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"West Kazakhstan Marat Ospanov Medical University"

ABSTRACT

of thesis for the degree of Doctor of Philosophy (PhD)
of doctoral student Aigerim Utegenova

Topic: **Improving early diagnosis of Parkinson's disease with detection of phosphorylated alpha-synuclein in skin biopsy of patients**
6D110100 – "Medicine"

Scientific supervisors:
Candidate of Medical Science, A.P.Yermagambetova
Scientific Advisor: MD, Professor, PhD Slawomir Michalak, (PUMS) Poland

Aktobe, 2023

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RELEVANCE OF THE RESEARCH TOPIC

Relevance of the problem. Parkinson's disease (PD) is the most common type of Parkinsonism. Parkinsonism is a term reflecting a group of neurological disorders in which movement disorders such as retardation, rigidity, and tremor are observed. PD is second only to Alzheimer's disease in terms of frequency among neurodegenerative diseases (NDD) (Armstrong M.J. 2020). The pathogenesis of PD is caused by the accumulation and aggregation of a pathological protein, alpha-synuclein. At the same time, the spatial structure of alpha-synuclein is disrupted with the formation of insoluble protein aggregates followed by neurodegeneration of dopaminergic neurons of the substantia nigra. Currently PD is diagnosed only clinically, there are no specific instrumental and laboratory markers of the disease (Abbas M. 2018).

PD has a preclinical stage in its development, manifested by non-motor symptoms (constipation, loss of smell, anxiety, depression); early (stable) and advanced stages, characterized by motor symptoms (tremor, bradykinesia, rigidity). It is most difficult to make a diagnosis in the preclinical stage, because non-motor manifestations of PD are mostly nonspecific (Barone P. 2017). Morphological verification of the diagnosis of PD is known to be based on the detection of Levi bodies in dopaminergic neurons of the substantia nigra in autopsy studies. However, an in vivo morphological diagnosis of PD using immunohistochemical methods of peripheral tissue biopsy to detect alpha-synuclein, which can act as a biomarker of PD, is now possible. A large multicenter study of alpha-synuclein detection in peripheral tissues has been supported by the Fox Foundation for Parkinson's Research since 2013 to integrate the results and further develop unified methods for detection of alpha-synuclein in peripheral tissue biopsy.

The potential of skin biopsy to detect alpha-synuclein in PD in living patients was first reported by Harvard researchers under the direction of R. Freeman. They performed a skin biopsy of the lateral surfaces of the upper and lower extremities. As a result, alpha-synuclein was detected in more than 90% of cases compared with healthy control subjects. Biopsy results for detection of alpha-synuclein from other anatomical areas such as the posterior surfaces of the neck, chest, abdomen and back revealed inconsistent results. The authors attribute the findings to the unequal loss of cutaneous autonomic nerves in which alpha-synuclein accumulates (Gibbons C.2016).

Analysis of the literature data studied by us has shown that at present the urgent problem is the development of criteria for diagnostics of non-motor manifestations of

PD at the preclinical stage and issues of differential diagnosis with clinically similar diseases. One of the methods contributing to the solution of these issues can be the detection of pathological protein alpha-synuclein in the intravital diagnosis, which was the reason for this study.

Purpose of the research:

On the basis of studying clinical manifestations of Parkinson's disease and results of immunohistochemical analysis with detection of phosphorylated alpha-synuclein in skin biopsy of patients, to develop approaches to early diagnosis of Parkinson's disease before manifestation of motor symptoms.

Objectives of the research:

1. To conduct clinical differential diagnostics of Parkinson's disease with clinically similar diseases in Aktobe patients.
2. To determine the correlation between alpha-synuclein level in skin biopsy specimen and severity of non-motor and motor manifestations of Parkinson's disease.
3. To work out algorithm of early diagnostics of Parkinson's disease before manifestation of motor manifestations.

Scientific novelty of the research:

For the first time in Kazakhstan immunohistochemical study on detection of alpha-synuclein in skin biopsy of patients with Parkinson's disease with the purpose of early and differential diagnostics with clinically similar diseases was carried out. The correlation of alpha-synuclein expression with the severity of clinical manifestations of Parkinson's disease was studied, allowing to use alpha-synuclein as a biomarker of Parkinson's disease.

Practical significance of the research:

1. The results of clinical and immunohistochemical studies will allow the development of criteria for early diagnosis of Parkinson's disease with an emphasis on non-motor symptoms of the disease.
2. Detection of alpha-synuclein by immunohistochemical study in skin biopsy of patients with suspected Parkinson's disease can be considered as a biomarker of Parkinson's disease.
3. The results of the study can serve as a basis for making additions to clinical protocols for diagnosis and treatment of Parkinson's disease and other clinically similar diseases.

The main provisions of the dissertation research for defense:

1. Differential diagnosis of Parkinson's disease with clinically similar diseases - essential tremor, vascular parkinsonism, parkinsonism syndrome, causes certain difficulties at the outpatient stage. To diagnose Parkinson's disease, it is necessary to take into account an objective assessment of non-motor and motor manifestations of

the disease using specific evaluation scales and questionnaires, as well as diagnostic criteria for extrapyramidal diseases.

2. Pathological alpha-synuclein is detected in 95.7% of cases in patients with Parkinson's disease in a dermal biopsy. The diagnostic test for the detection of alpha-synuclein in the skin of PD patients has a high sensitivity and specificity. The level of alpha-synuclein correlated with the severity of the disease. Patients with bradykinesia had higher levels of alpha-synuclein.

3. If Parkinson's disease is suspected, patients have autonomic dysfunction, hyposmia and the duration of the disease is less than three years, the risk of developing the disease ranges from 74% to 92.1%.

Personal contribution of the author. All the main sections of the research (collection of materials, their processing, analysis of research materials, interpretation of the results and their discussion) were carried out personally by the author.

Approbation and implementation of research results

1. "Abstracts of The IX Annual International Scientific-Practical Conference; Medicine Pressing Questions". Utegenova A.B. ISBN: 978-81-942709-5-9; DOI: 10.21467/abstracts.97 4. May 06- 08, 2020, Baku, Azerbaijan.
2. International Scientific and Practical Conference: "Neurodegenerative diseases: diagnosis, intensive therapy, comorbidity, rehabilitation". "Issues of differential diagnosis of Parkinson's disease and essential tremor ". Utegenova A.B. 10.12.2020. UFA
3. International Scientific and Practical Conference : "The Elderly Patient at the Outpatient Clinic" Report: "General features of diagnostics and treatment of elderly patient with Parkinson's disease: data of international hydrolines". Bashkortostan. 24.02.2021
4. Annals of Anatomy. «Severity of depression in Parkinson's disease on the example of patients in Aktobe». Aigerim B. Utegenova, Volume 230S-2020. Aktobe, Kazakhstan, May 27-31, 2021.

Publications on the dissertation topic:

11 scientific papers were published on the topic of the dissertation, including 2 articles - in the edition indexed in the Scopus information base - "Georgian Medical News" (26 percentile in 2019); 4 articles - in editions recommended by the Committee for Control in Education and Science of Kazakhstan; 4 theses - in collections of international conferences (including foreign - 3, including those indexed in the Scopus base - 1).

Implementation of study results.

Materials of the study are implemented in practical health care - in the work of outpatient clinic № 1. The results of the thesis research are used in the educational process of the Department of Neurology

MATERIALS AND METHODS OF THE RESEARCH

We performed a simple one-stage observational descriptive cross-sectional study. This scientific work was performed within the framework of intramural funding of the scientific and technical project "Determination of pathological proteins content in skin biopsy of patients with neurodegenerative diseases in order to develop an algorithm of differential diagnosis with clinically similar diseases". The state registration number: 0113RKI0244 of May 29, 2019.

Participation of patients in the study complied with the principles of the Declaration of Helsinki. The conclusion of the local bioethical commission of the West Kazakhstan Medical University named after Marat Ospanov No.6 dated 20.06.2022 was received.

Inclusion criteria for the study. The main group included persons with Parkinson's disease and with Parkinsonism syndrome over the age of 18 years.

The control group included relatively healthy individuals without a history of neurodegenerative diseases over 18 years of age.

Exclusion criteria from the study. Excluded from the main group were subjects suffering from other neurological diseases; with sub- and decompensated somatic pathology; patients with infectious diseases and oncological diseases; patients with diseases of blood and hemopoietic organs; pregnant women; subjects younger than 18 years.

Excluded from the control group were individuals with chronic nervous system diseases and severe somatic diseases in sub- and decompensation stages; patients with infectious diseases and cancer; patients with diseases of blood and hemopoietic organs; pregnant women; and individuals younger than 18 years.

We examined the study participants twice. The primary examination of the patients was performed at the Department of Neurology, during which an objective neurological examination with specific scales and questionnaires was performed. During the second visit, a skin biopsy was performed to determine alpha-synuclein by immunohistochemical analysis. According to the appeal, 169 patients with a directional diagnosis of PD were examined, who are on the outpatient register of neurologists of polyclinics in Aktobe. The diagnosis of PD was confirmed in 74% (n=125), essential tremor (ET) – in 20% (n=34), vascular parkinsonism (VP) in 4.7% (n=8) cases, progressive supranuclear palsy (PNP) and dementia with Lewy bodies (DTL) 1-occasion.

The average age of patients was: PD - 63.73±9.10; ET - 62.94±11.75; VP - 57.62±1.72. The age of patients with PNP and DTL was 66 years and 71 years, respectively. The duration of the disease of patients: PD - 3.72±3.63; ET - 9.71 ± 8.49; VP - 3.00 ± 2.39 years; PNP – 1 year; DTL – 1 year. The number of men and women at: BP - 50 and 75; ET – 10 and 24; SP – 5 and 3, respectively. Cases of PNP and DTL were presented by women.

Clinical methods of investigation:

In the course of clinical investigation (1st stage) there was conducted neurological examination according to the conventional scheme with evaluation of the

state of cranial-brain innervation, motor, sensory spheres, cerebellar system, higher cerebral functions.

PD was diagnosed on the basis of the United Kingdom Parkinson's Disease Society Brain Bank criteria and the 2015 MDS (Movement Disorder Society) diagnostic criteria.

The following scales were used to clinically evaluate PD patients:

- The Hoenh and Yahr scale determines the stage of PD;
- The Schwaab and England scale determines the level of daily activities and self-care ability of patients;
- Unified PD assessment scale modified by the International Society for the Study of Motor Disorders (MDS-UPDRS) has 4 blocks to determine the severity of non-motor and motor manifestations of PD;
- Montreal Cognitive Assessment Scale (MoCA) assesses the degree of cognitive impairment in PD patients;
- Hospital Anxiety and Depression Scale (HADS) assesses the level of anxiety and depression in PD patients;

The criteria for the diagnosis of essential tremor corresponded to the Classification of Tremors by the Task Force on Tremor of the International Parkinson and Movement Disorder Society (IPMDS):

1. isolated kinetic bilateral upper limb tremor syndrome;
2. duration of the disease at least 3 years;
3. with or without tremor in other localizations (e.g., head, vocal cords, or lower extremities);
4. absence of other neurologic signs such as dystonia, ataxia, or parkinsonism.

Diagnostic criteria for vascular parkinsonism corresponded to the criteria for the diagnosis of vascular parkinsonism: (Levin O.S., 2017):

1. The atypical nature of the motor disorder, which differs from the classical picture of Parkinsonism in neurodegenerative diseases (for example, PD) by motor pattern, localization, the presence of concomitant syndromes and reflects the logic of a vascular, not a degenerative or other process.
2. Characteristic course: development shortly after a stroke (for example, within 6 months), acute or subacute onset, long periods of stabilization, the possibility of regression of symptoms, a gradual increase in the severity of the syndrome with periods of stabilization and reverse development of symptoms.
3. The defeat of "strategic" areas for parkinsonism, according to CT or MRI.

The clinical study of patients with VP included an assessment on the scales: MoCA; HADS.

The criteria for the diagnosis of progressive supranuclear palsy (PSP) correspond to the criteria of the National Institute of Neurological Disorders and Stroke and the Society for PSP (NINDSSPSP):

1. Vertical gaze palsy;
2. progressive postural instability with falls;
3. proximal akinesia and rigidity;
4. retrocollis;

5. minor response to levodopae therapy;
6. early dysphagia; dysarthria;
7. early specific cognitive behavioral traits.

The clinical study of patients with PSP included an assessment on the scales: MoCA; HADS.

The criteria for diagnosis of dementia with Lewy body was made in accordance with the diagnostic criteria of I. McKeith et al. 2005:

1. Mandatory progressive disorder of cognitive functions, the degree of which is sufficient to disrupt the social and professional adaptation of the patient (dementia);

2. Mnestic disorders, optional at the initial stages of the disease and expressed in the presence of a detailed clinical picture;

3. The presence of a frontal-subcortical component of cognitive disorders (impaired attention, visual-spatial functions, thinking, regulatory changes in praxis and gnosis);

4. Fluctuation of cognitive and emotional-affective disorders;

5. Transient visual hallucinations, detailed, detailed, well-defined;

6. Parkinsonism, unrelated to previous use of neuroleptics.

The clinical study of a patient with DTL included an assessment on the scales: MoCA; HADS.

Immunohistochemical methods of research

Immunohistochemical studies were performed in a specialized laboratory of Poznan University of Medical Sciences (Poland). Written informed consent was obtained from all subjects to participate in the study.

Skin biopsy. All patients (n=56) and healthy subjects (n=20) underwent puncture skin biopsy of the proximal parts of the lower limb: in the thigh (15 cm above the kneecap). Biopsy with a 3-mm dermopunch (Miltex 33-33 Standard Biopsy Punch,3.0 mm) was performed under local anesthesia with 2% lidocaine. Skin samples were immediately fixed in 4% paraformaldehyde dissolved in 0.1 M phosphate buffer in Eppendorf tubes. Samples were then sent at room temperature to the Department of Neurochemistry and Neuropathology at Poznan University of Medical Sciences for immunohistochemical analysis of alpha-synuclein in paraffin-impregnated sections.

In the laboratory, skin biopsy specimens were cut into 5-mm sections using a Leica microtome (LeicaSM2010R). The slices were first deparaffinized using xylene and 99% ethyl alcohol. After washing with distilled water for 5 minutes, the sections were incubated in citrate buffer for 15 minutes at 90°C to extract the antigen. The citrate buffer consisted of 0.1 M sodium citrate and 0.1 M citric acid pH = 6.0. The sections were cooled for 2 h at room temperature and washed twice for 5 min in distilled water.

In the next step, the sections were washed twice for 10 minutes with phosphate buffered saline (PBS) containing 0.25% Triton X (Sigma-Aldrich). The sections were incubated for 60 minutes with 5% bovine serum albumin (BSA, Sigma-Aldrich) to block the binding of nonspecific antibodies. The sections were then incubated overnight at 4°C with antibody conjugated with anti-alpha-synuclein fluorescent

isothiocyanate (FITC, Bioss polyclonal rabbit antibody, bs-0968R-FITC) 1:100 in dilution buffer (1% BSA in PBS).

The slices were then washed three times for 5 minutes in phosphate buffered saline. When finished, the slices were washed for 5 minutes with distilled water and placed in glycerol.

Assessment of alpha-synuclein expression.

Laboratory diagnosis of immunohistochemical analysis of alpha-synuclein in skin biopsy specimens was performed based on fluorescence microscopy using a Z1 Axioimager microscope (Zeiss) and Axiovision licensed software (Zeiss). Fluorescence intensity representing alpha-synuclein expression was assessed using a 4-point system, where the total analyzed biopsy area at 20× magnification was scored as:

0 point - no specific fluorescence;

1 point - weak fluorescence - glow area up to 25%;

2 points - moderate fluorescence - glow area from 26% to 50%;

3 points - strong fluorescence - glow area over 50%.

Statistical methods. For quantitative variables: mean and standard deviation as "M±S"; frequency table, Spearman rank correlations, nonparametric Mann-Whitney test, comparisons of three or more groups by quantitative measures were performed using nonparametric Kruskal-Wallace test. The statistical reliability of group differences for binary and nominal scales was determined using the chi-square test.

Results of clinical research

Bradykinesia (100%) was manifested by slowing down the movements of patients. Muscle rigidity (55.2%) was characterized by hypertonicity of the "gear wheel" type in the involved half of the body. Patients noted pain and stiffness in large joints; stiffness in the lumbar spine; difficulty turning in bed and getting up.

Unilateral resting tremor of the "pill rolling" type (93.6%) was expressed in the distal parts of the hands. The tremor disappeared during sleep. Postural instability (18.5%) was observed in patients with stage 3 and higher on the Hoehn-Yar scale and was the cause of falls in patients. Postural deformities (4.8%) were manifested by abnormal axial postures (anterocollis, scoliosis, camptocormia). In 12.8% of cases, "freezing" of gait was detected (akinesia at the beginning of movement when walking). Vegetative dysfunction (65.6%) was manifested by constipation, orthostatic hypotension. Hyposmia or anosmia (50.4%) had bilateral localization.

Assessment on the **Hoenh and Yahr** scale (average score 1.69±0.93): 1st stage of PD (n=72); 2nd stage (n=30); 3rd stage (n=19); 4th stage (n=3); 5th stage (n=1).

The average score on the **Schwaab-England** scale was 81.12±15.72 points.

The total **MDS-UPDRS** scale is 33.71±23.62. The **MDS-UPDRS-1** scale was used to assess non-motor symptoms. The patients had a mild degree of non-motor disorders with an average score of 7.37±5.67. Cognitive impairment (89.5%) was expressed by bradyphrenia, violation of short-term memory, attention, visual-spatial and executive functions of memory, abstract thinking. Hallucinations and psychosis (2.2%) were manifested by visual hallucinations. Depressive disorders (12.6%) were manifested by

depressed mood, difficulties in making decisions. Anxiety (89.5%) was noted in the form of emotional lability, fearfulness, resentment. Apathy (35.8%) was manifested by isolation, limited communication, loss of appetite, lack of initiative. Dopamine dysregulation syndrome (4.4%) appeared with an independent increase in the dose of levodopa drugs without a doctor's appointment and was manifested by complaints from patients about the ineffectiveness of drugs, overeating and panding. Sleep disorders (47.7%) were expressed by insomnia, early awakenings and fragmentation of night sleep. Daytime sleepiness (35.8%) was due to insufficient amount of daytime sleep.

In 44.7% of cases, pains of various localization and severity were detected. Dysuria (2.9%) was manifested by urinary incontinence, a feeling of incomplete emptying of the bladder, imperative urge to urinate. Constipation (28.3%) was characterized by the absence of stool for more than 3 days and required the use of laxatives on a regular basis. Dizziness when getting up (22.3%) was accompanied by headache, muscle weakness, loss of coordination and tinnitus. Fatigue (47%) was expressed by extreme fatigue, weakness, exhaustion, loss of strength when performing the usual load for the patient and limiting his functional activity.

When assessing the motor aspects of patients' daily life on the **MDS-UPDRS-2** scale, the average score was 7.91±7.03. At the same time, there was a speech disorder in the form of dysarthria; salivation and deterioration of handwriting due to tremor. Also, patients noted difficulties when changing clothes, buttoning buttons due to oligokinesia.

When assessing the motor symptoms of PD in patients on the **MDS-UPDRS-3** scale the average score was 17.90±12.38. At the same time, there was a weakening of voice modulation, a decrease in the frequency of blinking, muscle rigidity, oligokinesia of the involved limb during finger tapping and hand movements.

When assessing motor complications on the **MDS-UPDRS-4** scale the average score was 0.53 ± 2.17. Dyskinesia was manifested by muscle twitching and spasms in the arms and legs. Motor fluctuations in the form of "inclusion" dystonia were described by patients as the time of the onset of action of antiparkinsonian drugs.

The assessment of cognitive functions was carried out on the **MoCA** scale. The average score was 21.04±4.76 points. 85.7% of patients had difficulties in performing tasks using visual-constructive and executive skills. Difficulties were noted in 71.4% of cases when performing the task "Copying the cube". When performing the clock drawing test, one or more errors were made in 64.3% of cases.

57.4% of patients could not name a rhinoceros when performing the task "Naming three animals". When assessing short-term memory immediately after naming words at the first attempt, 85.7% of patients could not recall 2 words out of 5 named; with delayed reproduction, 92.8% of patients found it difficult to recall 2 or more words.

The task of attention consisted in the correct naming of the voiced digital series in the direct order and caused difficulties in 64.3% of cases. When performing the task to name the digital series in reverse order, 87.1% of patients could not cope with the task and called the voiced digital series in direct order.

To assess attention, the researcher read a list of letters, and the subject had to clap his hands only when naming the letter "A". When performing the task, patients had a

delayed reaction to verbal commands - 28.5% of patients clapped their hands when naming another letter. Serial counting (sequential subtraction of 7 out of 100) caused difficulties in 50% of patients from the second cycle.

When repeating two sentences: "I know only one thing that Ivan is the one who can help today" and "The cat always hid under the sofa when the dogs were in the room", 84.2% of the patient skipped conjunctions or words, swapped words. The assessment of fluency of speech required to name the maximum number of words (at least 11) starting with the letter "L" within one minute. 82.8% of patients failed to complete the task (they called less than 6 words). Violation of abstract thinking (34.2%) was manifested by incorrect performance of the task to name a common feature between the words "train-bicycle", "watch-ruler". Violations of orientation in time and space (5.7%) revealed errors when specifying the current date and year.

The average score on the **HADS-1** and **HADS-2** scale was 5.98±4.00 and 7.89±4.26, respectively. The patients were diagnosed with subclinical depression (constant feeling of fatigue, guilt, emotional lability, longing, tension, fear of death).

The correlation of quantitative indicators of the scales, the duration of the disease with the age of the studied revealed the following types of relationships: moderate positive correlation of the severity of PD with the duration of the disease ($Rs=0.64$, $p\leq0.01$); strong positive correlation between non-motor and motor manifestations of PD and the severity of the disease ($Rs=0.74$, $p\leq0.01$).

The level of daily activity had a strong negative relationship with the severity of motor and non-motor manifestations of PD ($Rs=-0.8$, $p\leq0.01$); non-motor symptoms moderately positively correlated with the level of depression ($Rs=0.54$, $p\leq0.01$).

Motor disorders were moderately positively correlated with the severity of the disease ($Rs=0.69$, $p\leq0.01$). A moderately negative relationship was found between cognitive impairment and age ($Rs=-0.33$, $p\leq0.01$), as well as between cognitive impairment and the level of daily motor activity ($Rs=-0.42$, $p\leq0.01$).

There was a moderate positive correlation between the stage of the disease on the **Hoenh and Yahr** scale with the duration of the disease ($Rs=0.64$, $p\leq0.01$). A strong positive correlation was revealed between the quantitative indicators of motor and non-motor manifestations of PD (MDS-UPDRS scale) and the stage of the disease (Hyun-Yar) ($Rs=0.74$, $p\leq0.01$).

The daily activity of patients with PD (the Schwaab-England scale) strongly negatively correlated with quantitative indicators of motor and non-motor manifestations of PD (MDS-UPDRS) ($Rs=-0.8$, $p\leq0.01$). Non-motor symptoms (MDS-UPDRS-1) were moderately positively correlated with the level of depression (HADS 2) ($Rs=0.54$, $p\leq0.01$).

Motor disorders (MDS-UPDRS 3) were moderately positively correlated with the stage of the disease (Hyena) ($Rs=0.69$, $p\leq0.01$). A moderately negative relationship was found between cognitive impairment (MOS) and the age of patients with PD ($Rs=-0.33$, $p\leq0.01$). A negative correlation of weak closeness between cognitive indicators (Mos) and motor aspects of everyday life (MDS-UPDRS 2) was revealed ($Rs=-0.42$, $p\leq0.01$).

Results of clinical differential diagnosis
Parkinson's disease with clinically similar diseases

Diagnosis of ET. The diagnosis of ET was made in 34 patients out of 169 patients referred for investigation according to IPMDS diagnostic criteria. When studying this group of patients no motor symptoms forming clinical nucleus of PD in the form of bradykinesia, rigidity and postural instability were revealed in ET patients.

Tremor in patients with ET was detected in 100% of cases and was clinically manifested by symmetrical, kinetic or postural bilateral upper limb tremor in combination with a "yes-yes" or "no-no" head tremor.

Hyposmia was diagnosed in one patient with ET, which could be related to the presence of allergic rhinitis according to the history. Autonomic dysfunction was also observed in one case among ET patients, clinically manifested by constipation and urinary dysfunction.

On examination for anxiety and depression, the mean **HADS 1** and **HADS 2** scores were 4.03 ± 4.22 and 5.44 ± 3.92, respectively, which corresponded to normal values. Thus, clinically and subclinically significant anxiety and depression were not detected in ET patients.

The **MoCA** score in patients with ET demonstrated a decrease in cognitive function to 22.76 ± 5.79. A moderate negative correlation (Rs=-0.46, $p \leq 0.01$) was observed when examining the correlation between age of ET patients and cognitive scores (MoCA), that is, with increasing age there was a decrease in cognitive function.

As a result of clinical diagnosis, we diagnosed vascular parkinsonism in 8 patients. Analysis of the results of clinical observation showed the presence of bradykinesia in 100% of cases. Bradykinesia in patients with VP manifested itself clinically as slow walking, "magnetization" of the feet during walking initiation.
Tremor frequency in patients with VP was 75%. Tremor was observed in the lower extremities, so-called lower body parkinsonism.

Postural instability characterized by impaired balance, falls, and lateropulsion was detected in 50% of patients. Rigidity was detected in 62.5% of patients and was also characterized along with a plastic increase in muscle tone of the "cogwheel" type and hypertonicity of the "folding knife" type characteristic of pira-mid disorders.

When studying non-motor symptoms in patients with VP, autonomic dysfunction was detected in 62.5% of cases. It was clinically manifested by hypotension, urinary incontinence.

Hyposmia was observed in 37.5% of patients, due to the lack of special olfactory tests, we relied on the response of patients who believed that their sense of smell was markedly reduced.

On examination of anxiety and depression, the mean **HADS 1** and **HADS 2** scores were 5.75 ± 3.65 and 9.50 ± 4.57, respectively. Clinical depression was diagnosed. Symptoms of depression in those studied were expressed in the form of a constant feeling of fatigue, emotional lability, melancholy, tension, and fear of death.

The mean score on the **MoCA** scale was 21.50 ± 3.82, consistent with cognitive impairment.

Statistically significant differences p=0.0047 were detected between the measures of disease duration among patients with ET, VP, and PD, with disease duration being almost 3 times higher in ET patients than in PD and SP patients.

Statistically significant differences p=0.0020 were found in anxiety levels on the HADS 1 scale among ET, SP, and PD patients.

Statistically significant differences p=0.0012 were found for depression on the HADS 2 scale among ET, SP, and PD patients.

The results of the comparative analysis of multiple pairwise comparisons of patients by age and quantitative data of the scales and questionnaires are presented in Table 3.

Results of immunohistochemical study

This section describes the results of immunohistochemical examination (second stage) of 56 skin biopsy samples obtained from patients with PD (n=47), ET (n=6), VP(n=3) and 20 healthy control subjects.

The control group was recruited for immunohistochemical examination of skin biopsy specimens in order to detect differences in immunohistochemical indices and to determine the sensitivity and specificity of the diagnostic test in healthy subjects and patients. The control group was formed by screening the general population of volunteers who had no neurodegenerative diseases at the time of the study and in the history.

The number of women in the main group (n=56) was 36 (64%), and 20 men (36%). The number of women in the control group (n=20) was 15 (75%), men were 5 (25%). The mean age of the main and control groups was 61.41±8.45 and 61.45±10.2, respectively. The main and control groups were comparable in age (P=0.98).

To estimate clinical informativity of prognostic significance of qualitative and quantitative index of alpha-synuclein in skin biopsy of PD patients we performed ROC-analysis (ROC analysis). Expression of alpha-synuclein in skin biopsy showed AUC=0.993, P<0.001 with a sensitivity of 90.9% and specificity of 100% (Figure 1). The cut-off level (cut-of) of the alpha-synuclein indicator was 1. The Juden index =0.8571.

We found that patients with bradykinesia had higher alpha-synuclein levels than patients without bradykinesia, with values of 2.46±0.65 and 2.06±0.66, respectively. Statistically significant differences (p=0.041) were established when comparing the values using Student's t-test.

When assessing the expression of alpha-synuclein in skin biopsy specimens of the examined individuals, statistically significant differences in protein expression were found in PD (3; 1 - 3), SP (2; 2 - 3) and ET (2; 1 - 3) patients compared to the control group (0; 0 - 1) (P<0.000001). Moreover, alpha-synuclein expression was significantly (P<0.000001) higher in PD patients compared with ET patients. There were no differences in alpha-synuclein expression between patients with PD and SP, and between patients with SP and ET.

When comparing alpha-synuclein expression in patients with autonomic dysfunction with patients without autonomic dysfunction, there was a trend toward higher alpha-synuclein expression in patients with autonomic dysfunction (P = 0.0548)

When evaluating the results of correlation analysis, we found that alpha-synuclein expression in skin biopsy specimens had a direct statistically significant moderate close relationship (r Spearman = 0.307; P=0.0237) with the stage of disease progression according to the Hoenh and Yahr scale. We found no correlation between alpha-synuclein expression intensity and disease duration.

Correlation analysis revealed no correlation between alpha-synuclein expression and severity of non-motor and motor manifestations on the MDS-UPDRS scale, level of daily activities on the Schwaab-Ingland scale, severity of cognitive disorders on the MoCA scale, anxiety on the HADS-1 scale and depression on the HADS-2 scale and disease duration.

In our study, we observed a trend toward higher alpha-synuclein scores (3) in older patients (Me 65; IQR 57.5 to 69.5;), whereas younger patients (Me56; IQR41.5 to 63.2; P = 0.0571) showed low alpha-synuclein scores (2).

We found no differences in the MDS-UPDRS scale in PD patients with different expression levels of alpha-synuclein.

There was a trend toward higher alpha-synuclein expression (3) and (2) in PD patients on the Schwaab-Ingland scale (Me80; IQR 60.0-85.0) and (Me70; IQR 0.0-80.0) (P = 0.0542) respectively, meaning that the more daily activity patients had, the more pronounced the protein expression was.

Hoenh and Yahr scale scores (Me 1.25; IQR 1.0 to 2.0) were higher in patients with grade 3 alpha-synuclein expression than in patients with grade 2 alpha-synuclein expression (1.0; 0.0 to 1.5; P = 0.0273), that is, as the disease progressed, protein expression was more pronounced.

In our study, we classified the expression of alpha-synuclein in terms of qualitative assessment (is/is binary scores). The classification was based on control group analyses and statistical evaluation of normal values for pathological protein expression. The normal value was the intensity of the immunohistochemical response below the 95 confidence interval (CI) median in the controls. **The reference values for alpha-synuclein** expression in the epidermis and dermis were 0 points. The values presented represent the 95% CI for the median in the control group.

Immunohistochemical analysis of alpha-synuclein expression in biopsy samples of skin layers revealed the absence of alpha-synuclein in the epidermis, sweat glands, sebaceous glands, hair straightening muscles, and vessels. Expression of alpha-synuclein was observed only in the nerve endings of the dermal stroma.

Formation of risk classes of PD development

In order to develop an algorithm for early diagnosis of PD, a multifactorial prediction of the target variable – the "decision tree" was carried out. According to the results of the study, three influencing factors were identified, the combination of which determined the indicator "PD, yes": "Autonomic dysfunction", "Duration of the disease" and "Hyposmia".

Based on this method, 4 risk classes with risk levels from 20.0% to 92.1% were identified for the target indicator "PD, yes" with the help of three main influencing factors: "Autonomic dysfunction"; "Duration of the disease"; "Hyposmia". The highest

risk of 92.1% (n=89) was demonstrated by the indicator " PD, yes " in patients with the factor: "Autonomic dysfunction (yes)".

The lowest risk level of 20.0% (n=30) for the development of the indicator "PD, yes" was observed with a combination of factors: "Vegetative dysfunction (No)" and " Duration of the disease≥3.0". The predictive quality of this constructed model has a high level.

ROC analysis and predictive quality indicators of the constructed decision tree for the target indicator " PD, yes ". The AuROC value (0.85) indicated a high predictive quality of the simulated decision tree. When assessing the risk of ≥63.3%, a positive result was expected; otherwise, a negative result. Thus, a positive result was identified in 94.3% of cases and a negative result in 70.6% of cases.

Based on the decision tree method, we have developed a step-by-step algorithm for early diagnosis of PD.

CONCLUSIONS

Thus, on the basis of the results of our study we can draw the following conclusions:

1. As a result of clinical diagnosis using Brain Bank diagnostic criteria and MDS 2015 criteria, out of all investigated 169 patients with referral diagnosis of Parkinson's disease, this diagnosis was confirmed in 74% (125) cases. The remaining cases were clinically diagnosed as essential tremor in 20% (34), vascular parkinsonism in 4.7% (8), progressive supranuclear palsy, and dementia with Levy corpuscles in 1 case each.

2. Alpha-synuclein in skin biopsy of patients with Parkinson's disease was detected in 95.7% of cases, indicating a relationship between the development of Parkinson's disease and deposits of pathological alpha-synuclein in the skin of patients. The diagnostic test for detection of alpha-synuclein in the skin of PD patients has high sensitivity and specificity of 90.9% and 100% respectively, which allows to consider alpha-synuclein as a biomarker of Parkinson's disease. The results demonstrated a correlation of alpha-synuclein expression with bradykinesia (P=0.041*) and disease severity according to the Hen-Yar scale (r=0.307; P=0.0237).

3 On the basis of multifactorial forecasting, combinations of three influencing factors were identified: "Autonomic dysfunction (is)", "Disease duration < 3.0 years" and "Hyposmia (is)", in the presence of which the risk of developing Parkinson's disease ranges from 74.0% to 92.1%.

PRACTICAL RECOMMENDATIONS:

1. In order to improve the diagnosis and differential diagnosis of Parkinson's disease, it is necessary to conduct consultations and dynamic observation of patients in specialized offices / centers of degenerative diseases of the nervous system.

2. Detection of phosphorylated alpha-synuclein in the biopsy of patients' skin can consider alpha-synuclein as a biomarker in the diagnosis of Parkinson's disease.

3. In the initial treatment of patients with suspected PD, outpatient neurologists are recommended to use the "decision tree" diagram for the indicator "PD, is" based on a combination of three influencing factors: "Autonomic dysfunction (is)", "Disease

prescription < 3.0 years" and "Hyposmia (is)" and the developed a step-by-step algorithm for early diagnosis of PD.

4. The obtained results of scientific research can be used in the design of methodological recommendations for neurologists and in the educational program of the residency "Adult, children's neurology".