuations and complications. So, dopamine agonist is initially used in young-onset PD. A group of dopamine agonists include bromocriptine, pergolide, pramipexole, ropinirole, and apomorphine.

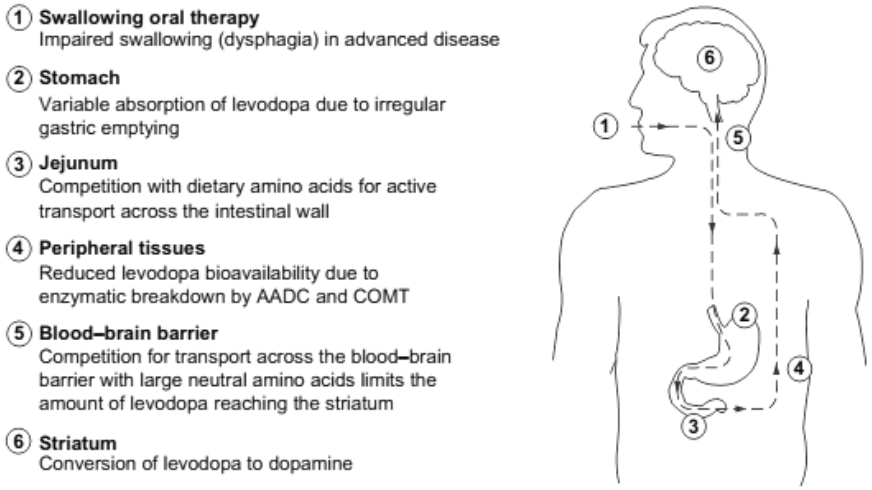


Figure 10 Oral levodopa therapy: from oral administration, ingestion, enter to the brain, and converse to DA

(57)

2) Non-dopaminergic medication

Many symptoms of PD are not improved and some of them are worsening after dopaminergic therapy, particularly non-motor symptoms. Depression is usually treated with a selective serotonin reuptake inhibitor (SSRI) or tricyclic antidepressants. Daytime somnolence is improved by reduced dopamine agonist. REM is treated by clonazepam.

3) Deep brain stimulation (DBS)

This technique employs an electrode implantation in deep brain area, such as subthalamic nucleus or Globus pallidus interna. High-frequency (100–200 Hz) electricity will then apply on that brain area.

CHAPTER III

MATERIALS AND METHODS

1. Materials

1) Chemicals

- HPLC complete kit for catecholamines in plasma (RECIPE, Munich, Germany)
- HPLC complete kit for serotonin in plasma (RECIPE, Munich, Germany)
- HPLC complete kit for VMA, HVA, and 5-HIAA in urine (RECIPE, Munich, Germany)
- HPLC complete kit for metanephrines in urine (RECIPE, Munich, Germany)

2) Equipment

- High performance liquid chromatography
  - Pump: CLC 300 (Chromesystems, Germany)
  - Electrochemical detector: CLC 100 (Chromesystems, Germany)
  - Sample injector: programmable autosampler CLC 200 (Chromesystems, Germany)
  - Column for catecholamines, serotonin, VMA, HVA, 5-HIAA, and metanephrine (Recipe, Germany)
  - Guard column
  - Data systemic control and analysis software: Easyline analysis software (Munich, Germany)
- Centrifuge
- Blood collection tubes: K3 EDTA (Hangzhou Healthaw Technology, China)
- Urine containers
- Disposable plastic syringes with 22G needles
- Pipettes, pipette tips
- Vortex mixer
- Sample Vials for HPLC (2 ml)

3) Tools

- Mini – Mental State Examination in Thai version (MMSE – Thai 2002)
- The Hospital Anxiety and Depression Scale in Thai version (Thai-HADS)
- The Modified Parkinson's Disease Sleep Scale in Thai version (Thai MPDSS)
- Non-motor Symptoms Questionnaire for Parkinson's disease in Thai language (Thai-NMSQ)
- The Arizona Sexual Experience Scale in Thai version (ASEX-Thai)
- The eight-item Parkinson's Disease Questionnaire (PDQ-8)

2. Methods

This study divided into 2 parts. For the first part, we aimed to investigate the differences of DA, NE, 5-HT and their metabolites levels in plasma, saliva and urine of PD and control groups and also evaluated their correlations with the pathogenesis of NMSs. In the second part, the purpose was to investigate the fluctuations of plasma DA, NE and 5-HT levels during medication ON/OFF periods in advanced-PD patients. The experimental protocols of this study were approved by the institutional ethic committee of faculty of Medicine, Chulalongkorn University. The details of each part were as follows:

Part I: The alteration of monoamines levels in peripheral body fluids and their correlations with NMSs in PD patients.

1) Research Design

Cross-sectional analytic study

2) Populations

Target Population

Male or female patients who were diagnosed as idiopathic Parkinson's disease (IPD), aged between 30-70 years old from the Movement Disorders Outpatient Clinic at Chulalongkorn Center of Excellence on Parkinson's Disease & Related Disorders, Thailand.

Control Population

Male or female volunteers aged between 30-70 years old who had no family history with PD.

3) Participants recruitment and experimental design

We performed this study at Chulalongkorn Hospital between July to December 2018. Male or female, aged between 30-70 years volunteers and PD patients participated in this project. We recruited IPD patients from the Movement Disorders Outpatient Clinic at Chulalongkorn Center of Excellence on Parkinson's Disease & Related Disorders, Thailand. These IPD patients were diagnosed by clinician according to the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria (UKPDSBB) (Table 1). All subjects signed a written informed consent form under the protocol approval by the Institutional Review Board of Faculty of Medicine, Chulalongkorn University, Thailand (approval number 451/60). All procedures were performed in accordance with the approved guidelines of the ethical standards of the institutional research committee.

Table 1 UK PD SOCIETY BRAIN BANK CLINICAL DIAGNOSTIC CRITERIA

Step 1. Diagnosis of Parkinsonian Syndrome
• Bradykinesia • At least one of the following - Muscular rigidity, 4-6 Hz rest tremor - postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction
Step 2. Exclusion criteria for Parkinson's disease
• history of repeated strokes with stepwise progression of parkinsonian features • history of repeated head injury, history of definite encephalitis • oculogyric crises • neuroleptic treatment at onset of symptoms • more than one affected relative, sustained remission • strictly unilateral features after 3 years • supranuclear gaze palsy, cerebellar signs • early severe autonomic involvement • early severe dementia with disturbances of memory, language, and praxis • Babinski sign • presence of cerebral tumor or communication hydrocephalus on imaging study • negative response to large doses of levodopa in absence of malabsorption • MPTP exposure
Step 3. supportive prospective positive criteria for Parkinson's disease
Three or more required for diagnosis of definite Parkinson's disease in combination with step one • Unilateral onset, Rest tremor present • Progressive disorder • Persistent asymmetry affecting side of onset most • Excellent response (70-100%) to levodopa • Severe levodopa-induced chorea, Levodopa response for 5 years or more • Clinical course of ten years or more

Reference: Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease. A clinico-pathological study of 100 cases. JNNP 1992; 55:181-184.

Principle personal data of participants were obtained from interview and medical records and dementia condition was evaluated by using Mini-Mental State Examination (MMSE-Thai 2002). The participants who had characteristics as inclusion criteria showing in following table were included. On the other hand, participants who had dementia (MMSE score < 22) or presented the one of characteristics as the exclusion criteria were removed (Table 2).

Table 2 Inclusion and exclusion criteria of control and PD groups

Groups of experiment	Inclusion criteria	Exclusion criteria
Control	- male or female volunteer - aged between 30-70 years old - understand and able to complete the Thai-questionnaire - no history of following conditions; CVD, TBI, brain tumor, encephalitis, Alzheimer's disease, spinal cord disease, peripheral neuropathy, and Schizophrenia	- had family history of PD - dementia (MMSE score < 22) - use medication that can interfere the concentrations of neurotransmitter or drink or eat coffee, tea, chocolate, and banana 12 hours before sample collection - present severe anxiety, depression, and sleep disturbance
PD	- male or female patients are diagnosed as idiopathic Parkinson' disease - aged between 30-70 years old - understand and able to complete the Thai-questionnaire - no history of following conditions; CVD, TBI, brain tumor, encephalitis, Alzheimer's disease, spinal cord disease, peripheral neuropathy, and Schizophrenia - present one of these NMSs including anxiety and depression, sleep disturbance, sexual dysfunction	- Dementia (MMSE score < 22) - Use medication that can interfere the concentrations of neurotransmitters Take serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, neuroleptic 12 hours before sample collection - drink or eat coffee, tea, chocolate, banana 12 hours before sample collection

Then participants were interviewed and physical examined by physician. They replied the questionnaires by themselves or partial assistance with the researcher. The questionnaire for control and PD groups consisted of 6 parts (91 questions) and 7 parts (100 questions), respectively. These questionnaires used to assess conditions of anxiety, depression, sleep disturbances, sexual dysfunction, general NMSs and quality of life. The parts of questionnaire included demographic data, Hospital Anxiety and Depression Scale (Thai-HADS), Modified Parkinson's Disease Sleep Scale (MPDSS), Arizona Sexual Experiences Scale (ASEX-Thai), non-motor symptoms questionnaire (NMSQ), the eight item of Parkinson's disease questionnaire (PDQ-8). Participants replied these questionnaires in private room and lasted about 30 minutes to complete them.

Considering from the result of physical examination and questionnaires, PD patients who presented at least one of these NMSs; including anxiety, depression, sleep disturbance, and sexual dysfunction were included. In contrast, participants in control group who presented severe anxiety, depression, and sleep disturbance conditions were excluded. Then blood, saliva, and urine were collected from all participants for determination of neurotransmitters and their metabolites concentrations by using high performance liquid chromatography (HPLC) with electrochemical detector (ECD). The levels of monoamines in plasma and saliva which were determined in this study including DA, NE, and 5-HT. We also measured urinary metabolites levels; including 3-MET, HVA, NM, VMA and 5-HIAA. The concentrations of these neurotransmitters and metabolites between PD and control groups were compared. Moreover, the correlation between neurotransmitter concentrations and score of NMSs questionnaires and clinical profiles in PD group were analyzed. The flow diagram of methods in part I show in fig. 11.

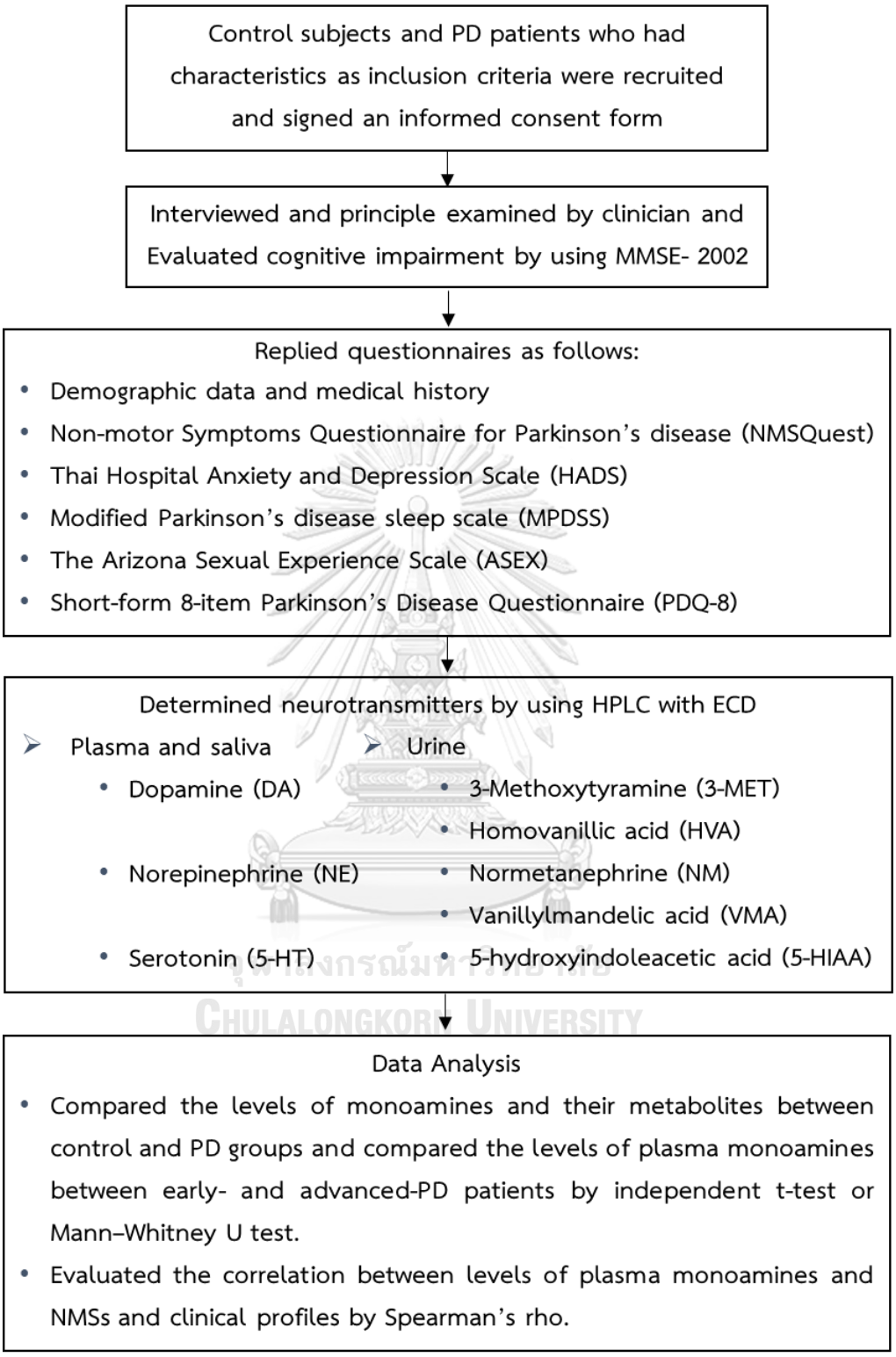


Figure 11 Flow diagram of procedure in part I

1) Non-motor symptoms assessment by using standard questionnaires

(1) Cognitive Impairment

Cognitive function was assessed by Mini Mental State Examination Thai 2002 (MMSE-Thai 2002) (Appendix 1). This questionnaire was translated and adjusted from the original version of MMSE (Folstein et al., 1975) by the Institute of Geriatric Medicine, Department of Medical Services, Ministry of Public Health, Thailand. The sensitivities and specificity ranges are vary from 71-92% and 56-96%, respectively (60). The MMSE 2002 composes of eleven items, which is divided into six categories; including time and place orientation, registration of words, attention, calculation, recall, and language/ visual constructions. This questionnaire requires 5-10 minutes to complete it. The range of score is 0-30. Zero score represent severe impairment, whereas thirty score represent no impairment of cognitive function. The cut point of this questionnaire shows as in table 3.

Table 3 The cut point of Mini Mental State Examination Thai 2002

Education levels	Cut point	Total score
Un-education	14	23 (do not test item 4, 9, 10)
Primary school	17	30
Secondary school or higher education	22	30

(2) Anxiety and Depression

Anxiety and depression were evaluated by the Hospital Anxiety and Depression Scale (HADS). This questionnaire was created by Zigmond and Snaith (1983) to assessment anxiety and depression conditions in non-psychiatric person at outpatient clinic. HADS is the brief self-screening questionnaire, which use short time to complete it. HADS consists of two divisions (14-items); including anxiety (odd items) and depression (even items). The range score of each item is 0-3 and the maximum score of each subdivision is 21. Anxiety and depression were indicated when the total scores

of odd and even items equal or higher than 11, respectively (Appendix 2). HADS in Thai version was translated by Nilchaikovit and colleagues (61). They tested the validation and reliability of this questionnaire in cancer patients. The findings showed the sensitivities of cut-off points in anxiety and depression equal to 100% and 85.71%, respectively.

(3) Sleep Disturbances

Sleep problems of PD patients were assessed by using the Modified Parkinson's Disease Sleep Scale (MDPSS). This questionnaire was modified by Tanasanvimon and team (62). They modified from PDSS original version of Chaudhuri and coworkers in 2002 (63). The MPDSS is a visual analog scale to ask about sleep problems in 19 items. The questions in MPDSS compose of several aspects; including quality/ onset/ maintenance of sleep, nocturnal restlessness/ psychosis/ motor symptoms, nocturia, sleep refreshment, daytime dozing, REM behavioral disorder (RBD), sudden onset of sleep, and snoring (Appendix 3). The visual analog scale was ranging from 0 to 10 that was judged by patients or caregivers. The scale was 10, when patients had never that sleep symptom. If the patients had that sleep symptom, they will mark to scale according to the frequency of symptom occurrence. The lower scale implicated to more frequency of abnormal sleep symptom. The cut-off point used for screening sleep problems was lower than 6 score.

(4) Sexual Dysfunctions

Sexual dysfunctions in PD were evaluated by the Thai version of Arizona Sexual Experiences Scale (ASEX-Thai). This questionnaire was translated and adapted from the original version of McGAHUEY and colleagues (64) by neurologist at Chulalongkorn Center of Excellence on Parkinson Disease & Related Disorder. ASEX is composed of five items in regard to sexual drive, arousal, penile erection/vaginal lubrication, ability to reach orgasm, and satisfaction from orgasm (Appendix 4). The scale is range in 1 to 6, which 1 and 6 mean hyper-function and hypofunction, respectively. The cutoff point

is equal or more than 16 points. The sensitivity and specificity of ASEX-Thai are 96.2% and 92.9%, respectively (65).

2) Blood, saliva, and urine collections

Blood samples (3 ml.) were collected during off period from the cubital vein at anterior forearm. The venipuncture was taken after local anesthesia with lidocaine cream. Blood was drawn into an ethylenediamine tetraacetic acid (EDTA) tube to prevent blood clot. Then blood samples were immediately centrifuged (within 60 minutes) at 3000xg for 10 minutes at 4°C. After that plasma was separated and stored at -80°C until analyzed.

Saliva samples (2 ml) were directly collected without exogenous stimulation after mouth washing. Participants spited the saliva into the container. This saliva was aliquoted and stored frozen at -80°C until further process.

A single sample of urine (10 ml.) was collected at the same period time of plasma and saliva collections. Urine samples were kept in container and added the 32% hydrochloric acid (HCl) in the concentration of 10 ml per liter urine. Samples were aliquoted and kept at the temperature below -80 °C until metabolite determination.

3) Neurotransmitters and their metabolites determination

All neurotransmitters and their metabolites were analyzed by using the high-performance liquid chromatography (HPLC) with electrochemical detector (ECD) (Chrome, Germany). Their concentrations were determined by Easyline Analysis software program (Munich, Germany). Number of neurotransmitters and metabolites were calculated with internal standard method by compared the correlations of retention times and peak areas of chromatograms between sample and its calibration curve.

The analytic concentration was calculated according to the following formula:

$$C_{(sample)(\mu g/l)} = \frac{Area_{(sample)} \times C_{(calibrator)(\mu g/l)}}{Area_{(calibrator)} \times REC}$$

Where: C = concentration (µg/l)

Area = peak area of chromatogram

REC = recovery rate that calculate from this formula;

$$REC = \frac{Area\ IS_{(sample)}}{Area\ IS_{(calibrator)}}$$

IS = internal standard

The detail of plasma neurotransmitter and urine metabolites measuring procedures and HPLC setting were described as below.

Determinations of plasma DA and NE

Plasma (1 ml) and internal standard (50µl) were pipetted into preparation tube containing the aluminum oxide suspension, respectively. After that they were mixed for 10 minutes, centrifuged for 1 minute at 1000xg and discarded the effluent. Subsequently, we added the washing solution (1 ml) into preparation tube, centrifuged for 1 minute, and discarded the effluent. This washing process was repeated for 3 times. Then the elution vial was inserted onto the preparation tube. Finally, 120 µl of eluting reagent was mixed for 1 minute and centrifuged at 1000xg for 1 minute. The eluted sample (40 µl) was injected into the HPLC system for DA and NE determination (fig. 12).

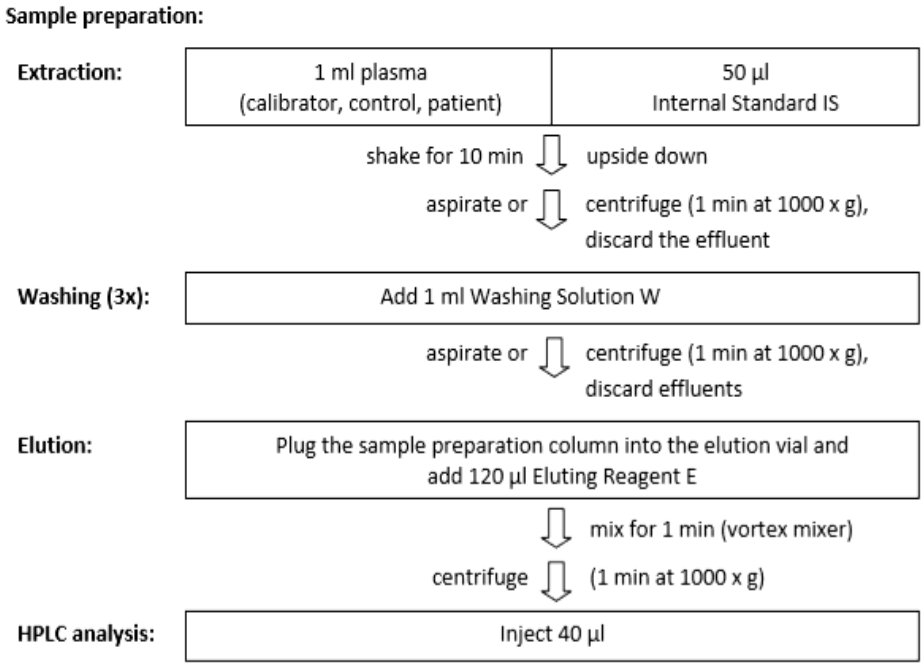


Figure 12 Flow diagram of plasma DA and NE determinations

For determinations of DA and NE, HPLC system, autosampler, and ECD conditions were set as in table 4. The retention times of DA and NE extraction were approximate 5 and 12 minutes, respectively (fig. 13).

Table 4 Conditions of HPLC and ECD for plasma DA and NE determinations

HPLC pump	flow rate 1.0 ml/min
HPLC column	25°C
Injection volume	40 µl for condition sample and 10 µl for standard solution
Injection time	15 minutes
ECD	- potential 500 mV, sensitivity 10 nA - filter setting 0.2 Hz The basic current should be within range of 0.2 and 2.5 nA.

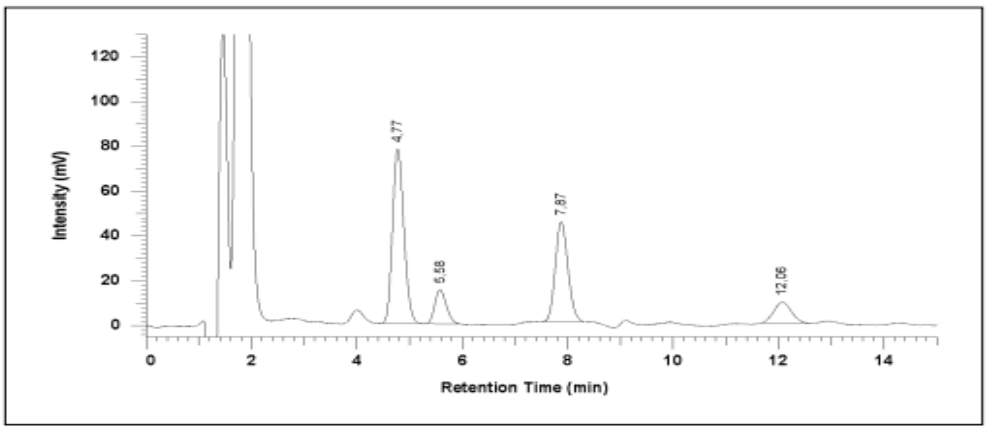


Figure 13 Chromatogram with retention times of plasma NE and DA calibrator (NE: 4.77 min, EPI: 5.58 min, internal Standard: 7.87 min, and DA: 12.06 min)

Determination of plasma 5-HT

Plasma (200 µl) and internal standard solution (10 µl) were pipetted into preparation tube followed by mix them on vortex mixer for 5 seconds. Then precipitant P solution (200 µl) was put into preparation tube, subsequently mixed for 5 seconds and centrifuged at 10,000xg for 1 minute. The 20 µl of centrifuged supernatant was injected into the HPLC system for 5-HT measurement (fig. 14).

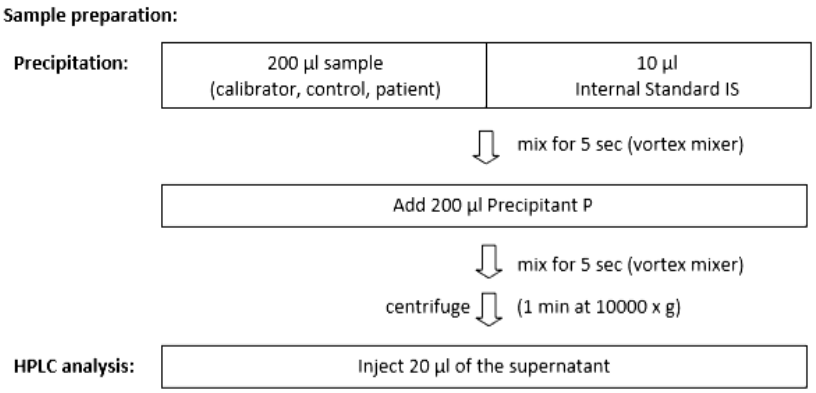


Figure 14 Flow diagram of 5-HT determination

To quantification of 5-HT, we set the HPLC system, autosampler, and ECD conditions as in table 5. The retention times of 5-HT extraction were approximate 6.2 minutes (fig. 15).

Table 5 Conditions of HPLC and ECD for 5-HT determination

HPLC pump	flow rate 1.0 ml/min
HPLC column	30°C
Injection volume	20 µl for condition sample and 10 µl for standard solution
Injection time	10 minutes
ECD	- potential 450 mV, sensitivity 20 nA - filter setting 0.2 Hz - the basic current should be within the range of 0.2 and 2.5 nA

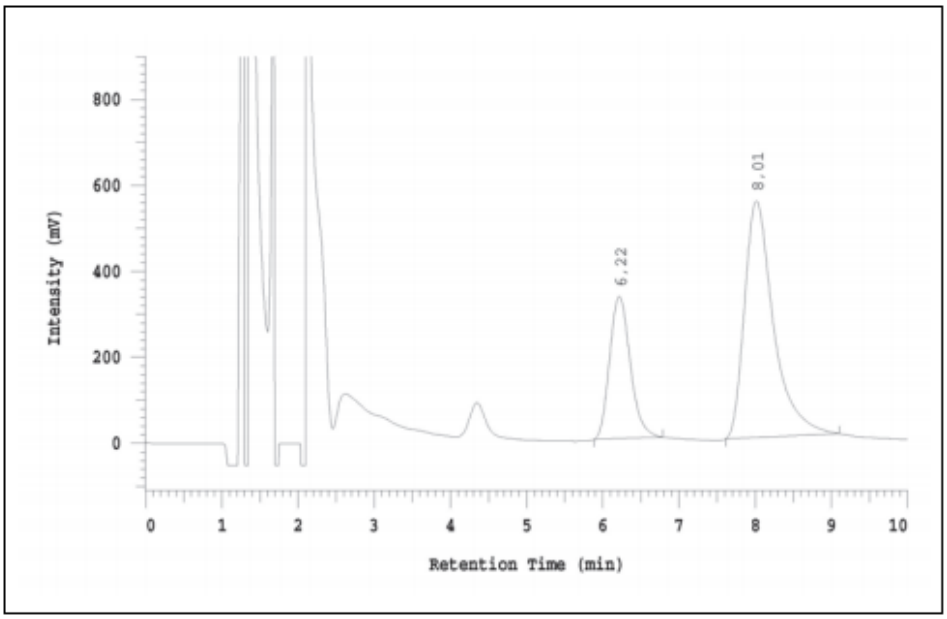


Figure 15 Chromatogram and retention times of plasma 5-HT calibrator (5-HT 6.2 min and internal standard: 8.0 min)

Determinations of VMA, HVA, 5-HIAA in urine

This process started from mix the 50 µl of urine sample and 1 ml of internal standard in the preparation tube, followed by centrifuge at 800xg for 1 minute, and discarded the effluent. Next, the solution was washed by 1 ml of ammonia (1 time) and boric acids (2 times). Between these washing steps, they were centrifuged at 800xg for 1 minute and discarded effluent. The last step, we added 2 ml of elution reagent into tube and centrifuged at 800xg for 1 minute. The eluted solution (20 µl) was collected for injected into HPLC system (fig. 16). To measure the concentration of these metabolites, we set the parameters of HPLC, autosampler, and ECD as in table 6. The retention time of VMA, HVA, and 5-HIAA were approximately 3.7, 7.9, and 11.6 minutes, respectively (figure 17).

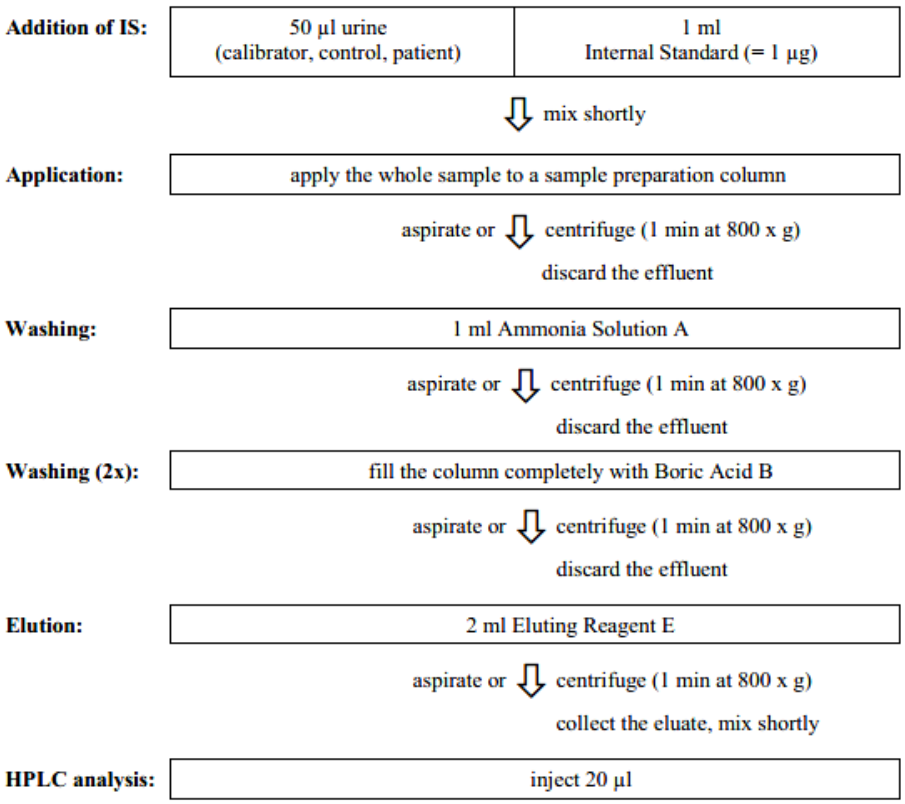


Figure 16 Flow diagram of urinary VMA, HVA, and 5-HIAA determinations

Table 6 Conditions of HPLC and ECD for VMA, HVA and 5-HIAA determinations

HPLC pump	flow rate 0.9 ml/min
HPLC column	30°C
Injection volume	20 µl for condition sample and standard solution
Injection time	15 minutes
ECD	- potential 800 mV, sensitivity 50 nA - filter setting 0.2 Hz - the basic current should be within the range of 0.2 and 2.5 nA

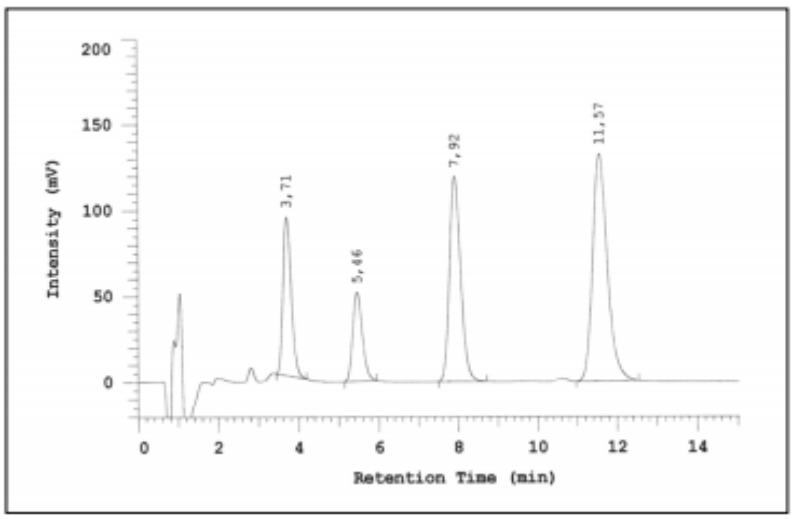


Figure 17 Chromatogram and retention times of VMA, HVA and 5-HIAA calibrator (VMA: 3.7 min, internal standard: 5.5 min, HVA: 7.9 min and 5-HIAA: 11.6 min)

Determination of urinary NM

Urine (1 ml) and internal standard (20 µl) were put and mixed into the vial. Then pH-value was evaluated and adjusted in the range of 0.5-1.0 with HCl or NaOH. Urine samples were incubated at 90-100°C for 30 minutes. After pH adjustment, whole

samples were put into preparation column contained the solid phase extraction. Samples were drained into the collecting tray and the effluents were discarded. The next step, HPLC water was filled into the column, followed by washing solution (4 ml), and HPLC water again. In every step, the solution was drained through the column and discarded the effluent. Finally, the stabilizing reagent (5 ml) was added and mixed with vortex mixer. Then the eluted solution was drained through the column into the vial. This eluted solution (20 µl) was injected to HPLC system (fig. 18). For NM extraction, we set the HPLC parameters as in the table7. The retention time was approximately 5.18 minute (fig 19).

Table 7 Conditions of HPLC and ECD for urinary NM determination

HPLC pump	flow rate 1.0 ml/min
HPLC column	30°C
Injection volume	20 µl for condition sample and standard solution
Injection time	17 minutes
ECD	- potential 650 mV, sensitivity 50 nA - filter setting 0.2 Hz - the basic current should be not over than 5.0 nA

Sample preparation:

Hydrolysis: 1 ml urine (calibrator, control, patient) | 20 µl Internal Standard IS (= 200 ng)

Check the pH-value. If necessary, ⇩ adjust with conc. HCl (approx. 20 µl) to a pH value between pH = 0.5 – 1.0

Incubate for 30 min ⇩ at 90 - 100 °C

pH Adjustment:
1.) Add 4 ml Diluting Solution D to the hydrolysed, cooled (room temperature) sample
2.) If necessary, add 1 M NaOH dropwise, until the colour indicator switches to yellow (pH > 6)

⇩

Extraction: Apply the entire sample to the sample preparation column

⇩ discard the effluent

Washing: Wash the sample preparation column with
1.) HPLC water (fill up the column)
2.) 4 ml Washing Solution W
3.) HPLC water (fill up the column)

⇩ discard the effluents

Elution: Pipette 1 ml Stabilising Reagent S into a sample preparation vial. Put a sample preparation column upon the vial and apply 5 ml Eluting Reagent E.

collect the eluate ⇩ and mix shortly (vortex mixer)

HPLC analysis: inject 20 µl

Figure 18 Flow diagram of NM determination

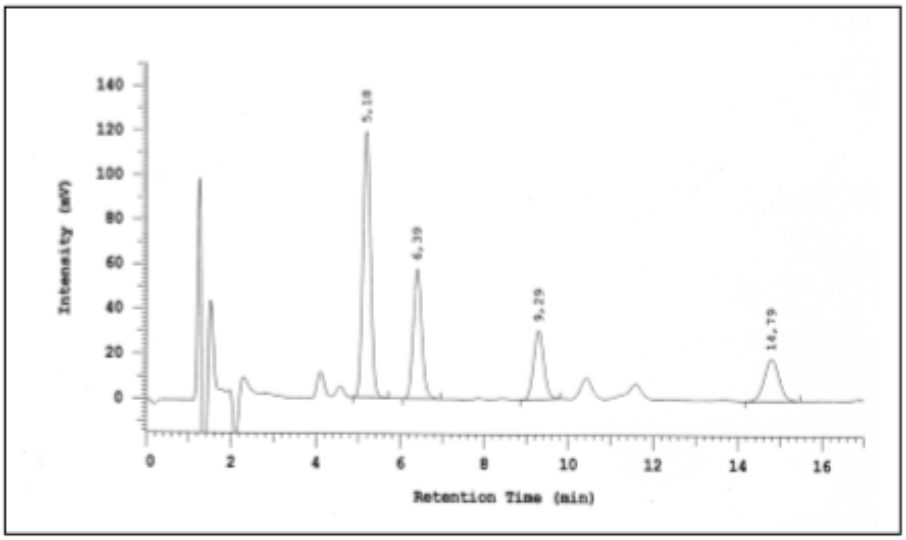


Figure 19 Chromatogram and retention time of urinary NM calibrator (NM: 5.2 min, MET: 6.4 min, internal standard: 9.3 min, 3-MET: 14.8 min)

4) Sample Size Determination

The sample size was calculated by reference the study of Qing Tong and colleagues in 2015. They measured plasma 5-HT level of 82 PD patients compared with 64 controls. The mean concentrations of plasma 5-HT in PD and control groups were 26.53±15.07 μg/l and 42.58±16.99 μg/l, respectively.

Sample size formula:

$$n/group = \frac{\left(Z_{\alpha/2} + Z_{\beta}\right)^2 2\sigma^2}{\left(d^2\right)}$$

Where: n= sample size per group

Z = Z statistic for a level of confidence

σ^2 = pool variance

d = difference ($\bar{x}_1$-$\bar{x}_2$)

At the 95% confidence; α = 1-0.95 = 0.05, $Z_{\alpha/2}$= 1.96 (2-tail)

At power 90%; β = 0.1, Z_{β} = 1.28

We first specify σ^2, $\sigma^2 = s_p^2 = \frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2-2}$

For *n* therefore, $n = \frac{2(1.96+1.28)^2 \left[\frac{(82-1)(15.07^2)+(64-1)(16.99^2)}{82+64-2}\right]}{(26.53-42.58)^2}$

$$n = \frac{2(10.49)\left[\frac{(18395.1+18185.58)}{144}\right]}{257.60} = \frac{5,329.55}{257.60} = 20.69$$

n = 20.69 (≈ 21)

To adjust the dropout rate of 20%,

$$n_{adj} = \frac{n}{1-d} = \frac{21}{1-0.2} = 27$$

Thus, the number of participants that needed to detect the clinical parameters should be at least 27 in each group. However, we determined neurotransmitter levels and find their correlations with NMSs of 40 participants per group.

5) Data Analysis and Statistics

We performed statistics analysis with SPSS statistics 22.0 (IBM corporation, New York, USA). All data were tested normal distribution by using Kolmogorov-Smirnov. The continuous variables were presented as mean ± standard deviation (SD) and mean ± standard error of mean (SEM). The demographic data, NMSs, levels of neurotransmitter and metabolite were compared between PD and control groups by using independent t-test (2-tail) or Mann-Whitney U-test for parametric and nonparametric data, respectively. The correlations between scale of NMSs and neurotransmitter levels were tested by using Spearman's rho. Statistical significance was considered when *p*-value less than 0.05.

Part II: The fluctuations of plasma monoamines during medication "ON/ OFF" periods of advanced-PD patients

1) Research Design

Cross-sectional analytic study

2) Populations

Target Population

Male or female PD patients who were diagnosed as idiopathic Parkinson's disease (IPD) in advance stage, aged below 70 years old from the Movement Disorders Outpatient Clinic at Chulalongkorn Center of Excellence on Parkinson's Disease & Related Disorders, Thailand.

3) Patients and experimental design

We performed the second part at Chulalongkorn hospital between July to December 2018. The advanced-PD patients, aged below 70 years were recruited from the Movement Disorders Outpatient Clinic at Chulalongkorn Center of Excellence on Parkinson's Disease & Related Disorders, Thailand. Like in part I, patients who had other neurological diseases such as cerebrovascular disease, traumatic brain injury, brain tumor, encephalitis, Alzheimer's disease, spinal cord disease, peripheral neuropathy and Schizophrenia were excluded. Patients signed an informed consent form. After that, advanced-PD patients were principle interviewed and physical examined by physician. Patients who had unstable medical conditions or recently eat some food or beverage, or take medicine which can interfere level of neurotransmitter were excluded. These food and drug included banana, coffee, tea, chocolate, serotonin-norepinephrine reuptake inhibitors, tricyclic antidepressants, and neuroleptic drugs. Patients were collected blood (3 ml.) from the median cubital vein at antecubital fossa at three time points for monoamines determination, including 5 minutes before taking next dose of antiparkinsonian drug (represented OFF time), 45 and 75 minutes after taking antiparkinsonian drug (represented ON time) (fig. 20). Blood samples were drawn into EDTA tube and immediately centrifuged at 3000xg for 10 minutes, and kept the plasma at below -80°C until analysis.

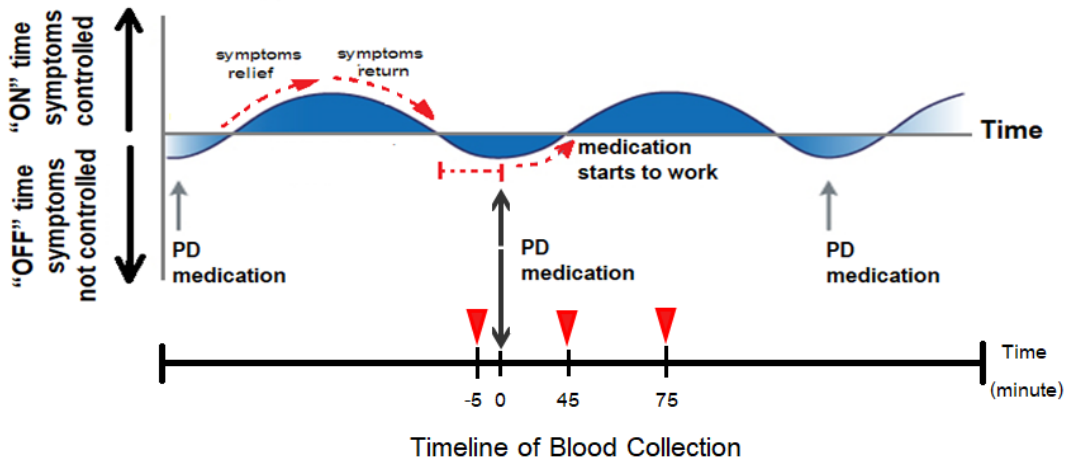


Figure 20 Timeline of blood collections in part II

4) Neurotransmitters determination

We compared the levels of plasma DA, NE, and 5-HT among 3 time points. Plasma monoamines levels were determined by using HPLC with ECD and the details of methods are similar to the one described in the first part.

5) Sample size determination

The sample size was calculated by reference the study of Tolosa and colleagues in 1975 (66). They determined serial plasma dihydroxyphenylalanine (dopa) levels (16 times in a day) of 16 PD patients who received chronic L-dopa therapy and found the relation with motor fluctuations. An average plasma dopa level was 1.47±1.65 µg/ml.

Sample size formula:

$$n/\text{group} = \frac{N\left(Z_{\alpha/2}\right)^2 \sigma^2}{\left(\left(e^2(N-1)\right)+\left(Z^2_{\alpha/2} * \sigma^2\right)\right)}$$

Where: n= sample size per group

N = number of population

Z = Z statistic for a level of confidence

σ^2 = pool variance

e = precision level

N = 16, σ = 1.65

At the 95% confidence; α = 1-0.95 = 0.05, $Z\alpha_{/2}$= 1.96 (2-tail)

At 5% of precision level; e = 0.05

For *n* therefore,

$$= \frac{16(1.96^2)(1.65^2)}{(0.05^2)(16-1)+(1.96^2)(1.65^2)}$$

$$= \frac{16(3.84)(2.72)}{(0.0025)(15)+(3.84)(2.72)}$$

$$= \frac{167.11}{10.48} = 15.94 \approx 16$$

Thus, the number of participants that needed to detect the clinical parameters should be at least 16. Because of some limitations, there were only 10 advanced-PD patients who enrolled in this study.

6) Data analysis and statistics

Data were analyzed with SPSS statistics 22.0 (IBM corporation, New York, USA). Normal distribution of data was tested by using Kolmogorov-Smirnov. The concentrations of neurotransmitter were presented as mean ± SEM and compared among 3 time points by using repeated measure analysis of variance (RM). The statistically significant when *p*-value was less than 0.05.

Ethical Consideration

The experimental protocol was approved by the institutional ethic committee of Faculty of Medicine, Chulalongkorn University (Protocol number 451/60).

Venue of the Study

- This study conducted at Chulalongkorn Center of Excellence for Parkinson's disease and Related Disorders, the 7th floor of SoThor building, King Chulalongkorn Memorial Hospital, the Thai Red Cross Society, 1873 Rama IV Road, Pathumwan, Bangkok, Thailand.

- The neurotransmitters and metabolites were analyzed at Center for Medical Diagnostic Laboratories, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, AorPorRor Building, 11st floor, 1873 Rama IV Road, Pathumwan, Bangkok, Thailand.

CHAPTER IV

RESULTS

This study consisted of two parts. The first part investigated the difference in concentrations of plasma and salivary monoamine neurotransmitters and their metabolites in urine between PD and control group and also evaluated their correlations with anxiety, depression, sleep disturbances, and sexual dysfunction in PD patients. Levels of neurotransmitters in peripheral body fluids were determined by HPLC with ECD. Standard questionnaires were used to evaluate NMSs. In part II, we aimed to investigate the fluctuations of plasma monoamine levels during medication ON/OFF periods in advanced-PD patients. Levels of DA, NE and 5-HT were determined at three time points in ON period (5 minutes before drug taking) and OFF period (45 and 120 minutes after drug taking). The results in each part were described in more detail below.

Part I: The alteration of monoamines levels and their correlations with NMSs

1. Demographic and Clinical Characteristics

Table 8 shows the demographic data and clinical characteristics of control and PD patients in Part I. Eighty participants, half of whom were PD patients, and another half were control subjects. Mean age of control subjects and PD patients was 55.5±6.3 and 57.6±8.5 years, respectively (p=0.22). The majority of participants were male in both PD (55.0%) and control (67.5%) group (p=0.25). Histories of essential hypertension, type 2 diabetes mellitus and hypercholesterolemia were documented in both control subjects and PD patients.

In PD group, average age onset and duration of disease were 44.38±7.97 and 13.18±7.11 years, respectively. The severity of PD was evaluated by clinician according to modified H&Y staging. Mean modified H&Y stage was 2.76±1.06. The number of patients in each stage were difference, which the most of them were categorized in modified H&Y stage 2.5 (32%). An average levodopa equivalent daily dose (LEDD) was 1055.3 ± 656.9 mg. Quality of life in patients with PD was assessed by PDQ-8. An

average PDQ-8 score was approximately 11.53 points, which increasing of this score indicated poor health-related quality of life.

Table 9 presents the characteristics of PD patients in early- and advanced-stages. The number of PD patients were different in the two-disease severity sub-groups; 24 (60%) patients were classified as early stage (modified HY stage 1-2.5) and 16 (40%) were in advance stage (modified HY stage 3-5). Early stage patients were younger than the advance stage ones (54.3±8.5 versus 62.5±5.7 years old, p=0.001). Proportion of male were not significantly different between the early and advance sub-groups (75.0% and 56.2%, respectively, p=0.31). Comparing to the advance stage sub-group, early stage patients had shorter disease duration (10.8±5.7 versus 16.8±7.7 years, p=0.014) and lower daily LED (886.6±472.1 versus 1,338.3±798.3 mg, p=0.045).

Table 8 Demographic and clinical characteristics of control and PD group

Characteristics	Control (n=40)	Parkinson (n=40)	*p*-value
Age [years, mean±SD]	55.50±6.33	57.55±8.48	0.22[a]
Males: females [N (%)]	22(55%):18(45%)	27(67.5%): 13 (32.5%)	0.30[b]
MMSE score [mean ± SD]	28.08±1.94	28.50±1.52	0.46[a]
Underlying disease [N (%)]			
- Hypertension	5 (12.5%)	4 (10.0%)	-
- Hypercholesterolemia	7 (17.5%)	3 (7.5%)	-
- Type 2 diabetes mellitus	3 (7.5%)	2 (5.0%)	-
Age onset of PD [years, mean±SD]	NA	44.38±7.97	-
Disease duration [years, mean±SD]	NA	13.18±7.11	-
Modified H&Y stages of PD			
- Average modified H&Y stage [mean±SD]	NA	2.76±1.06	-
- Frequency of patients [N (%)]			
Stage 1	NA	3 (7.5%)	-
Stage 1.5	NA	2 (5%)	-
Stage 2	NA	6 (15%)	-
Stage 2.5	NA	13 (32%)	-
Stage 3	NA	8 (20%)	-
Stage 4	NA	4 (10%)	-
Stage 5	NA	4 (10%)	-
LEDD [mg]	NA	1055.3±656.9	-
PDQ-8	NA	11.53±5.35	-

MMSE, Mini-Mental State Examination; LEDD, levodopa-equivalent daily dose;

[a] Independent t-test; [b] Chi-square test

Table 9 Characteristics of PD patients in early- and advanced-stages

Characteristics	Early stage (n=24)	Advanced stage (n=16)	*p*-value
Age [years, mean±SD]	54.3±8.5	62.5±5.7	0.001[a]
Males: females	18(75%):6(25%)	9(56.2%):7(43.8%)	0.31[b]
Disease duration [years, mean±SD]	10.8±5.7	16.8±7.7	0.014[a]
LEDD [mg, mean±SD]	886.6±472.1	1,338.3±798.3	0.045[a]

LEDD, levodopa-equivalent daily dose; [a] Independent t-test; [b] Chi-square test

2. Comparisons of monoamine and metabolite levels between PD and control

2.1 Plasma monoamine levels

In this part, levels of monoamine neurotransmitter and their metabolite were expressed in value of mean ± standard error of the mean (SEM). The statistically significant differences were analyzed by Mann-Whitney test (two-tailed) (p<0.05).

Figure 21 represents HPLC chromatograms of plasma monoamines levels comparing between control (dash line) and PD (solid line) group. Plasma DA was slightly higher in PD patients compared to control subjects (389.85±48.06 versus 346.45±37.37 ng/L, p=0.864). Plasma NE levels were significantly higher in PD patients than in control group (1,336.72±235.87 versus 295.48±31.14 ng/L, p<0.001). Compared to control subjects, PD patients had significantly lower plasma 5-HT levels (14.81±3.11 versus 31.20±6.15 µg/L, p=0.014) (Fig. 22A-C).

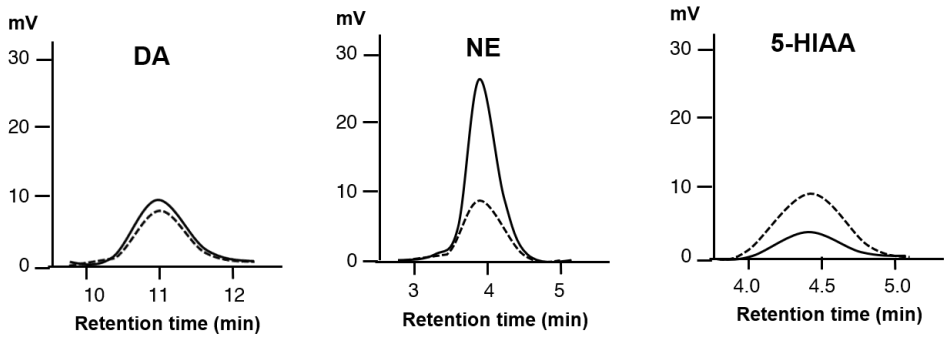


Figure 21 HPLC chromatograms show the alterations of plasma DA, NE and 5-HT comparing between control (dash lines) and PD (solid lines) group.

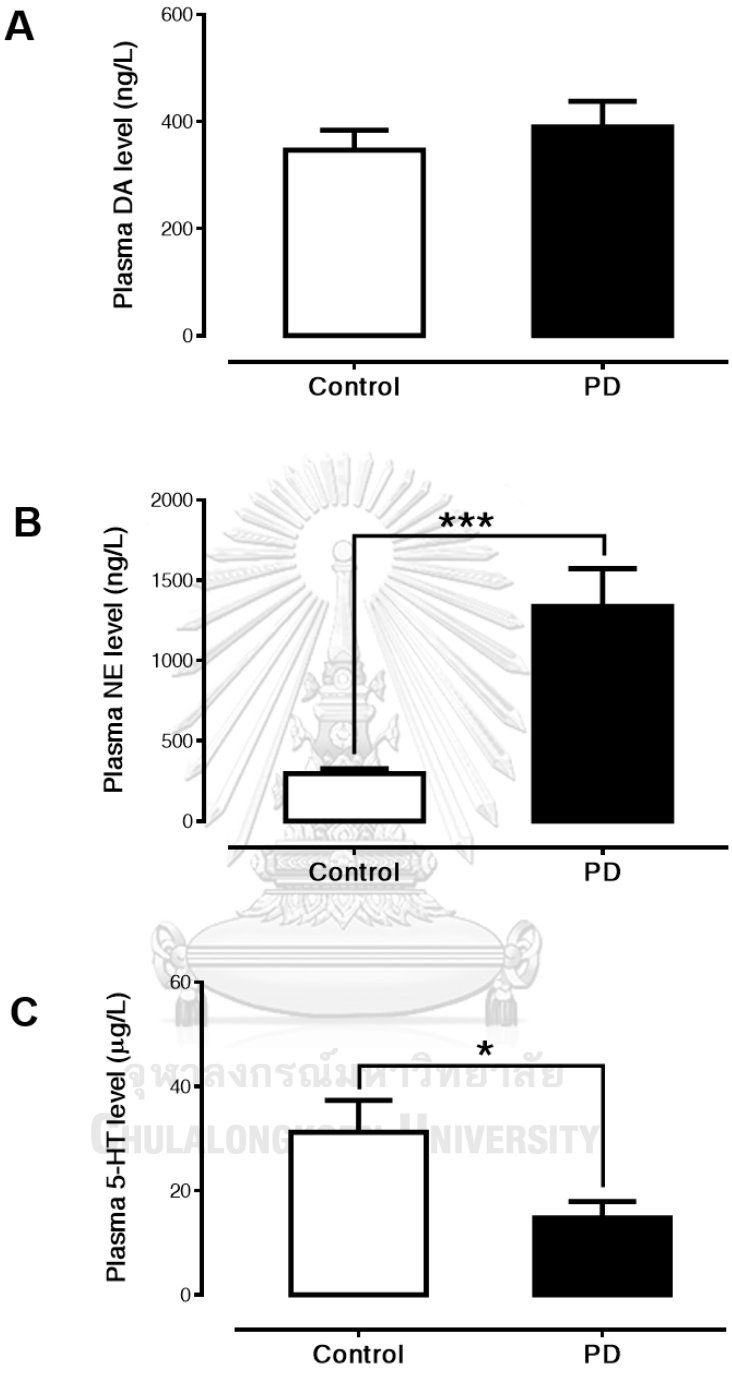


Figure 22 Comparisons of plasma DA (A), NE (B) and 5-HT (C) levels between control subjects and PD patients.

Data are presented as mean ± SEM. (*p<0.05, ***p<0.001)

2.2 Salivary monoamine levels

All participants were collected saliva but only 10 control subjects and 10 PD patients provided an adequate volume for monoamines analysis. Figure 23 represents HPLC chromatograms of salivary DA and NE comparing between control and PD group. In this study, salivary 5-HT level was also determined but its chromatographic peak did not present. Salivary DA and NE levels are displayed in fig. 24A and 24B, respectively. Salivary DA and particularly NE levels tend to increase in PD patients when compared to the control, however the analysis did not show any significant differences (649.8±347.9 vs 814.7±301.5 ng/L and 1184±691.8 vs 3165±2095 ng/L).

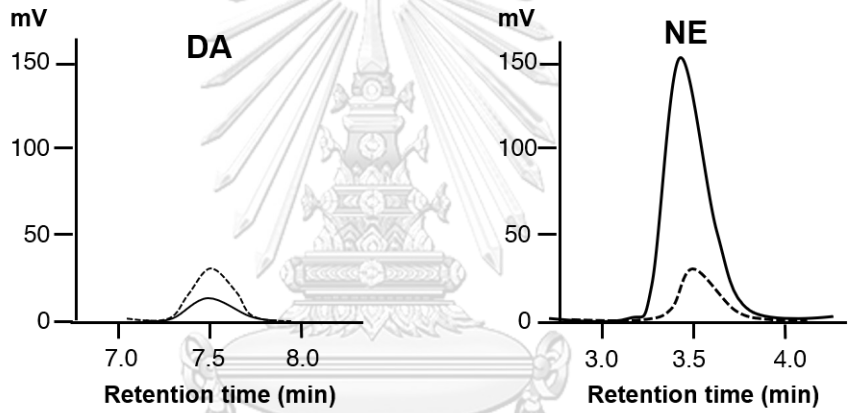


Figure 23 HPLC chromatograms show the alterations of salivary DA and NE comparing between control (dash lines) and PD (solid lines) group.

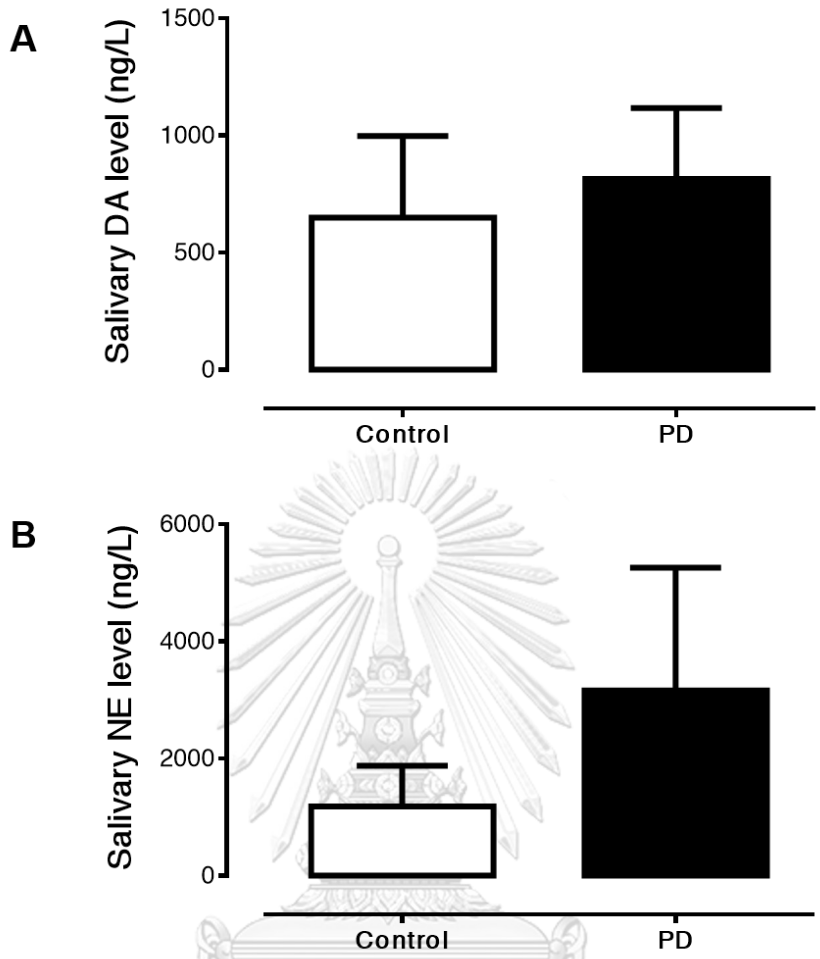


Figure 24 Comparisons of salivary DA (A) and NE (B) levels between control subjects and PD patients.

Data are presented as mean ± SEM.

2.3 Urinary metabolite levels

Figure 25 represents HPLC chromatograms of urinary 3-MET, HVA, NM, VMA and 5-HIAA levels comparing between PD and control group. Urinary 3-MET (1757.20±305.84 mg/L versus 134.70±18.39, $p<0.001$, fig. 26A) and HVA levels were significantly higher in PD patients compared to control subjects (12.94±1.78 versus 4.43±0.45 mg/L, $p<0.001$, fig. 26B).

For noradrenergic system, NM levels were significantly lower in PD patients than in control group (277.49±41.39 versus 537.35±81.62 mg/L, $p<0.001$, fig. 26C), whereas urinary VMA levels were not significantly different from controls (14.26±2.94 versus 9.36±1.10 mg/L, $p=0.917$, fig. 26D).

In serotonergic system, urinary 5-HIAA levels were significantly lower in PD patients than in the control group (1.54±0.27 versus 4.14±0.63 mg/L, $p<0.001$, fig. 26E).

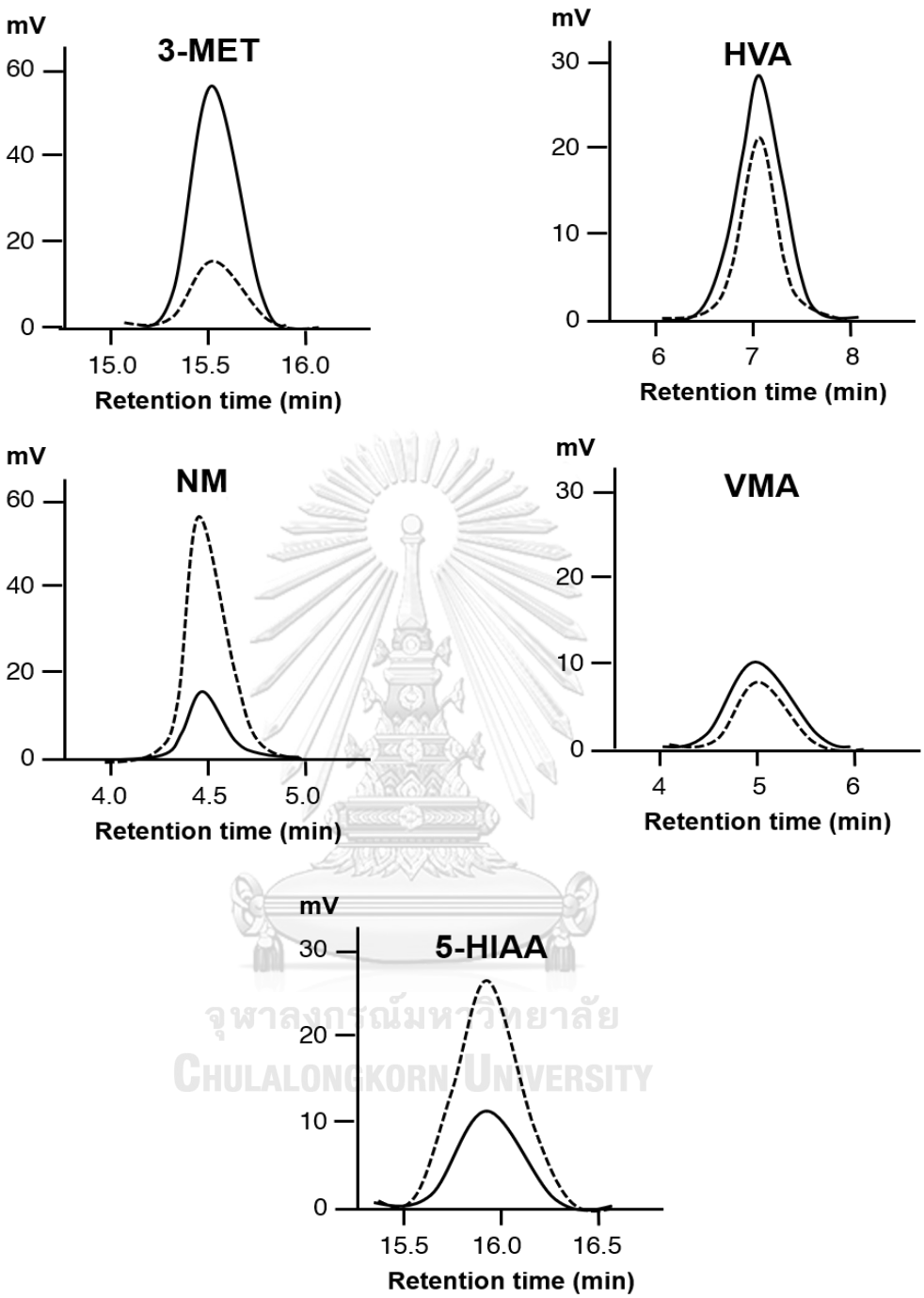


Figure 25 HPLC chromatograms show the alterations of urinary 3-MET, HVA, NM, VMA and 5-HIAA comparing between control (dash lines) and PD (solid lines)

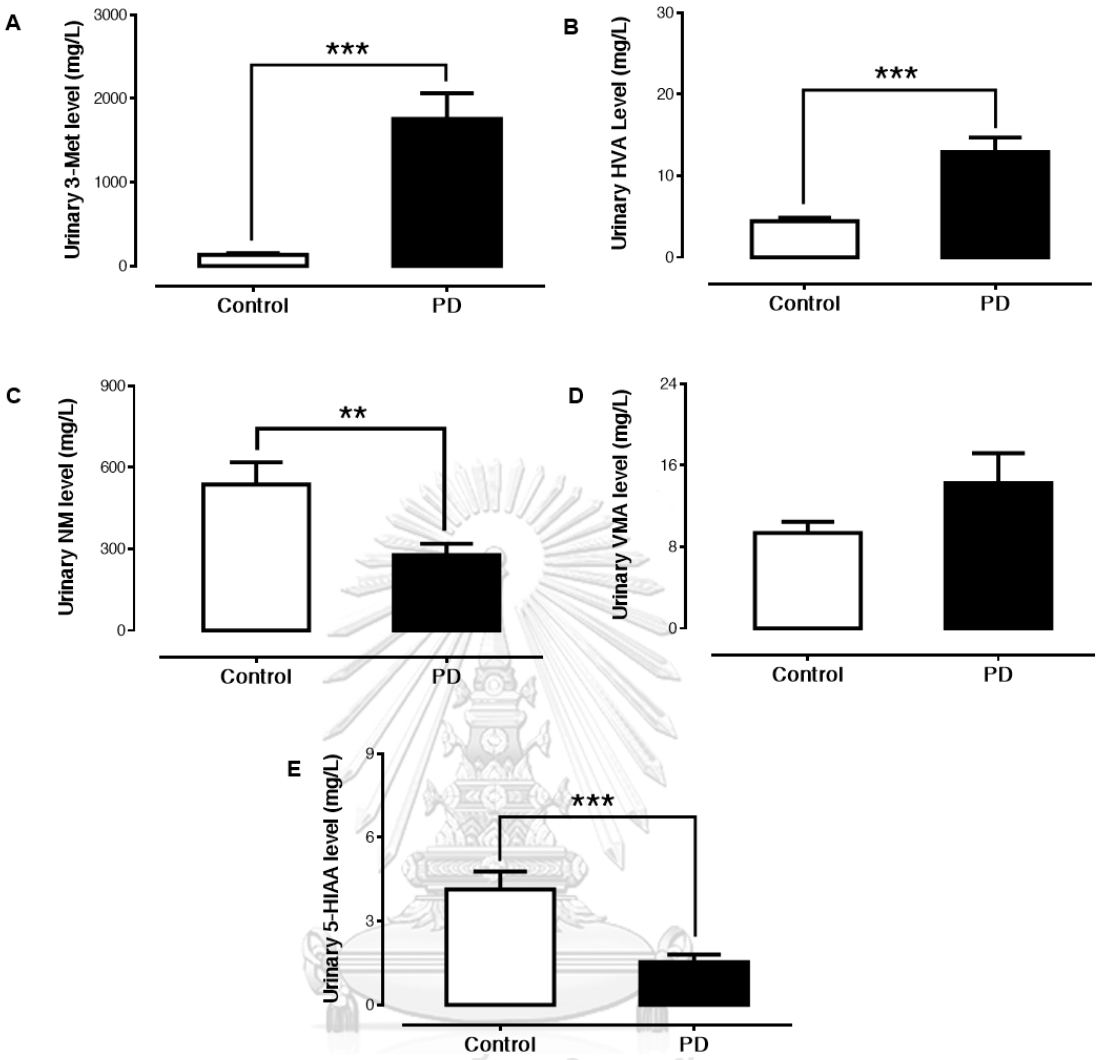


Figure 26 Comparisons of urinary 3-MET (A), HVA (B), NM (C), VMA (D), and 5-HIAA (E) levels between control subjects and PD patients.

Data are presented as mean ± SEM. (**$p < 0.01$, ***$p < 0.001$)

3. Associations between plasma monoamines and clinical profiles

Figure 27A-C present the levels of plasma DA, NE and 5-HT in early- and advanced-PD patients, respectively. Between these two sub-groups, there were no significant differences in plasma levels of DA (393.12±64.12 versus 384.94±74.52 ng/L, p=0.984) or NE (1,317.84±298.83 versus 1,365.05±395.14 ng/L, p=0.624). However, plasma levels of 5-HT in advanced-PD patients were significantly lower compared to early-PD patients (7.44±2.11 versus 19.72±4.77 µg/L, p=0.024).

Correlation analyses was also performed between plasma monoamine levels and clinical profiles, including disease duration and modified HY staging. Plasma DA levels had a significant negative correlation with disease duration (ρ=-0.32, p=0.04), but levels of other monoamines had no correlation to disease duration (fig. 28A-C). Plasma 5-HT levels were found to have a significant negative correlation with the motor severity classified by modified HY staging (ρ=-0.35, p=0.03), but levels of other monoamines had no correlation to the modified HY staging (fig. 28D-F). Concerning the possible influences of dopaminergic medications on these results, additional correlation analyses between LED and modified HY staging and disease duration were performed. LED was found to have a significant positive correlation to modified HY staging (ρ=0.40, p=0.01), without correlation to disease duration (ρ=0.04, p=0.81) (Table 10).

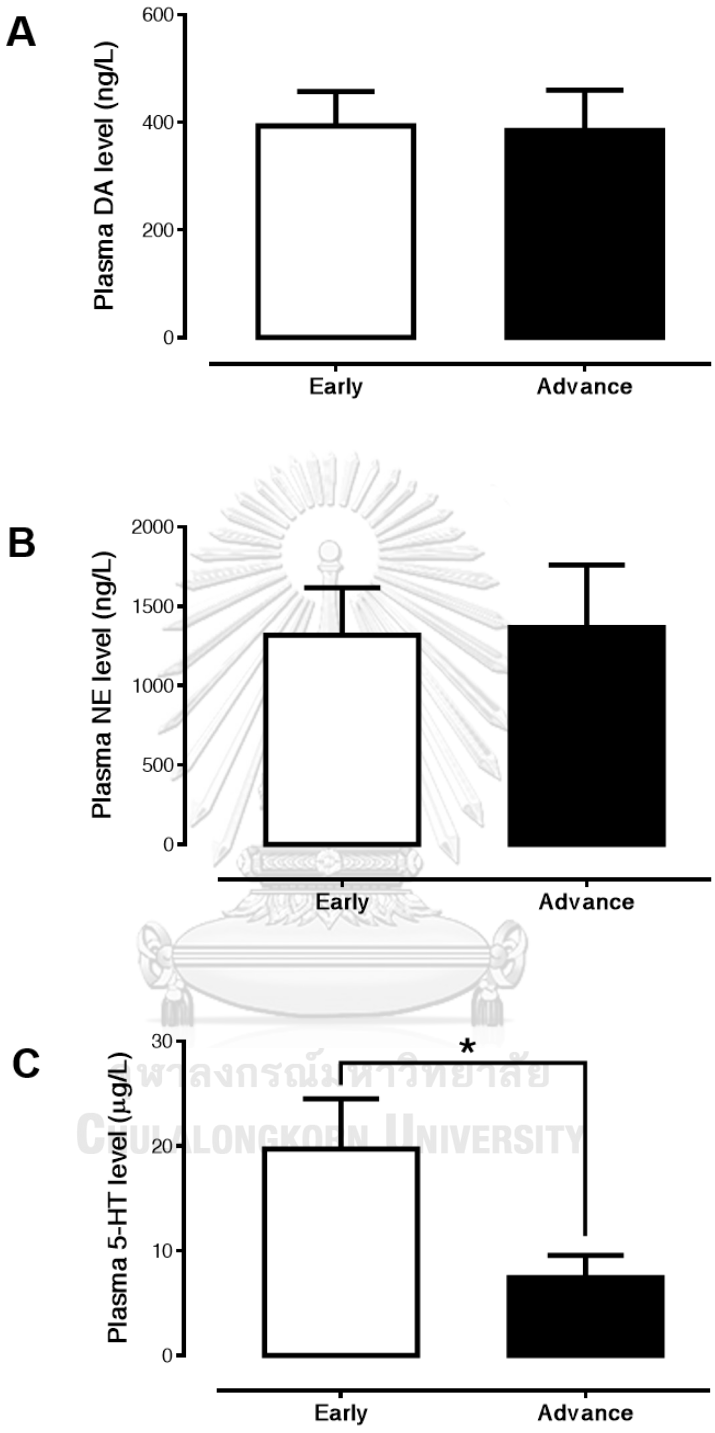


Figure 27 Levels of plasma DA (A), NE (B) and 5-HT (C) in early and advanced-PD patients.
Data are presented as mean ± SEM. (*p<0.05)

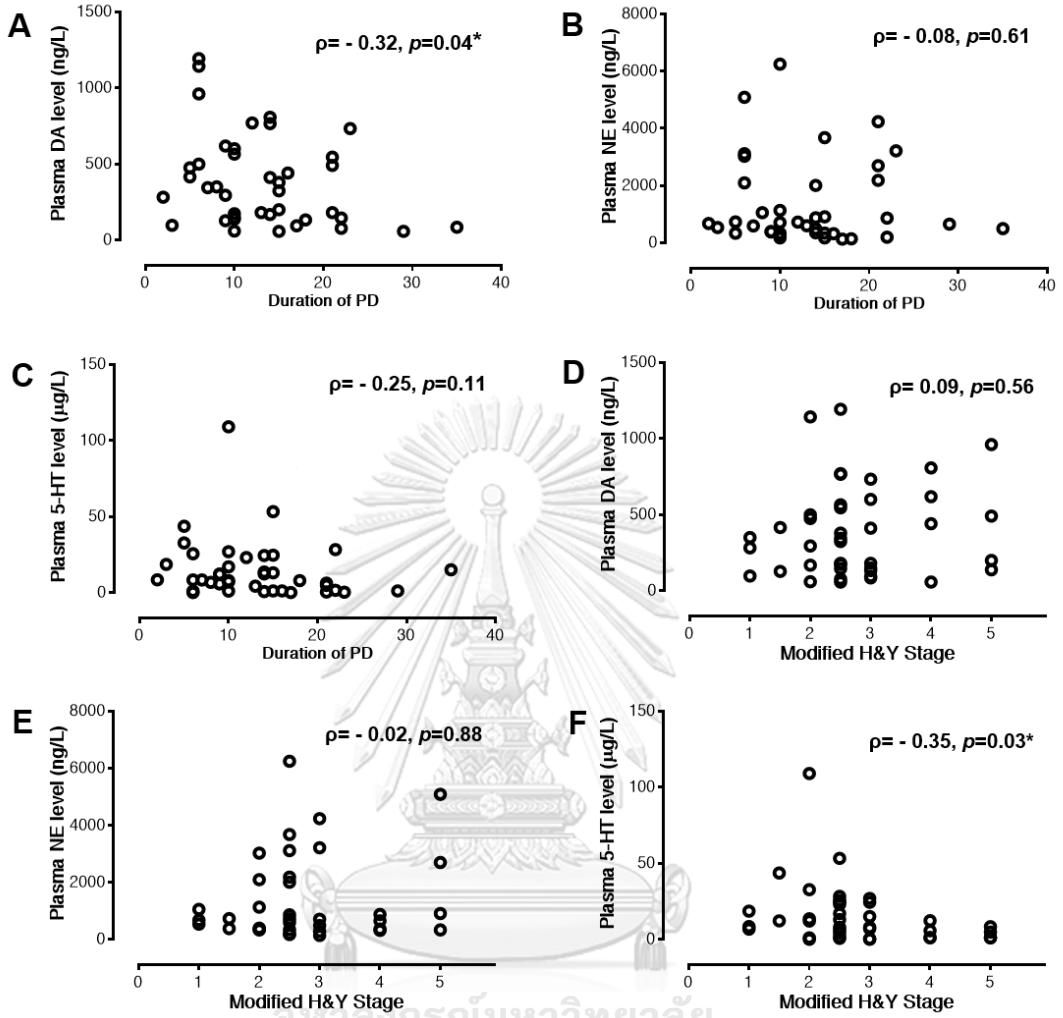


Figure 28 Scatterplots showing correlations of DA, NE and 5-HT levels to disease duration (A-C) and to modified HY stage (D-F) in PD patients

Table 10 Correlations between LED and disease duration and severity

Parameter	Disease duration		Modified H&Y staging	
	Spearman's rho	*p*-value	Spearman's rho	*p*-value
LEDD [mg]	0.04	0.81	0.40	0.01**

4. Correlation between plasma monoamine levels and NMSs in PD patients

1) Prevalence of Non-motor symptoms in PD

NMSs of PD patients were screened by using the non-motor symptoms questionnaire (NMS-quest) (Table 11). The Majority of NMSs of PD patients were the problems in sleep disturbances, sexual dysfunctions, urinary and gastrointestinal dimensions. Sleep disturbances, which commonly occur in patients including difficulties falling asleep (60%), acting out a dream (60%), vivid dream (42.50%), excessive daytime sleepiness (37.5%), and restless leg syndrome (15%). Prevalence of sexual dysfunction and loss of libido were 60% and 70%, respectively. Common problems in dimension of urinary and gastrointestinal were urinary urgency (62.5%), night time urinary incontinence (82.5%), and constipation (75%). In addition, the important non-motor problems were sad (42.5%) and anxiety (27.5%). The other common NMSs, which exhibited in patients including drooling (48%), impaired sensations (42.5%), swallowing difficulties (60%), nausea (20%), fecal incontinence (10%), incomplete bowel emptying (45%), abnormal pain (20%), remembering problem (50%), loss of interesting (37.5%), poor concentration (42.5%), abnormal weight change (25%), orthostatic hypotension (37.5%), excessive sweating (47.5%), leg edema (12.5%), double vision (15%), hallucination (15%), and delusion (15%) (fig. 29).

Table 11 Prevalence of non-motor symptoms in PD patients screening by the non-motor symptoms questionnaire (NMS-quest)

Dimensions	Items for screening NMSs	Frequency N [%]
Gastrointestinal tract	Drooling during the daytime	19 [48%]
	Difficulty swallowing food or problems with choking	24 [60%]
	Nausea	8 [20%]
	Constipation	30 [75%]
	Fecal incontinence	4 [10%]
	Incomplete bowel emptying	18 [45%]
Urinary tract	Urinary urgency	25 [62.50%]
	Night-time urinary incontinence	33 [82.50%]
Pain	Unexplained pain	8 [20%]
Attention and memory	Problems remembering things	20 [50%]
	Loss of interesting	15 [37.50%]
	Poor concentration	17 [42.50%]
Sad/ blue/ anxiety	Feeling sad	17 [42.50%]
	Feeling anxious, frightened or panicky	11 [27.50%]
Sexual function	Loss of libido	28 [70%]
	Sexual dysfunctions	24 [60%]
Cardiovascular	Orthostatic hypotension	15 [37.50%]
	Falling	23 [57.50%]
Sleep and fatigue	Daytime sleepiness	15 [37.50%]
	Difficulties sleep and frequent awakenings	24 [60%]
	Intense, vivid or frightening dreams	17 [42.50%]
	Acting out a dream	26 [60%]
	Restless legs syndrome	6 [15%]
Miscellaneous	Impaired ability to taste or smell	17 [42.5%]
	Abnormal weight change	10 [25%]
	Legs edema	5 [12.50%]
	Excessive sweating	19 [47.50%]
	Double vision	6 [15%]
Hallucination and delusion	Seeing or hearing things that you know are not there	6 [15%]
	Delusion	6 [15%]

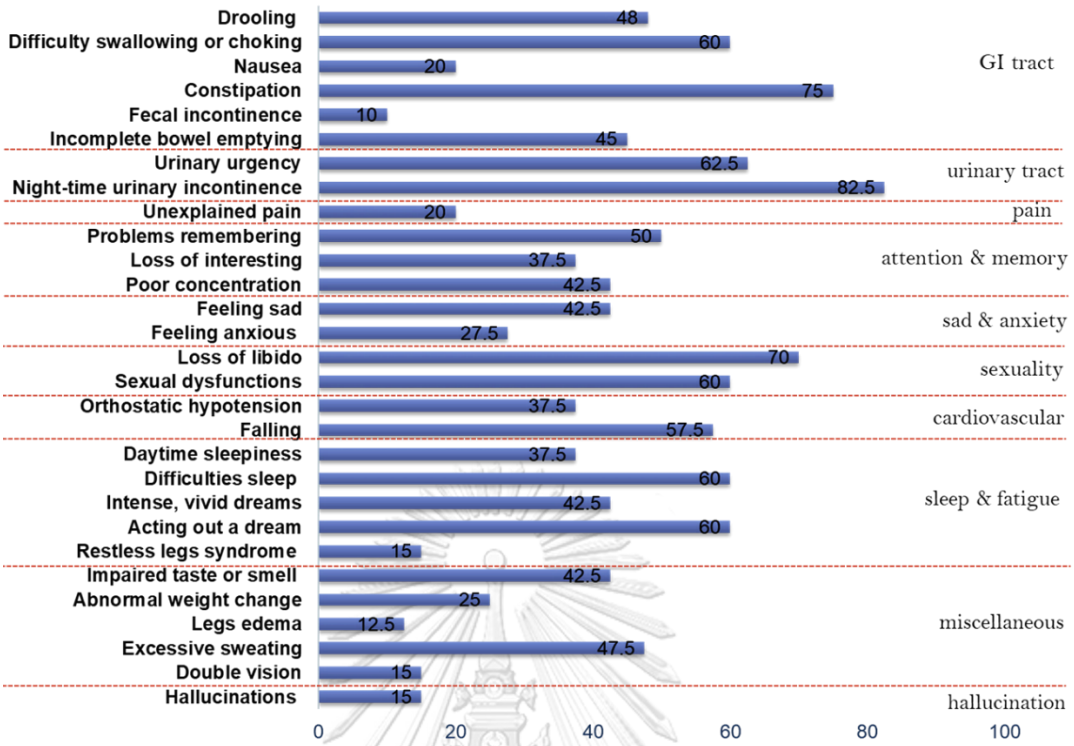


Figure 29 Prevalence of NMSs in PD patients

In this study, we focused on psychiatric problems (anxiety and depression), sleep disturbances, and sexual dysfunctions in PD patients. So, these NMSs were assessed again by using the specific standard questionnaires (i.e. HADS, MDPSS, and ASEX, respectively) for an accuracy. Figure 30 presents the scores of HADS in anxiety and depression items. Mean scores in anxiety (6.33±3.48 versus 4.38±2.10, p=0.003) and depression (6.60±3.57 versus 2.95±2.25, p=0.001) items of PD group were significantly higher than in control group. It seemed that PD patients suffer from anxiety and depression more than age-match control subjects. Figure 31 shows the score of MPDSS, which is used for evaluate sleep problems. Mean scores of MPDSS were significantly lower in PD than in control group (136.30±28.92 versus 156.70±21.72, p=0.001). The decreasing of MPDSS score indicated more frequency of sleep problems in PD patients. The ASEX scores of PD and control present in figure 32. Mean ASEX score of PD group was significantly higher than control group (20.35±6.12 versus 16.93±4.16, p=0.02). This suggested that PD patients have more sexual impairments.

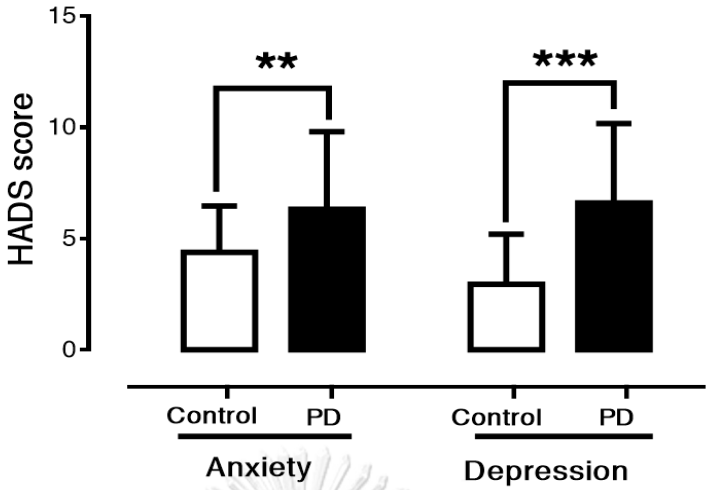


Figure 30 Comparison of the HADS scores between control and PD group

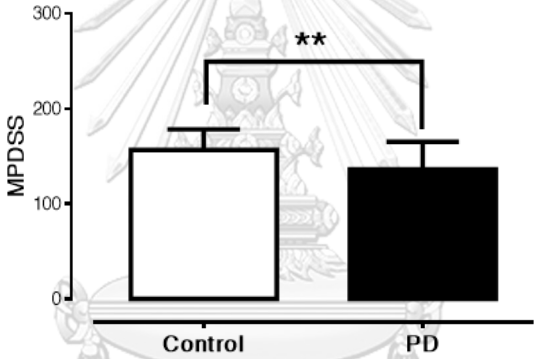


Figure 31 Comparison of the MPDSS scores between control and PD group

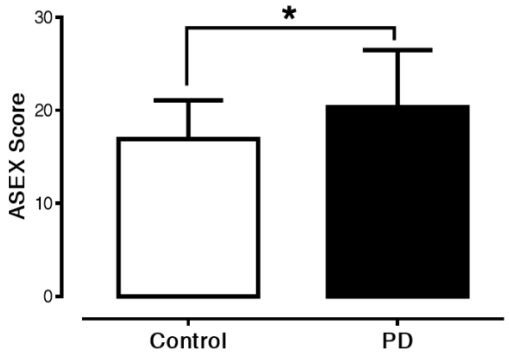


Figure 32 Comparison of the ASEX scores between control and PD group

2) Correlation between NMSs and clinical profiles of PD patients

In this part, we evaluated the relationship between NMSs (anxiety, depression, sleep disturbances, and sexual dysfunctions) and clinical profiles (disease duration and severity).

PD duration had positive correlation with depression (p=0.001) and ASEX scores (p=0.007). The correlation coefficient value between disease duration and anxiety, depression, MPDSS, and ASEX scores were 0.15, 0.49, -0.20, and 0.42, respectively (fig. 33A-D).

Similar to disease duration, PD patients had positive correlation between modified H&Y stage with depression (p=0.003) and ASEX scores (p=0.004). The correlation coefficient value between modified H&Y staging and anxiety, depression, MPDSS, and ASEX scores were 0.19, 0.45, -0.30, and 0.45, respectively (fig. 33E-H).

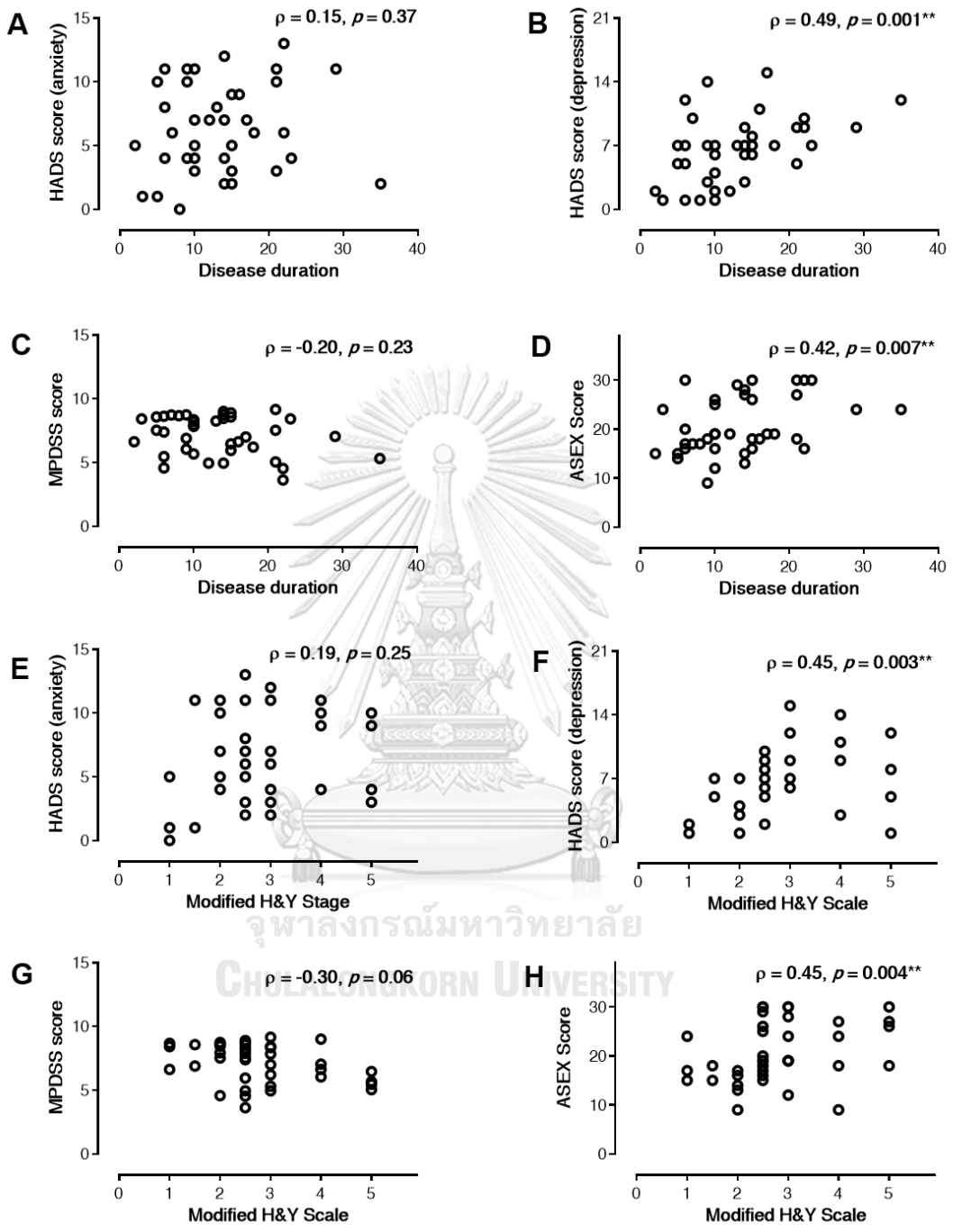


Figure 33 Scatterplots showing correlations of NMS scores (anxiety, depression, MPDSS and ASEX scores) and disease duration (A-D) and modified H&Y stage (E-H) in PD patients.

3) Correlation between plasma monoamine levels and NMSs

The associations between plasma monoamine levels and score of HADS, MPDSS, and ASEX were analyzed. All of these values were non-parametric data, so the relationships were tested by using Spearman's rho correlation.

Figure 34A-F present the correlations between HADS scores in anxiety and depression items with levels of plasma DA, NE and 5-HT, respectively. The correlation coefficient values (ρ) of anxiety were 0.10, -0.09 and -0.15, and depression were -0.17, -0.19 and -0.19, respectively. However, the results did not show any relationship between plasma DA levels and both anxiety and depression in PD patients.

Figure 34G-I show the relationship between MPDSS score and levels of plasma DA, NE and 5-HT, respectively. Similar to HADS scores, plasma monoamine levels did not show any correlation with MPDSS score. The Spearman's rho coefficient values of these were 0.07, 0.05 and -0.04, respectively.

Figure 34J-L present the correlations between ASEX score and levels of plasma DA, NE and 5-HT, respectively. The correlation coefficient values were 0.01, 0.16 and -0.08, respectively. However, there were no significant correlation between these parameters.

It assumes that monoamine levels in plasma may not associate to genesis of psychiatric problems, sleep disturbances and sexual dysfunctions.

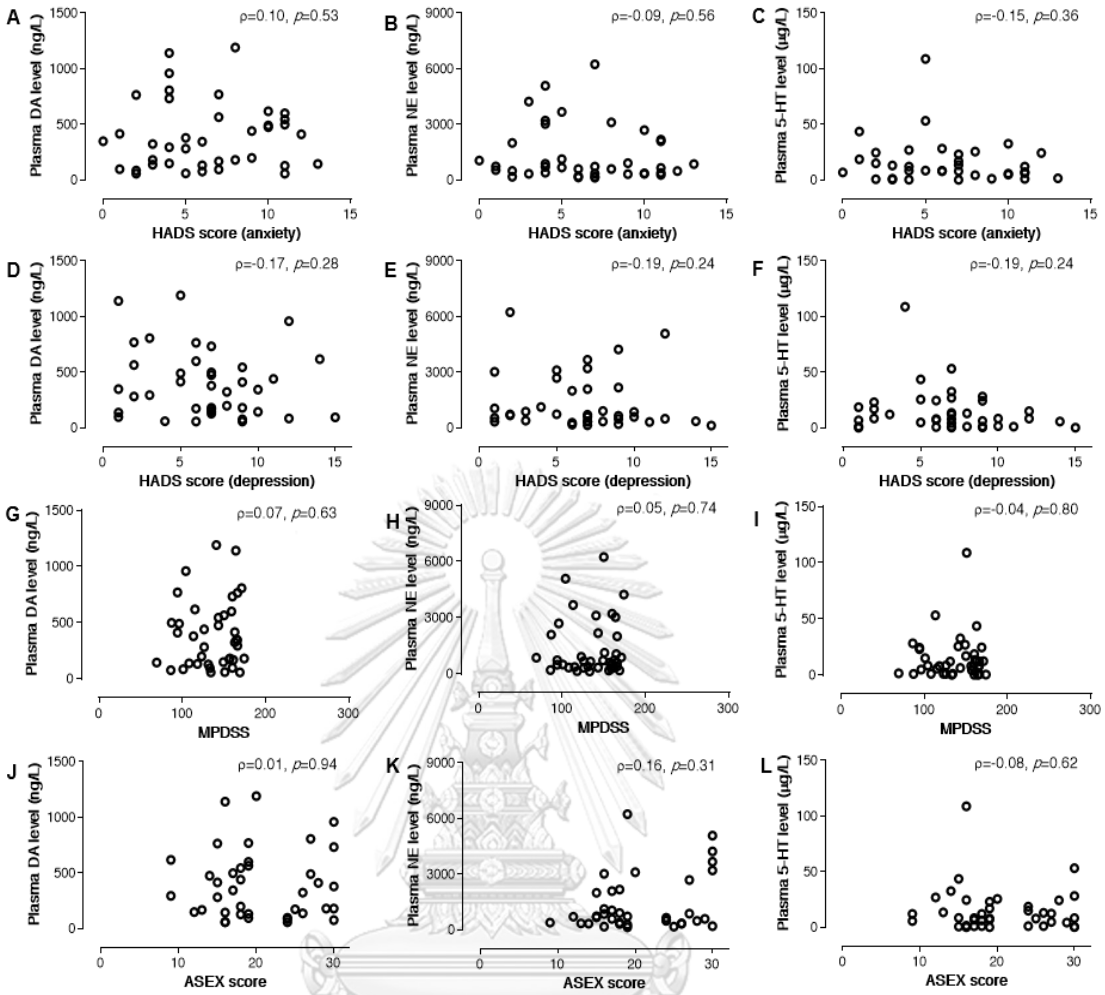


Figure 34 Scatterplots showing correlations of DA, NE and 5-HT levels to anxiety (A-C), depression (D-F), MPDSS (G-I) and ASEX (J-L) scores in PD patients

Part II: The fluctuations of plasma dopamine, norepinephrine, and serotonin during medication ON/OFF periods of advanced-Parkinson's disease patients

1. Demographic data and clinical profiles

In part II, all participants were advanced-PD patients. An average age, disease duration and modified H&Y stage were 56.40±10.79 years, 13.60±6.97 years and 3.25±1.27, respectively (Table 12).

Table 12 Demographic data and clinical profiles of advanced-PD patients

Characteristics	Advanced-PD patients (n=10)
Age [years, mean±SD]	56.40±10.79
Males: females [N]	6: 4
Disease duration [years, mean±SD]	13.60±6.97
Modified H&Y stages [mean±SD]	3.25±1.27

2. Fluctuations of plasma monoamines during ON and OFF periods

Figure 35A-C display mean values of plasma DA, NE and 5-HT at 3 time points. The white, grey, and black bars represent levels at 5 minutes before drug taking (ON period), 45 minutes and 120 minutes after drug taking (OFF) period), respectively. The mean plasma DA level in OFF period (5 minutes before drug taking) was 659.1±103.4 ng/L. After medication taking for 45 minutes, plasma DA increased to 1415±624 ng/L. Conversely, plasma DA decreased to 1093±208.8 ng/L after drug taking for 120 minutes (fig. 35A). The alteration pattern of plasma NE levels were slightly different from plasma DA (fig. 35B). In "OFF" period, an average plasma NE level was 2024±551.9 ng/L. It increased into 2611±608.6 ng/L after drug taking for 45 minutes. Contrast to DA, plasma NE still continuously increased into 2927±854.7 ng/L after drug taking for 120 minutes. The fluctuation pattern of plasma 5-HT looks similar to plasma DA. Level of plasma 5-HT in OFF period was 10.24±3.92 µg/L and slightly increased to 11.76±4.79 µg/L after drug taking for 45 minutes (fig. 35C). Then, it decreased into 7.91±3.31 µg/L after drug taking for 120 minutes, which this level was lower than in OFF period. However, there were no significant difference among 3 time points of these plasma monoamine levels.

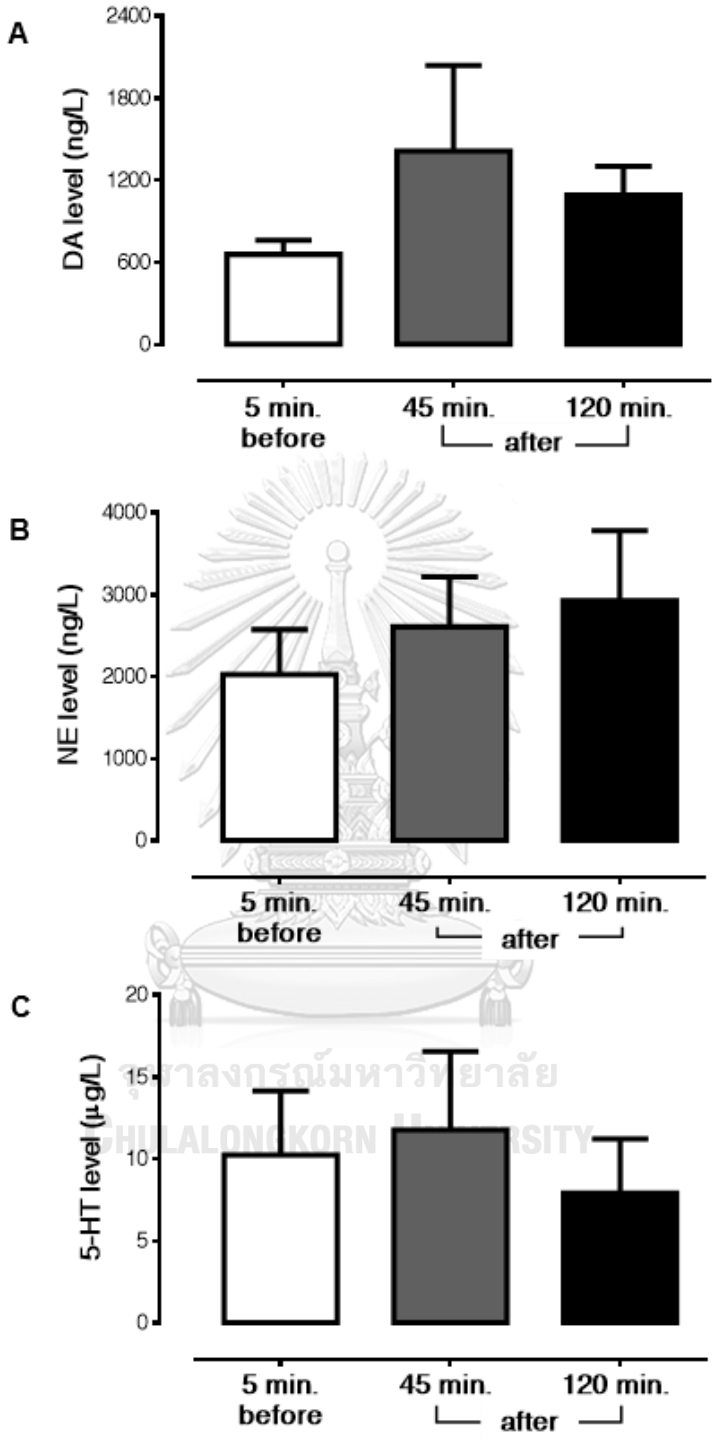


Figure 35 Levels of plasma DA (A), NE (B), and 5-HT (C) at "ON" (5 minutes before drug taking) and "OFF" period (45 and 120 minutes after drug taking)

CHAPTER V
DISCUSSION

Part I: The alteration of monoamines levels and their correlations with NMSs

The alterations of monoamines and their metabolites in plasma and urine

- **Dopaminergic system**

Even though the degeneration of dopaminergic neurons is the crucial pathophysiology of PD, this study has revealed that levels of other monoamine transmitters are altered in peripheral body fluids, by quantifying monoamine levels in plasma, saliva and their metabolites in urine of PD patients. For the dopaminergic system, our analyses show that PD patients have slightly higher plasma DA levels, urinary 3-MET and HVA levels than control. These findings are consistent with former studies which reported several folds increasing of urinary excretion of DA, 3-MET and HVA levels in patients with PD (67). Similarly, Andersen and colleagues (68) found that DA and HVA in CSF were greatly increased in PD patients treated with levodopa, while significantly decreased in untreated PD patients. Lunardi and Stefani (69) reported that HVA level as well as HVA/DA ratio were higher in the advanced stage versus the early stage of PD. When nigrostriatal degeneration occurs in PD, the surviving dopaminergic neurons compensate the loss by increasing DA synthesis, storage, release, and turnover through upregulation of aromatic amino acid decarboxylase (AADC) and vesicular monoamine transporter type 2 (VMAT2), (70). This response may explain why DA and HVA were higher in PD patients than in normal control subjects. In addition, the activity of monoamine oxidase (MAO) in PD patients is also increased (70). The rise in HVA thus appears to be greater than the rise in DA. L-DOPA administration may be an additional factor causing abnormal increases in DA and its metabolites with several researches showing that L-DOPA treatment may induce multi-neurotransmitters dysregulation. L-DOPA can be taken up by non-dopaminergic neurons, particularly serotonergic, noradrenergic neurons and astrocytes, which could lead to the production of DA as these neurons possess plentiful AADC and VMAT2 which are essential for DA synthesis

and storage (71-73). Furthermore, long term use of L-DOPA also stimulates angiogenesis and changes permeability of the blood brain barrier to increase its diffusion into the brain (74). All the above mentioned factors may be implicated in the raising and dysregulation of DA levels in the synaptic cleft, extracellular fluid and peripheral circulation (75). However, the acute effect of L-DOPA therapy should be minimal in our study as the patients discontinued their medications for 12 hours prior to specimen collection, which is much longer than the half-life of L-DOPA.

- **Noradrenergic system**

Information regarding the alteration of NE remains controversial. This study demonstrated significant increases in plasma NE with decrease in urinary NM in PD patients. in line with a study of Chia and coworkers (76) that showed plasma NE level higher in PD patients than age-matched control subjects. On the other hand, a study by Eldrup (77) revealed no differences in plasma DA, NE and EPI between PD patients and control subjects. However, in an electrophysiology study, it was reported that the firing rate of noradrenergic neurons in locus coeruleus was increased in PD rats compared to normal ones (78). Normally, DA is converted into NE by the catalyzed activities of dopamine beta-hydroxylase (DBH). The study by Kopp (79) found that PD patients had enhanced activity of DBH in the brainstem. This may be the reason why PD patients in our study had increased NE levels.

- **Serotonergic system**

For the serotonergic system, we found that PD patients had remarkably decreased plasma 5-HT and urinary 5-HIAA. These results agree with several previous studies which reported that 5-HT and 5-HIAA levels were significant lower in PD patients compared to healthy controls, in both CSF and peripheral blood circulation. (80, 81). The decrease of 5-HT may result from Lewy bodies deposition and subsequent destruction of raphe nuclei (82). Moreover, many studies suggest that L-DOPA could inhibit 5-HT production. As mentioned earlier, serotonergic neurons are susceptible to uptake of L-DOPA, and this agent might act as a competitive inhibitor of L-tryptophan, therefore diminishing 5-HT synthesis, transport, and metabolism (83).

The associations between plasma monoamine levels and clinical profiles

When analyzing the association between monoamine levels and clinical profiles of PD, we found that lower plasma 5-HT levels were associated with higher modified HY staging. This association is similar to that reported by Politis et al. who studied the staging of serotonin dysfunction with positron emission tomography (PET) study (84). They revealed a greater loss of serotonergic terminals at raphe nuclei and striatum in advanced PD compared to early PD patients. Another two PET studies also reported that decreasing serotonin transporter binding markers and 5-HT_{1A} receptors in raphe nuclei, caudate, and putamen correlated with an increasing tremor severity in PD patients (85, 86). Moreover, Coppen et al. (87) showed L-DOPA co-treatment with tryptophan is more effective in improving motor symptom. Thus, 5-HT depletion may contribute to the severity of motor impairment in PD. As there was a positive correlation between the modified HY staging and LED, it should be noted that the lower 5-HT level in the patients with more advance modified HY stage might arise from the influence of L-DOPA as discussed above.

We also found a significant negative correlation between plasma DA levels and disease duration. This result is consistent with Lunardi et al. who showed CSF-DA levels decreasing in parallel with increasing PD duration (69). Therefore, it can be assumed that progressive degeneration of dopaminergic neurons leads to a decrement in plasma DA levels. As additional analysis did not show any correlation between disease duration and LED, the effect of dopaminergic medications should not have an interference to this finding at all.

The alterations of monoamines in saliva

Currently, it has been identified salivary alpha-synuclein and DJ-1 proteins as disease diagnostic biomarkers of PD (88). However, there has not been research focus on change in salivary monoamines in patients with PD. In this study, we tried to quantify monoamines in saliva to further develop as biomarker in laboratory setting because saliva collection is noninvasive method, which is easy and safe to obtain. Additionally, the alterations of salivary monoamines may explain the mechanism of some oro-pharyngeal symptoms such as dysphagia and drooling. Saliva is produced from salivary glands and consist of water, electrolytes, mucus, enzymes, and many types of hormone such as steroids, peptides, and monoamines. The exact source of salivary monoamines is obscure, it may be derived from blood stream or the direct salivary sympathetic nerves. Salivary glands are regulated by parasympathetic released acetylcholine to increase flow of saliva as well as sympathetic released NE to induce protein secretion with saliva flow reduction (89). Study of Fedorova revealed PD patients decrease saliva flow rate with increased level of salivary acetylcholinesterase (AChE). These AChE, hydrolytic enzyme is synthesized in the cholinergic neurons and considered as a biomarker of parasympathetic dysfunction. They suggested that the increasing of AChE activity reflects to salivary parasympathetic denervation in PD patients (90). Our study found that salivary NE tends to increase in PD patients. NE is catecholamine which is main neurotransmitter releasing from sympathetic nerves. This finding may clarify the mechanism of xerostomia or dry mouth in PD. Although PD patients usually had drooling, many researches revealed saliva flow lower in PD than normal control (91, 92). They suggest that drooling in PD do not involve excessive saliva, but results from facial muscle rigidity, swallowing reduction, and flexed head posture. In this study, 5-HT did not detect in saliva. It has been reported that salivary 5-HT might be derived from blood circulation because there is no evidence of serotonergic nerve innervated at salivary glands (93). In peripheral blood circulating has very low levels of free 5-HT and it is rapidly degraded (half-life as a few minutes) (94), hence 5-HT derived from blood may difficult to detect in saliva.

Correlations between plasma monoamine levels and NMSs in PD patients

Our findings showed PD patients exhibit higher degree of anxiety, depression, sleep problems and sexual dysfunctions than in age-matched control.

The scores of HADS in anxiety and depression item of PD group were significantly higher than control subjects. In addition, HADS score in depression item had positive correlation with disease duration and severity. According to a study of Rodriguez-Blazquez, HADS scores in both anxiety and depression item increased with disease duration and H&Y staging (95). However, there was no relationship between HADS scores and plasma monoamine levels in our study. Consistent with Olivola report, lower 5-HT in CSF did not correlate to depression in PD patients (81). Even though our result differs from study of Tong, they found that decreasing of plasma 5-HT and 5-HIAA correlate to more severe depression assessed by Hamilton Depression Scale (80). Lian et al. revealed PD patients with depression had lower DA and 5-HT in CSF than control but depression score correlate only with decreasing of DA level (96). Nevertheless, Peacock and colleagues found plasma DA, NE, and 5-HT levels increase in depression patients and speculated that prolong stress may activate activity of AAAD enzyme leading to more monoamines synthesis (97). Moreover, there are controversial in PET scan study about the association of depression and transporter of monoamines especially change in number of DAT binding in striatum of PD patients. Remy et al. reported decreasing of both DA and NE transporters in locus coeruleus, limbic, cortex and thalamus of PD patients with depression (41). However, Richard reported that depressed patients were significant higher in level of plasma NE when compared to control and suggested as noradrenergic system dysregulation in depressive disorders (98). In contrast to our report, study of Park revealed that striatal dopaminergic depletion is not associate to depression assessed by Beck depression inventory and Beck anxiety inventory (99).

In this study, MPDSS scores of PD group were lower than control group. It seems that PD patients had more frequency of sleep problems, particularly difficult to sleep, frequent awake, RBD and daytime sleepiness. However, MPDSS score did not relate to disease duration and motor severity. This is in good agreement with study of Suzuki

and coworkers (100). They suggest that sleep problems occur at initial stage of PD and involve pons and medulla impairments which precede substantia nigra dysfunction and motor symptoms. So, cause of sleep problems may result from multi-factors including degeneration of central sleep regulation center, psychiatric symptoms (anxiety and depression), autonomic dysfunctions (abnormal temperature control and sweating) and medication effect. At present, there has been little study about relationship between sleep disturbances and monoamines level in body fluids. In this study, we found that there is no association between MPDSS score and altered plasma monoamine levels. Agreement with Olivola study, they found decreasing of 5-HT in CSF do not associate to sleep problems in PD (81). Study of Park also showed disassociation of striatal dopaminergic depletion and PDSS score (99). In contrast with PET scan study of Wilson that revealed sleep disturbances correlate with reduced serotonergic functions in the midbrain raphe, basal ganglia and hypothalamus (101). RBD is a common sleep problem in PD. There were studies revealed NE play a key role in arousal and wakefulness induction. NE also regulate sleep cycle by prevented REM generation, so it is identified as "REM-OFF" neurotransmitter (102). Study of Rasch found plasma NE significantly decreased during REM sleep cycle (103). Our work found PD had high level of plasma NE, which could inhibit REM cycle and may cause of RBD symptoms. Moreover, study of Chung demonstrated that RBD in PD patients relate to striatal DAT depletion (104).

In addition to psychiatric and sleep problems, PD patients also suffer from sexual dysfunctions. The finding showed ASEX scores of PD group were higher than control group that indicate PD patients had more level of sexual impairment. Moreover, Sexual dysfunctions progress with disease duration and motor severity. This confirm by prior studies that demonstrated PD patients had higher total ASEX score than in control subjects; furthermore, ASEX score had positive correlation with H&Y staging (105, 106). It is the well-known that 5-HT, DA and NE are important neurotransmitters regulated sexual behavior and desire by controlling the

hypothalamic-hypophyseal-gonad system (51). Prevalence of sexual dysfunction is higher in male than women and the major problems are erectile dysfunction and loss of libido that are regulated by hypothalamus. It has been reported that there is dopamine deficiency in hypothalamus, which may be the main cause of sexual impairments in PD (107). Study of Becker revealed that increasing of NE levels in plasma and cavernous in erectile dysfunction patients may indicate to a sympathetic dysregulation leading to erectile function impairment (108). However, many works point out that sexual dysfunctions in PD result from a broad of factors including depression, anxiety, motor impairment and side effect of medication (109). This is a reason why ASEX score do not associate to change in levels of monoamines.

Part II: The fluctuations of plasma dopamine, norepinephrine, and serotonin during medication ON/OFF periods of advanced-Parkinson's disease patients

In this section, we investigated the fluctuations of plasma monoamine levels in advanced PD patients. Our findings did not show statistically significant differences of all plasma monoamine levels during ON and OFF periods. However, plasma DA showed prominent change along with drug administration period. The spectrum of previous reports demonstrated oscillating features of CSF and plasma L-DOPA after intermittent oral levodopa drug administration. This oscillating results from short half-life of L-DOPA (approximately 1.5 hours) and low ability of dopaminergic neurons to store DA, which contribute pulsatile stimulated to dopaminergic receptors and subsequent to develop motor fluctuations and complications (110-112). Additionally, L-DOPA-derived DA releasing from serotonergic terminals and other neurons possibly induce DA fluctuation as a result of lacking endogenous feedback inhibition in these non-dopaminergic neurons (71, 72). Aspect of NE, it seemingly upregulated along with DA level after L-DOPA taking for 45 minutes, which is possibly because of DA being their precursors. However, NE downregulated slower than DA that may due to delay in their synthetic and metabolic processes. For plasma 5-HT levels, they slightly increased after parkinsonian drug taking for 45 minutes. Interestingly, level of 5-HT at 120 minutes after drug taking tends to decrease lower than in OFF period. This evidence might result from competitive effect of L-DOPA inhibited 5-HT synthesis (83, 113) as described in part I.

In addition, fluctuations of plasma monoamine levels in PD patients probably result from age, menstrual cycle in female and their circadian variations. Both DA and NE release peaked during daytime and down during nighttime. DA also known as neuromodulator, its alteration could disrupt releasing of other neurotransmissions (114).

This study is limited by the small number of participants. In addition, a concurrent study on the activities of enzymes related to monoamine metabolism may lead to a better understanding of the monoamine system changes in PD. To evaluate

fluctuations of monoamine levels during medication ON/OFF periods, advanced-PD patients should be controlled LEDD in the same level. Furthermore, the potential use of monoamine level measurement in peripheral body fluids as PD biomarkers should be investigated in future studies.

CHAPTER VI
CONCLUSION

In conclusion, our findings demonstrated dysregulations of overall monoamine levels in peripheral body fluids. Moreover, this study revealed PD patients more suffer from anxiety, depression, sleep disturbances and sexual dysfunctions. Although these altered monoamine levels disassociate with these NMSs genesis, they exhibited some correlations with clinical profiles of PD patients. In addition, this study is the first work that justify the fluctuation patterns of plasma monoamines during ON/OFF periods of advanced-PD patients. This information contributes to our wider knowledge of multi-neurotransmitter dysfunctions and fluctuations in PD, thus enhancing evaluation of neurotransmitter status, prediction of subsequent symptoms, planning of appropriate disease management, and monitoring the effectiveness of treatments.

APPENDIX

APPENDIX 1. ข้อมูลพื้นฐานและข้อมูลด้านสุขภาพ

1. อายุ .....ปี .....เดือน
2. น้ำหนัก .....กิโลกรัม ส่วนสูง .....เซนติเมตร
3. เพศ ☐ ชาย ☐ หญิง
4. สถานภาพ

   ☐ โสด ☐ คู่ ☐ หย่าร้าง ☐ แยกกันอยู่ ☐ หม้าย
5. โรคประจำตัว (ตอบได้มากกว่า 1 ข้อ)

   ☐ ภูมิแพ้ ☐ ความดันโลหิตสูง ☐ ไขมันในเลือดสูง ☐ เบาหวาน

   ☐ โรคมะเร็ง ☐ โรคระบบทางเดินหายใจ ☐ โรคระบบหมุนเวียนเลือดและหัวใจ

   ☐ อื่นๆ ระบุ.....
6. เคยได้รับการวินิจฉัยจากแพทย์ว่าเป็นโรคไมเกรนหรือไม่

   ☐ ไม่เคย ☐ เคย เมื่อตอนอายุ.....ปี
7. ดื่มแอลกอฮอล์

   ☐ ไม่ดื่ม

   ☐ ดื่ม: ความถี่ของการดื่ม: [ ] 1-2 ครั้ง/สัปดาห์ [ ] 3-4 ครั้ง/สัปดาห์ [ ] ทุกวัน
8. สูบบุหรี่

   ☐ สูบ ☐ ไม่สูบ ☐ เคยสูบ
9. ระดับการศึกษา

   ☐ ไม่ได้รับการศึกษา ☐ ประถมศึกษา ☐ มัธยมศึกษา

   ☐ อนุปริญญา ☐ ปริญญาตรี ☐ สูงกว่าปริญญาตรี
10. อาชีพ

    ☐ ไม่ประกอบอาชีพ ☐ ค้าขาย ☐ รับจ้าง ☐ รับราชการ

    ☐ รัฐวิสาหกิจ ☐ เกษตรกรรม ☐ อื่นๆ ระบุ.....
11. ได้รับการวินิจฉัยว่าเป็นโรคกลุ่มพาร์กินโซนิซึม เมื่ออายุ.....ปี

    เป็นโรคพาร์กินโซนิซึมมานาน .....ปี
12. มีประวัติบุคคลในครอบครัวเป็นโรคพาร์กินโซนิซึมหรือไม่

    ☐ ไม่มี ☐ มี โปรดระบุ.....
13. ประวัติการใช้ยา

    .....

APPENDIX 2. Mini Mental State Examination Thai 2002 (MMSE-Thai 2002)

ในกรณีที่ผู้ถูกทดสอบอ่านไม่ออกเขียนไม่ได้ ไม่ต้องทำข้อ 4,9 และ 10

1. Orientation for time (5 คะแนน) (ตอบถูกข้อละ 1 คะแนน)	บันทึกคำตอบไว้ทุกครั้ง (ทั้งคำตอบที่ถูกและผิด)	คะแนน
1.1 วันนี้วันที่เท่าไร	.....	☐
1.2 วันนี้วันอะไร	.....	☐
1.3 เดือนนี้เดือนอะไร	.....	☐
1.4 ปีนี้ปีอะไร	.....	☐
1.5 ฤดูนี้ฤดูอะไร	.....	☐

2. Orientation for place (5 คะแนน) (ตอบถูกข้อละ 1 คะแนน)

2.1 กรณีอยู่ที่สถานพยาบาล

2.1.1 สถานที่ตรงนี้เรียกว่าอะไร <u>และ</u>....ชื่อว่าอะไร	.....	☐
2.1.2 ขณะนี้ท่านอยู่ที่ชั้นที่เท่าไรของตัวอาคาร	.....	☐
2.1.3 ที่อยู่ในอำเภอ - เขตอะไร	.....	☐
2.1.4 ที่นี่จังหวัดอะไร	.....	☐
2.1.5 ที่นี่ภาคอะไร	.....	☐

3. Registration (3 คะแนน)

ต่อไปนี้เป็นการทดสอบความจำ ดิฉันจะบอกชื่อของ 3 อย่าง คุณ (ตา, ยาย...) ตั้งใจฟังให้ดีนะ เพราะจะบอกเพียงครั้งเดียว ไม่มีก ารบอกซ้ำอีก เมื่อผม(ดิฉัน) พูดจบให้ คุณ(ต า,ยาย...) **พูดทบทวนตามที่ได้ยินให้ครบ ทั้ง 3 ชื่อ** แล้วพยามจำไว้ให้ดี เดี๋ยวดิฉันจะถามซ้ำ

*การบอกชื่อแต่ละคำให้ห่างกันประมาณหนึ่งวินาที ไม่ช้าหรือเร็วเกินไป (ตอบถูก 1 คำได้ 1 คะแนน)

◯ ดอกไม้ ◯ แม่น้ำ ◯ รถไฟ ..... ☐

4. Attention/Calculation (5 คะแนน) (ให้เลือกข้อใดข้อหนึ่ง)

4.1 ข้อนี้เป็นการคิดเลขในใจเพื่อทดสอบสมาธิ คุณ (ตา, ยาย....) คิดเลขในใจเป็นไหม?

"ข้อนี้คิดในใจเอา 100 ตั้ง ลบออกทีละ 7 ไปเรื่อยๆ ได้ผลเท่าไรบอกมา

... . ☐ บันทึกคำตอบตัวเลขไว้ทุกครั้ง (ทั้งคำตอบที่ถูกและผิด)

ทำทั้งหมด 5 ครั้ง ถ้าลบได้ 1,2,หรือ3 แล้วตอบไม่ได้ ก็คิดคะแนนเท่าที่ทำได้

4.2 "ผม (ดิฉัน) จะสะกดคำว่า มะนาว ให้คุณ (ตา ,ยาย....) ฟังแล้วให้คุณ(ตา, ยาย....) สะกดถอย หลังจากพยัญชนะ ตัวหลังไปตัวแรก คำว่ามะนาว สะกดว่า

มอม้า-สระอะ-นอหนู-สระอา-วอแหวน ไหนคุณ(ตา,ยาย....) สะกดถอยหลังให้ฟังซิ

.... ☐ บันทึกคำตอบไว้ทุกครั้ง (ทั้งคำตอบที่ถูกและผิด)

ว า น ะ ม

5. Recall (3 คะแนน) เมื่อสักครู่ที่ให้จำของ 3 อย่างจำได้ไหมมีอะไรบ้าง" (ตอบถูก 1 คำได้ 1 คะแนน)

◯ ดอกไม้ ◯ แม่น้ำ ◯ รถไฟ ..... ☐

6. Naming (2 คะแนน)

6.1 ยื่นดินสอให้ผู้ถูกทดสอบดูแล้วถามว่า “ของสิ่งนี้เรียกว่าอะไร” ..... □

6.2 ชี้นาฬิกาข้อมือให้ผู้ถูกทดสอบดูแล้วถามว่า “ของสิ่งนี้เรียกว่าอะไร” ..... □

7. Repetition (1 คะแนน) (พูดตามได้ถูกต้องได้ 1 คะแนน) ตั้งใจฟังผม (ดิฉัน) เมื่อผม (ดิฉัน) พูดข้อความนี้ แล้วให้คุณ (ตา, ยาย) พูดตามผม (ดิฉัน) จะบอกเพียงครั้งเดียว

“ใครใคร่ขายไก่ไข่” ..... □

8. Verbal command (3 คะแนน) ข้อนี้ฟังคำสั่ง “ฟังดีๆ นะเดี๋ยวผม (ดิฉัน) จะส่งกระดาษให้คุณ แล้วให้คุณ (ตา, ยาย....) รับด้วยมือขวา พับครึ่งกระดาษ แล้ววางไว้ที่.....”(พื้น,โต๊ะ,เตียง) ผู้ทดสอบแสดงกระดาษเปล่าขนาดประมาณ เอ-4 ไม่มีรอยพับ ให้ผู้ถูกทดสอบ

□ รับด้วยมือขวา □ พับครึ่ง □ วางไว้ที่”(พื้น,โต๊ะ,เตียง) ..... □

9. Written command (1 คะแนน) ต่อไปเป็นคำสั่งที่เขียนเป็นตัวหนังสือ ต้องการให้คุณ (ตา, ยาย....) อ่าน แล้วทำตาม (ตา, ยาย....) จะอ่านออกเสียงหรืออ่านในใจ

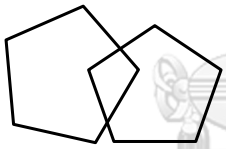
ผู้ทดสอบแสดงกระดาษที่เขียนว่า “หลับตาได้”◯ หลับตาได้..... □

10. Writing (1 คะแนน) ข้อนี้จะเป็นคำสั่งให้ “คุณ (ตา, ยาย....) เขียนข้อความอะไรก็ก็ที่อ่านแล้วรู้เรื่องหรือมีความหมายมา 1ประโยค”

.....

◯ ประโยคมีความหมาย ..... □

11. Visuoconstruction (1 คะแนน) ข้อนี้เป็นคำสั่ง “จงวาดภาพให้เหมือนภาพตัวอย่าง” (ในช่องว่างด้านขวาของภาพตัวอย่าง) ..... □



เต็ม 30 คะแนน

APPENDIX 3. The Hospital Anxiety and Depression Scale (HADS)

อารมณ์ความรู้สึกเป็นส่วนสำคัญส่วนหนึ่งของการเจ็บป่วย ถ้าผู้ดูแลรักษาผู้ป่วยเข้าใจสภาพอารมณ์ความรู้สึกเหล่านี้ของท่าน ก็จะสามารถให้การช่วยเหลือและดูแลท่านได้ดียิ่งขึ้น

แบบสอบถามชุดนี้มีจุดมุ่งหมายที่จะช่วยให้ผู้ดูแลรักษาท่านเข้าใจอารมณ์ความรู้สึกของท่าน

ในขณะเจ็บป่วยได้ดีขึ้น กรุณาอ่านข้อความแต่ละข้อ และทำเครื่องหมายถูก ในช่องคำตอบที่ใกล้เคียงกับความรู้สึกของท่าน ในช่วง 1 สัปดาห์ที่ผ่านมา มากที่สุด และกรุณาตอบทุกข้อ

	คะแนน		คะแนน
1. ฉันรู้สึกตรึงเครียด		2. ฉันรู้สึกเพลิดเพลินใจกับสิ่งต่างๆ ที่ฉันเคยชอบได้	
( ) เป็นส่วนใหญ่	3	( ) เหมือนเดิม	3
( ) บ่อยครั้ง	2	( ) ไม่มากเท่าแต่ก่อน	2
( ) เป็นบางครั้ง	1	( ) มีเพียงเล็กน้อย	1
( ) ไม่เป็นเลย	0	( ) เกือบไม่มีเลย	0
3. ฉันมีความรู้สึกกลัว คล้ายกับว่ากำลังจะมีเรื่องไม่ดีเกิดขึ้น		4. ฉันสามารถหัวเราะและมีอารมณ์ขันในเรื่องต่างๆได้	
( ) มี และค่อนข้างรุนแรง	3	( ) เหมือนเดิม	3
( ) มี แต่ไม่มากนัก	2	( ) ไม่มากนัก	2
( ) มีเพียงเล็กน้อย และไม่ทำให้กังวลใจ	1	( ) มีน้อย	1
( ) ไม่มีเลย	0	( ) ไม่มีเลย	0
5. ฉันมีความคิดวิตกกังวล		6. ฉันรู้สึกแจ่มใสเบิกบาน	
( ) เป็นส่วนใหญ่	3	( ) ไม่มีเลย	3
( ) บ่อยครั้ง	2	( ) ไม่บ่อยนัก	2
( ) เป็นบางครั้ง แต่ไม่บ่อย	1	( ) เป็นบางครั้ง	1
( ) นาน ๆ ครั้ง	0	( ) เป็นส่วนใหญ่	0

7. ฉันสามารถทำตัวตามสบาย และรู้สึกผ่อนคลาย

( ) ได้ดีมาก 3
( ) ได้โดยทั่วไป 2
( ) ไม่บ่อยนัก 1
( ) ไม่ได้เลย 0

8. ฉันรู้สึกว่าตัวเองคิดอะไร ทำอะไร เชื่องช้าลงกว่าเดิม

( ) เกือบตลอดเวลา 3
( ) บ่อยมาก 2
( ) เป็นบางครั้ง 1
( ) ไม่เป็นเลย 0

9. ฉันรู้สึกไม่สบายใจ จนทำให้ปั่นป่วนในท้อง

( ) ไม่เป็นเลย 3
( ) เป็นบางครั้ง 2
( ) ค่อนข้างบ่อย 1
( ) บ่อยมาก 0

10. ฉันปล่อยเนื้อปล่อยตัว ไม่สนใจตนเอง

( ) ใช่ 3
( ) ไม่ค่อยใส่ใจเท่าที่ควร 2
( ) ใส่ใจน้อยกว่าแต่ก่อน 1
( ) ยังใส่ใจตนเองเหมือนเดิม 0

11. ฉันรู้สึกกระสับกระส่าย เหมือนกับจะอยู่นิ่งๆ ไม่ได้

( ) เป็นมากทีเดียว 3
( ) ค่อนข้างมาก 2
( ) ไม่มากนัก 1
( ) ไม่เป็นเลย 0

12. ฉันมองสิ่งต่างๆในอนาคต ด้วยความเบิกบานใจ

( ) มากเท่าที่เคยเป็น 3
( ) ค่อนข้างน้อยกว่าที่เคยเป็น 2
( ) น้อยกว่าที่เคยเป็น 1
( ) เกือบจะไม่มีเลย 0

13. ฉันรู้สึกผวาหรือตกใจขึ้นมาอย่างกะทันหัน

( ) บ่อยมาก 3
( ) ค่อนข้างบ่อย 2
( ) ไม่บ่อยนัก 1
( ) ไม่มีเลย 0

14. ฉันรู้สึกเพลิดเพลินไปกับการอ่านหนังสือ ฟังวิทยุ หรือดูโทรทัศน์ หรือกิจกรรมอื่นๆ ที่เคยเพลิดเพลินได้

( ) เป็นส่วนใหญ่ 3
( ) เป็นบางครั้ง 2
( ) ไม่บ่อยนัก 1
( ) น้อยมาก 0

APPENDIX 4. The Modified Parkinson's Disease Sleep Scale (MDPSS)

การนอนหลับของคุณมีลักษณะอย่างไร ในช่วงหนึ่งสัปดาห์ที่ผ่านมา
(โปรดกากบาทบนเส้นแทนคะแนน 0-10 ตามความถี่ของอาการนั้น ๆ)

1. คุณภาพการนอนหลับตอนกลางคืนโดยรวม — แย่มาก / ดีมาก


2. มีอาการนอนหลับยากตอนกลางคืนหรือไม่ — เป็นประจำ / ไม่เคย


3. มีอาการนอนหลับไม่สนิทต้องตื่นมากลางดึกบ่อยหรือไม่ — เป็นประจำ / ไม่เคย


4. มีอาการแขนขาอยู่ไม่นิ่งชอบขยับไปมาหรือกระตุกจนทำให้หลับไม่สนิทหรือไม่ — เป็นประจำ / ไม่เคย


5. ขณะอยู่บนเตียงคุณรู้สึกงุ่นง่าน กระสับกระส่ายหรือไม่ — เป็นประจำ / ไม่เคย


6. รู้สึกทรมานกับการฝันร้ายตอนกลางคืนจนต้องตื่นกลางดึกหรือไม่ — เป็นประจำ / ไม่เคย


7. รู้สึกทรมานกับการได้ยินหรือเห็นในสิ่งที่มีคนอื่นไม่เห็นหรือไม่ได้ยิน — เป็นประจำ / ไม่เคย


8. ต้องตื่นมาปัสสาวะกลางดึกหรือไม่ — เป็นประจำ / ไม่เคย


9. มีการกลั้นปัสสาวะไม่อยู่เพราะไม่สามารถเคลื่อนไหวไปเข้าห้องน้ำได้หรือไม่ — เป็นประจำ / ไม่เคย


10. มีอาการชาหรือรู้สึกระยิบระยับตามแขนขาทำให้ต้องตื่นกลางดึกหรือไม่ — เป็นประจำ / ไม่เคย


11. มีการปวดเกร็งกล้ามเนื้อแขนหรือขาขณะนอนหลับหรือไม่ — เป็นประจำ / ไม่เคย


12. มีการตื่นตอนเช้าในท่าที่ปวดแขนหรือขาหรือไม่ เป็นประจำ ไม่เคย

13. ขณะตื่นตอนเช้ามีอาการสั่นหรือไม่ เป็นประจำ ไม่เคย

14. รู้สึกอ่อนเพลียและง่วงนอนไม่สดชื่นหลังตื่นนอนตอนเช้าหรือไม่ เป็นประจำ ไม่เคย

15. เคยหลับโดยไม่มีอาการง่วงนำมาก่อนระหว่างวันหรือไม่ เป็นประจำ ไม่เคย

16. มีการนอนละเมอที่อาจทำร้ายหรือเป็นอันตรายต่อตนเอง หรือผู้ดูแลหรือไม่ เป็นประจำ ไม่เคย

17. พบบาดแผลหรือรอยฟกช้ำหลังตื่นนอนโดยไม่ทราบเหตุหรือไม่ เป็นประจำ ไม่เคย

18. เคยนอนหลับขณะทำกิจกรรมที่ต้องใช้สมาธิ เช่น ขับรถ หรือไม่ เป็นประจำ ไม่เคย

19. นอนกรนหรือไม่ เป็นประจำ ไม่เคย

เอกสารอ้างอิง Tanasanvimon S, Ayuthaya NI, Phanthumchinda K. Modified Parkinson's Disease Sleep Scale (MPDSS) in Thai Parkinson's disease patients. J Med Assoc Thai. 2007;90(11):2277-83

APPENDIX 5. The Thai version of Arizona Sexual Experiences Scale (ASEX-Thai)

แบบประเมินประสบการณ์ทางเพศของอริโซน่า ASEX© ฉบับภาษาไทย

สำหรับคำถามแต่ละข้อ กรุณาระบุระดับ**โดยรวม**ในช่วง**อาทิตย์ที่ผ่านมา** รวมทั้งใน**วันนี้**ด้วย

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1. คุณมีความต้องการทางเพศมากแค่ไหน

1. มากที่สุด
2. มาก
3. ค่อนข้างมาก
4. ค่อนข้างน้อย
5. น้อยมาก
6. ไม่ต้องการเลย

2.ความต้องการทางเพศของคุณถูกกระตุ้นได้ง่ายแค่ไหน

1. ง่ายมากที่สุด
2. ง่ายมาก
3. ค่อนข้างง่าย
4. ค่อนข้างยาก
5. ยากมาก
6. ไม่รู้สึกถูกกระตุ้นเลย

3ก. (สำหรับผู้ชาย) อวัยวะเพศคุณสามารถแข็งตัวและรักษาการแข็งตัวได้ง่ายหรือไม่

1. ง่ายมากที่สุด
2. ง่ายมาก
3. ค่อนข้างง่าย
4. ค่อนข้างยาก
5. ยากมาก
6. ไม่แข็งตัวเลย

3ข. (สำหรับผู้หญิง) ช่องคลอดคุณมีน้ำหล่อลื่นหรือเปียกได้ง่ายแค่ไหน

1. ง่ายมากที่สุด
2. ง่ายมาก
3. ค่อนข้างง่าย
4. ค่อนข้างยาก
5. ยากมาก
6. ไม่มีน้ำหล่อลื่นเลย

4. คุณสามารถถึงจุดสุดยอดได้ง่ายแค่ไหน

1. ง่ายมากที่สุด
2. ง่ายมาก
3. ค่อนข้างง่าย
4. ค่อนข้างยาก
5. ยากมาก
6. ไม่เคยถึงจุดสุดยอดเลย

5.คุณพึงพอใจกับการถึงจุดสุดยอดของคุณหรือไม่

1. มากอย่างที่สุด
2. มาก
3. ค่อนข้างมาก
4. ค่อนข้างน้อย
5. น้อยมาก
6. ไม่พึงพอใจเลย

ความคิดเห็น :

APPENDIX 6. แบบสอบถามอาการนอกเหนือจากปัญหาด้านการเคลื่อนไหวในผู้ป่วยพาร์กินสัน (Non-motor symptoms questionnaire; NMSQ)

<u>คำชี้แจง</u> ต่อไปนี้เป็นอาการนอกเหนือจากปัญหาด้านการเคลื่อนไหวที่มักพบได้ในผู้ป่วยพาร์กินสัน ซึ่งควรแจ้งให้แพทย์ได้ทราบเพื่อวางแผนการรักษาที่เหมาะสมต่อไป
โดยให้ท่านเติมเครื่องหมาย ✓ ที่ช่อง “ใช่” หากท่านมีอาการดังกล่าวในช่วงเวลา 1 เดือนที่ผ่านมา หรือเติมเครื่องหมาย ✓ ที่ช่อง “ไม่ใช่” หากท่านไม่มีอาการดังกล่าวในช่วง 1 เดือนที่ผ่านมา

ในช่วงเวลา 1 เดือนที่ผ่านมาท่านมีอาการต่อไปนี้หรือไม่

คำถาม	“ใช่”	“ไม่ใช่”
1. มีน้ำลายไหลหยดจากปากในช่วงเวลากลางวัน	☐	☐
2. การรับรสชาติหรือการดมกลิ่นผิดปกติ	☐	☐
3. กลืนอาหาร หรือดื่มน้ำลำบาก หรือมีอาการสำลัก	☐	☐
4. รู้สึกคลื่นไส้ หรืออาเจียน	☐	☐
5. ท้องผูก (ถ่ายอุจจาระน้อยกว่า 3 ครั้งต่อสัปดาห์) หรือถ่ายยากต้องเบ่ง	☐	☐
6. กลั้นอุจจาระไม่อยู่	☐	☐
7. รู้สึกว่ายังถ่ายไม่สุดหลังจากที่อุจจาระเสร็จแล้ว	☐	☐
8. รู้สึกว่ากลั้นปัสสาวะไม่อยู่ต้องรีบไปเข้าห้องน้ำ	☐	☐
9. ต้องตื่นเพื่อลุกมาปัสสาวะในตอนกลางคืนเป็นประจำ	☐	☐
10. มีอาการเจ็บปวดตามร่างกายที่ไม่สามารถอธิบายได้ ไม่มีสาเหตุ และไม่ใช่อาการปวดจากโรคที่เป็นอยู่แล้วเช่น ข้ออักเสบ	☐	☐
11. น้ำหนักตัวเพิ่มขึ้น หรือลดลงโดยไม่ทราบสาเหตุ และไม่ได้เกิดจากการควบคุมอาหาร	☐	☐
12. มีปัญหาเรื่องความจำ เช่นจำเรื่องที่เพิ่งเกิดขึ้นไม่ได้ หรือลืมว่ากำลังจะทำอะไร	☐	☐
13. ขาดความสนใจในสิ่งต่างๆที่เกิดรอบตัว หรือสิ่งที่กำลังทำอยู่	☐	☐
14. เห็นหรือได้ยินในสิ่งที่ไม่มีอยู่จริงในตอนนั้น	☐	☐
15. ไม่มีสมาธิจดจ่อกับสิ่งที่ทำอยู่	☐	☐

คำถาม	“ใช่”	“ไม่ใช่”
16. รู้สึกเศร้า หดหู่ สิ้นหวัง	☐	☐
17. รู้สึกวิตกกังวล หวาดกลัว หรือตื่นตระหนก	☐	☐
18. มีความรู้สึกทางเพศลดลงหรือเพิ่มมากกว่าปกติ	☐	☐
19. มีความยากลำบากในการมีเพศสัมพันธ์	☐	☐
20. รู้สึกหน้ามืด เวียนศีรษะขณะเปลี่ยนจากท่านนอนหรือนั่งมาเป็นท่ายืน	☐	☐
21. หกล้ม	☐	☐
22. ไม่ค่อยตื่นตัว รู้สึกง่วงขณะกิจกรรมต่างๆ เช่น ทำงาน ขับรถ หรือรับประทานอาหาร	☐	☐
23. หลับยากในตอนกลางคืน หรือตื่นง่ายในช่วงกลางดึก	☐	☐
24. ฝันร้าย ฝันน่ากลัวเสมือนจริง	☐	☐
25. ละเมอ พูดหรือแสดงท่าทางต่างๆ ขณะที่หลับอยู่	☐	☐
26. มีความรู้สึกผิดปกติที่ขาขณะนอนพักผ่อน หรือนอนตอนกลางคืน เช่นรู้สึกเหมือนมีอะไรมาไต่หรือทิ่มขา ต้องสะบัดขาไป-มา จึงรู้สึกดีขึ้น	☐	☐
27. ขาบวม	☐	☐
28. เหงื่ออกมากผิดปกติ	☐	☐
29. มองเห็นภาพซ้อน	☐	☐
30. คิดว่าสิ่งต่างๆ ที่คนอื่นบอกว่ามันเกิดขึ้นกับคุณไม่ใช่เรื่องจริง	☐	☐

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VITA

NAME	นางสาวภัสสร วิชิต
DATE OF BIRTH	25 กุมภาพันธ์ 2528
PLACE OF BIRTH	ลพบุรี
INSTITUTIONS ATTENDED	1. มหาวิทยาลัยมหิดล พ.ศ. 2550-2553 2. มหาวิทยาลัยศรีนครินทรวิโรฒ พ.ศ. 2546-2549
HOME ADDRESS	82/261 หมู่บ้านซีเมนต์ไทย ซอย8 แขวงลาดยาว เขตจตุจักร กรุงเทพฯ 10900
PUBLICATION	1. Wichit P, Chuncharunee A, Charoenphandhu N, Jariyawat S, Suksamran A, Piyachaturawat P. "Protection of Bone in Ovariectomized Rats by Curcumin" at the 39th Physiological Society of Thailand's Annual Conference, Chonburi, Thailand, April 5-8, 2010. 2. Tantikanlayaporn D, Wichit P, Weerachayaphorn J, Chairoungdua A, Chuncharunee A, Suksamrarn A, et al. Bone sparing effect of a novel phytoestrogen diarylheptanoid from Curcuma comosa Roxb. in ovariectomized rats. PLoS One. 2013;8(11):e78739. 3. Thongon N, Boonmuen N, Suksen K, Wichit P, Chairoungdua A, Tuchinda P, et al. Selective Estrogen Receptor Modulator (SERM)-like Activities of Diarylheptanoid, a Phytoestrogen from Curcuma comosa, in Breast Cancer Cells, Pre-osteoblast Cells, and Rat Uterine Tissues. J Agric Food Chem. 2017; 65(17):3490-6.