Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2018;43(2):305-311
DOI: 10.17826/cumj.341649

ARAŞTIRMA / RESEARCH

Serum neutrophil-lymphocyte ratios, C-reactive protein and sedimentation levels in Parkinson's disease

Parkinson hastalığında nötrofil/lenfosit oranları, C reaktif protein ve sedimantasyon hızları

Volkan Solmaz[1], Elmas Pekdaş Genç[2], Dürdane Aksoy[2], Betül Çevik[2], Semiha Gülsüm Kurt[2], İsmail Benli[3]

[1] Trakya University Faculty of Medicine, Department of Neurology. Edirne, Turkey
[2]Gaziosmanpasa University Faculty of Medicine, Department of Neurology. [3]Department of Biochemistry. Tokat, Turkey

Cukurova Medical Journal 2018;43(2):305-311

Abstract

Purpose: We aimed to investigate the systemic inflammation status by analyzing the Neutrophil/Lymphocyte (N/L) ratio, Erythrocyte Sedimentation Rate (ESR), and C - reactive protein (CRP) in patients with Parkinson's disease (PD).

Materials and Methods: 101 PD patients, and 60 healthy subjects of similar age and gender were include in this study. The demographic data and duration of the disease, the drugs, duration of usage of Parkinson drugs, Hoehn-Yahr stage were noted. After full neurological examination blood samples are collected and neutrophil, lymphocyte counts, high sensitive CRP, ESR levels are detected.

Results: The N/L ratio and the CRP levels were statistically higher in Parkinson's group. There was a statistically significant but weak positive correlation between the CRP levels and the N/L ratio with the disease duration. Similarly, there was a statistically significant but weak positive correlation between the CRP levels and the N/L ratios with Hoehn-Yahr stages. When the N/L rates of the Parkinson's patients were correlated with the CRP and the ESR levels separately, it was observed that there was a weak but statistically significant correlation between the CRP and the N/L ratio

Conclusion: N/L ratio, and CRP levels, which are very important indicators of peripheral inflammation, was higher in PD. Our findings suggest that these biochemical markers may have a predictive value for the diagnosis of PD.

Key words: Idiopathic Parkinson disease, neutrophil-lymphocyte ratio, CRP, peripheral inflammation.

Öz

Amaç: Parkinson hastalığı (PH) olan kişilerin kanlarında nötrofil/lenfosit (N/L) oranları, sedimentasyon hızı (ESR), C-reaktif protein (CRP) düzeylerini sağlıklı kişilerle karşılaştırarak değerlendirmeyi amaçladık.

Gereç ve Yöntem: Çalışmamıza benzer yaş ve cinsiyetten 101 PH ve 60 sağlıklı gönüllü katılımcı aldık. Hastaların sosyodemografik verileri, hastalık süreleri, kullandığı ilaçlar ve Hoehn-Yahr evreleri kaydedildi. Tam bir nörolojik muayeneden sonar hastaların kan örnekleri alınarak, nötrofil ve lenfosit sayılar, CRP ve ESR düzeyleri incelendi.

Bulgular:N/L oranları ve CRP düzeyleri sağlıklı kişilerle kıyaslandığında PH olanlarda istatistiksel olarak anlamlı şekilde yüksek olduğu görüldü, ayrıca N/L oranları ile CRP düzeyleri arasında istatistiksel olarak anlamlı ancak zayıf bir korelasyonun olduğu tespit edildi. Benzer şekilde CRP düzeyleri ile N/L oranları ve Hoehn-Yahr evreleri arasında istatistiksel olarak anlamlı ancak zayıf bir korelasyonun olduğu görüldü. Hasta grubunda N/L oranları ayrı ayrı CRP ve ESR ile korele edildiğinde sadece N/L ile CRP arasında istatistiksel olark anlamlı zayıf bir korelasyon vardı.

Sonuç: Çalışmamızda periferik inflamasyonun çok iyi birer göstergeleri olan N/L oranları ve CRP düzeylerinin PH' larında anlamlı olarak daha yüksek olduğunu tespit ettik. Bulgularımız bu markerların PH için tanısal değeri olabilceğini düşündürmektedir

Anahtar kelimeler: İdiopatik Parkinson hastalığı, nötrofil-lenfosit oranı, CRP, periferik inflamasyon.

Yazışma Adresi/Address for Correspondence: Volkan Solmaz, Trakya University Medical Faculty, Department of Neurology Edirne, Turkey E-mail address: solmaz.volkan85@gmail.com
Geliş tarihi/Received: 16.03.2017 Kabul tarihi/Accepted: 09.06.2017

INTRODUCTION

Parkinson's disease (PD) is one of the most common primary progressive movement disorder observed all over the world and accounts for 80% of Parkinsonism etiology[1]. Over the 65 years old population the incidence of Parkinson's disease is approximately 1%[2-4]. The basic mechanism of pathogenesis is the degeneration in the nigrostriatal pathway; however, it is unknown what exactly causes the degeneration in this area. Recent studies have reported increased inflammation could have an important role in the pathogenesis in this neurodegenerative disease[5,6].

In some experimental studies, it has been reported that systemically or directly injected neurotoxic molecules such as rotenone, lipopolysaccharides into substansia nigra in rats causes parkinsonism and decreased neuronal survival due to the inflammatory effects[7,8]. Moreover, some studies reported that in the early stages of Parkinson's disease, increased α-synuclein (α-syn) levels are observed in intestinal tissue[9-11]. As it is already known; α-syn plays an important role in histopathological diagnosis of PD. Furthermore, previous studies have shown that α-syn inclusions are related to increased inflammation[12,13], therefore; increased inflammation (occurs) not only in peripheral nervous system but also in central nervous system too.

A new study on this topic reported that neutrophil/lymphocyte (N/L) ratio (is higher in PD patients compared to healthy individuals) was higher in the group with PD than healthy controls[14]. Blood Neutrophil and lymphocyte counts and their ratios is a practical method used to determine the level of systemic inflammation in previous studies[15]; this parameter has been shown to be increased in neurodegenerative diseases like Alzheimer's disease and cerebrovascular diseases[16, 17]. C-Reactive protein (CRP) and Erythrocyte Sedimentation Rate (ESR) are also other practical methods indicating the level of the systemic inflammation.

In the light of these data, we aimed to investigate the systemic inflammation status by analyzing the N/L ratio, ESR, and CRP in patients with PD. In this wise, the possible association between diagnosis of early stage Parkinson's disease and N/L ratio which is a good indicator of the systemic inflammation will be revealed.

MATERIALS AND METHODS

101 patients diagnosed with PD, and 60 healthy subjects of similar age and gender, who were admitted to the neurology clinic in Gaziosmanpasa University, Faculty of Medicine, were included in our study. Local ethic committee approved our study (approvel number:13-KAEK-124).

After a full neurological examination, the diagnosis of PD was made according to UK Parkinson's Disease Brain Bank's criteria (UKPDBC), UKPDBC defines Parkinson' s as; bradykinesia with at least one of the: muscular rigidity, 4-6 Hz rest tremor, postural instability (not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction) and supportive prospective positive criteria (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 exclusion criterias) [18]. The demographic data, such as age, gender, height and weight of the individuals were recorded.

In addition, the duration of the disease, the drugs, duration of usage of Parkinson drugs, Hoehn-Yahr (H&Y) stage were noted, H&Y scale defined by Hoehn and Yahr examines Parkinson' s disease in 5 stage; 1- Unilateral involvement only usually with minimal or no functional disability, 2- Bilateral or midline involvement without impairment of balance, 3- Bilateral disease: mild to moderate disability with impaired postural reflexes; physically independent, 4- Severely disabling disease; still able to walk or stand unassisted, 5- Confinement to bed or wheelchair unless aided [19]. Active infection, tumor, chronic, systemic (rheumatic, cardiac and endocrine diseases) or other neurological diseases were exclusion criteria for our study. We collected the blood samples in tubes containing ethylene-diamine-tetra-acetic acid for the measurements of the white blood cells, hemoglobin, hematocrit, mean corpuscular volume, platelet, neutrophil, lymphocyte counts and ESR. The samples were collected at 08:00-12:00 a.m. and analyzed within 60 min after the collection by using a CELLDYN 3,700 Automated Hematology Analyzer (Abbott Laboratories, Abbott Park, IL, the USA). The high sensitivity CRP (hs-CRP) was measured in our hospital laboratory by the Immuno-Turbidometric

Method (Cobas 6000, Roche Diagnostics, Mannheim, Germany

Statistical analysis

All statistical analyses were conducted using SPSS for Windows version 20.0 (SPSS Inc. Chicago, IL, USA). Prior to performing calculations on the non-qualitative data, the Kolmogrov Smirnov test was used to determine the conformity with the normal distribution. The Chi-Square test was used to compare the qualitative data, including the symptoms and the results of the physical examination. The nonparametric "Mann Whitney U" and "Kruskal-Wallis" tests were used, while the parametric "Student *t*-test" and "Unilateral Variance Analysis" (ANOVA) and Post-Hoc Tukey tests were used. The results are presented as average±standard deviation for numeric values, and as "n" and "%" for the qualitative values. The values of $p<0.05$ were accepted as significant. The Spearman Correlation Analysis was used for the correlation of the continuous variables. The ROC analysis was used to evaluate the diagnostic performance of the, CRP and N/L variant.

RESULTS

The mean age of the patients was 66.43±10.9, 58 of them were male (57.4%) and 43 of them were female (42.6%). The mean age of the control group was 63.34±8.9, 35 were male (59.4%) and 25 were female (41.6%), the average duration of Parkinson's disease were 6,21 years (max:25, min:1 years) the other sociodemographic characteristics of groups are shown in table 1. There were no significant difference between the two groups in terms of age and gender ($p>0.05$). Taken into account for comparison the N/L ratio, white blood cells, platelets, eosinophil, basophil, hemoglobin, ESR, and the CRP values were compared between the patients and the healthy subjects, the N/L ratio and the CRP levels were statistically higher in Parkinson's group ($p = 0.0001$, $p = 0.04$).

There were no significant differences in white blood cells, platelets, eosinophil, basophil, hemoglobin and ESR between patient and control groups (Table 1). There was a statistically significant but weak positive correlation between the CRP levels and the N/L ratio with the disease duration. Similarly, there was a statistically significant but weak positive correlation between the CRP levels ($p=0.04$ and $r=0.38$) and the N/L ratios ($p=0.038$, $r=0.27$) with Hoehn-Yahr stages. When the N/L rates of the Parkinson's patients were correlated with the CRP and the ESR levels separately, it was observed that there was a weak but statistically significant correlation between the CRP and the N/L ratio ($p=0.034$, $r=0.24$); and no correlation was observed between the N/L ratio and the ESR levels ($p=0886$, $r=0.015$) (Table 2). The mean BMI of the Parkinson's and control groups were respectively 26.74 ± 3.03, and 25.82 ± 3.1. There was no significant relationship between the patient and the control group in terms of BMI values ($p=0.066$). There were no statistically significant correlation between BMI and CRP values ($p = 0.364$, $r = 0.091$), ESR levels ($p = 0.088$ $r = 0.171$), N/L ratios ($p = 0649$, $r = 0.046$), Hoehn-Yahr stage ($p = 0.345$, $r = 0.95$) and disease duration ($p = 0.169$, $r = 0.138$).

29 Parkinson's patients were using monotherapy, 16 of whom used levodopa+benserazside; 11 patients were using dopamine agonists (pramipeksol, ropirinol, piribedil), 2 patients were using monoamine oxidase-B inhibitors (MAOI) (rasajilin). 13 patients were using triple combination therapy (MAOI, Levodopa+benserazide and dopamine agonists). 41 patients were using levodopa+benseraside and dopamine agonists. 8 patients were using levodopa+ benserazide and MAOI. 3 patients were using MAOI and dopamine agonists. There were no statistically significant differences between patients receiving combination (two and three medications) and monotherapy in terms of N/L ratios (Table 3). When the duration of using levodopa+benserazide and dopamine agonists combination (n=41) was compared with the CRP levels and the N/L ratio, there were no correlations between them. In our study, the ROC curve analysis showed that when the diagnostic performance of the N/L ratios was evaluated, over 2.39 values was classified as Parkinson's Disease with 65% sensitivity and 75% specificity ($AUC=0.714$; $p<0.001$) and CRP values above 8.7 is classified as PD with 65% sensitivity and 70% specificity ($AUC=0.683$; $p<0.0001$) (Figure 1).

Table 1. General characteristics of the groups

Characteristics	Idiopathic Parkinson's Disease (n=101)	Control (n=60)	*p* value
Age (years)	66.43 ±10.9	63.34±8.9	0.33
Gender (female/male)	43/58	25/35	0.54
BMI (kg/m²)	26.74±3.03	25.82±3.1.	0.066
e-SR (mm/hour)	13.19	10.85	0.302
c-RP (mg/L)	10.89	4.65	0.04
N/L	3.07	2.17	0.0001
Neutrophil (K/UL)	4.75	4.47	0.183
Lymphocyte(K/UL)	1.173	2.27	0.0001
Plt (10ʌ3µL)	240.28	248.55	0.161
Eosinophil (K/UL)	0.17	0.19	0.754
Basophil (K/UL)	0.56	0.54	0.810
Hgb (gr/dl)	12.8	13.1	0.328
H&Y stage	2.24		

BMI: Body Mass Index; e-SR: erythrocyte-Sedimentation Rate; c-RP: c-Reactive Protein; N/L: Neutrophil/Lymphocyte; Plt: Platelet; Hgb: Hemoglobin; H&Y: Hoehn-Yahr; gr:gram; dl; deciliter; mm: millimeter; mg: milligram; µL:microliter; kg:kilogram; m²: square meters

Table 2.Correlation results of some variables with neutrophil/lymphocyte ratios in Parkinson's group

Correlation with N/L	r	p
Duration of Parkinson's	**0.204**	**0.041**
Hoehn Yahr stage	**0.27**	**0.038**
e-SR	0.004	0.968
c-RP	**0.24**	**0.034**

N/L; neutrophil/lymphocyte ratios. e-SR; erythrocyte sedimentation rate. c-RP; C reactive protein. r=correlation coefficient. p= significance values.

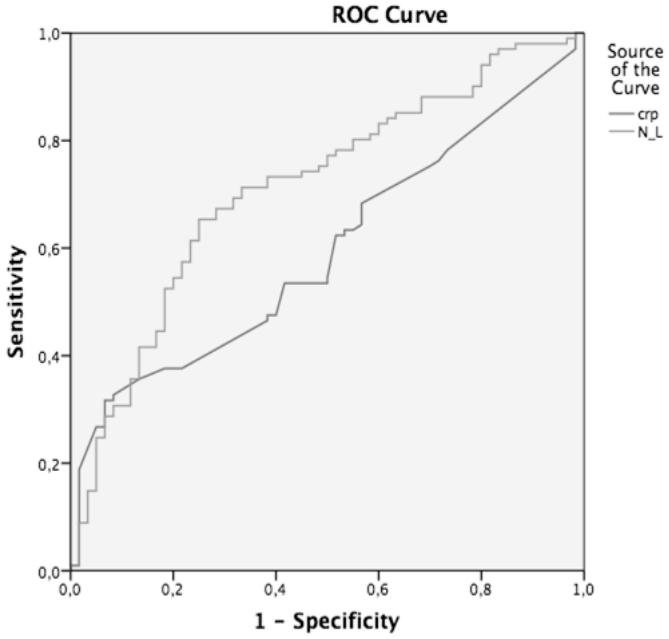

Figure 1. The ROC curve analysis of the N/L Ratio and the CRP levels for the prediction of Parkinson's Disease

Table 3. The N/L ratios of the treatment groups (monotherapy or polytherapy)

Treatment	Treatment	p	n	F
Only LD	Only D-agonist	0.887	11	1.561
	Only MAO	0.799	2	
	LD+D-agonist	1.000	41	
	LD+MAO	0.656	8	
	MAO+D-agonist	0.785	3	
	LD+MAO+D-agonist	1.000	13	

D-agonist: Dopamine Agonist; MAO: Monoamine Oxidase Inhibitor; LD: Levodopa.

DISCUSSION

We found that the N/L ratio and CRP levels, which are very good indicators of systemic inflammation, may have a predictive value for Parkinson's disease. In addition, we have detected another important finding that there was a statistically significant positive correlation between the N/L ratio and the CRP levels with the duration of the disease and the Hoehn-Yahr stages. ESR, another indicator of inflammation, was found to have no relationship either with Parkinson's disease or with N/L ratios.

A great deal of studies in recent years has reported that PD was associated with systemic inflammation. Akıl et al. reported that the N/L ratio, ESR, CRP and high sensitive Carcinoembryonic Antigen (CEA) levels were found to be high in 51 Parkinson's patients, and they also reported that the cut-off value for the N/L ratio was 2.25[14].

In our study, we found that there was a relationship between the PD, CRP levels and the N/L ratio, which is consistent with this study; however, we did not detect any association between the PD and ESR values. As a difference from Akıl et al.'s study, we have more than two-fold number of patients than this study, in addition, we also compared the N/L ratios with drug using and duration, but we could not find any relationship. The N/L ratio is used frequently in neurological diseases owing to being a good indicator of systemic inflammation. Rembach et al. found that the N/L ratio was higher in 18th, 36th, 54th months in Alzheimer disease, and they did not find any relationship between the N/L ratio and age, gender, and APOE ε4 allele. In addition, there was no cross-sectional relationship between the cortical amyloid burden and the N/L ratio[15]. According to Kuyumcu et al. [16], the N/L ratio was higher than the healthy subjects in Alzheimer's patients. Köklü et al. showed that the N/L ratio was reported to be an independent risk factor for symptomatic and asymptomatic carotid artery stenosis[17]. We found that the predictive value of the N/L ratio is possibly important for the diagnosis of Parkinson's disease, which is the most common movement disorder.

CRP which is produced by the liver and the adipose tissue is commonly used in clinical practice as an acute or chronic inflammation biomarker. The relationship between the CRP and the PD was investigated in previous studies. De Farias et al. reported that the CRP levels were higher in PD than healthy subjects; they also reported that the Superoxide Dismutase (SOD) and Malone Dialdehyde (MDA) levels which are lipid peroxidation biomarkers were also significantly different from the control group. Whereas PD drugs do not affect the stress of the reactive oxygen and nitrogen species (ions), and levodopa + carbidopa combinations were found to increase the CRP levels in PD[20]. In our study there were no correlation between duration of levodopa+benserazide and dopamine agonists combination usage (n=41) and CRP, N/L ratios. In the other mono or polytherapy groups we could not made correlation analyses because there were not enough patients for powerful statistical analyses. Umemura et al. conducted a retrospective study and found out that the CRP level was associated with the motor function during 5 different follow-up periods in PD patients; and reported that this relationship was independent from drug usage and gender[21]. Another retrospective study by same research group revealed that the CRP levels were independent from the disease duration and the incidence of the disease-related mortality[22]. Song et al. found CRP levels are higher in PD patients [23]. Similar to this study, the CRP levels were higher in PD group, and higher CRP levels showed a positive correlation with the disease duration and the Hoehn-Yahr stages in our study. In addition, a positive correlation was found between the serum CRP levels and the N/L ratios, which were independent of age and gender. We have not identified any association between the CRP

levels and the N/L ratios in patients taking levodopa+benserazide. These data are in accordance with the study of Hassin-Bauer et al., who did not identify any correlation between the CRP levels and the levodopa dosage[24]. But it is not possible to claim that there is no relationship between the CRP, N/L ratios and drug usage, due to inadequate methodological methods.

Our study has some limitations, we are unable to assess the causal relationship between drug usage N/L ratios and CRP levels due to the heterogeneous distribution of the drug used. Consequently in our study, we found that the N/L ratio, and CRP levels, which are very important indicators of peripheral inflammation, was higher in PD. Our findings suggest that these biochemical markers may have a predictive value for the diagnosis of PD. However, further studies must be performed prospectively with large number of participants.

As a result we found that the N/L ratio, and CRP levels, which are very important indicators of peripheral inflammation, was higher in PD. Our findings suggest that these biochemical markers may have a predictive value for the diagnosis of PD.

REFERENCES

1. Evlice AT, Aslan K, Bozdemir H, Demirkiran M, Unal I, Bicakcı S. Nörolojik hastalıklarda özürlülük. Cukurova Medical Journal. 2014;39:566-71.
2. Fahn S PS. Parkinsonism. In Merritt's Textbook of Neurology, 10th edition (Ed LP Rowland). Philadelphia, Lippincott Williams Wilkins, 2000
3. Watts RL, Koller WC. Movement Disorders: Neurologic Principles and Practice, 2nd edition. New York, McGraws Hill, 1997.
4. Adams RD VM, Ropper AH. Principles of Neurology, 6th Ed. New York, NY, McGraw-Hill. 1997.
5. Li JY, Ma SS, Huang QY, Li MT. The function of neuroinflammation in Parkinson disease. Sheng Li Ke Xue Jin Zhan. 2015;46:175-9.
6. Lopez Gonzalez I, Garcia-Esparcia P, Llorens F, Ferrer I. Genetic and transcriptomic profiles of inflammation in neurodegenerative diseases: Alzheimer, Parkinson, Creutzfeldt-Jakob and Tauopathies. Int J Mol Sci. 2016;17:86-7.
7. Erbas O, Cinar BP, Solmaz V, Cavusoglu T, Ates U. The neuroprotective effect of erythropoietin on experimental Parkinson model in rats. Neuropeptides. 2015;49:1-5.
8. Blesa J, Phani S, Jackson-Lewis V, Przedborski S. Classic and new animal models of Parkinson's disease. J Biomed Biotechnol. 2012;2012:845618.
9. Devos D, Lebouvier T, Lardeux B, Biraud M, Rouaud T, Pouclet H et al. Colonic inflammation in Parkinson's disease. Neurobiol Dis. 2013;50:42-8.
10. Villaran RF, Espinosa-Oliva AM, Sarmiento M, De Pablos RM, Arguelles S, Delgado-Cortes MJ et al. Ulcerative colitis exacerbates lipopolysaccharide-induced damage to the nigral dopaminergic system: potential risk factor in Parkinson`s disease. J Neurochem. 2010;114:1687-700.
11. Shannon KM, Keshavarzian A, Mutlu E, Dodiya HB, Daian D, Jaglin JA et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. Mov Disord. 2012;27:709-15.
12. Gustot A, Gallea JI, Sarroukh R, Celej MS, Ruysschaert JM, Raussens V. Amyloid fibrils are the molecular trigger of inflammation in Parkinson's disease. Biochem J. 2015;471:323-33
13. Lawand NB, Saade NE, El-Agnaf OM, Safieh-Garabedian B. Targeting alpha-synuclein as a therapeutic strategy for Parkinson's disease. Expert Opin Ther Targets. 2015;19:1351-60.
14. Akil E, Bulut A, Kaplan I, Ozdemir HH, Arslan D, Aluclu MU. The increase of carcinoembryonic antigen (CEA), high-sensitivity C-reactive protein, and neutrophil/lymphocyte ratio in Parkinson's disease. Neurol Sci. 2015;36:423-8.
15. Rembach A, Watt AD, Wilson WJ, Rainey-Smith S, Ellis KA, Rowe CC et al. An increased neutrophil-lymphocyte ratio in Alzheimer's disease is a function of age and is weakly correlated with neocortical amyloid accumulation. J Neuroimmunol. 2014;273:65-71.
16. Kuyumcu ME, Yesil Y, Ozturk ZA, Kizilarslanoglu C, Etgul S, Halil M et al. The evaluation of neutrophil-lymphocyte ratio in Alzheimer's disease. Dement Geriatr Cogn Disord. 2012;34:69-74.
17. Koklu E, Yuksel IO, Arslan S, Bayar N, Cagirci G, Gencer ES et al. Is elevated neutrophil-to-lymphocyte ratio a predictor of stroke in patients with intermediate carotid artery stenosis? J Stroke Cerebrovasc Dis. 2016;25:578-84.
18. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry. 1992;55:181-4.
19. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology. 1967;17:427-42.
20. de Farias CC, Maes M, Bonifacio KL, Bortolasci CC, de Souza Nogueira A, Brinholi FF et al. Highly specific changes in antioxidant levels and lipid peroxidation in Parkinson's disease and its progression: Disease and staging biomarkers and new drug targets. Neurosci Lett. 2016;617:66-71.
21. Umemura A, Oeda T, Yamamoto K, Tomita S, Kohsaka M, Park K et al. Baseline plasma c-reactive protein concentrations and motor prognosis in Parkinson disease. PloS One. 2015;10:0136722.

22. Sawada H, Oeda T, Umemura A, Tomita S, Kohsaka M, Park K et al. Baseline c-reactive protein levels and life prognosis in Parkinson Disease. PloS one. 2015;10:0134118.
23. Song IU, Cho HJ, Kim JS, Park IS, Lee KS. Serum hs-CRP levels are increased in de Novo Parkinson's disease independently from age of onset. Eur Neurol. 2014;72:285-89.
24. Hassin-Baer S, Cohen OS, Vakil E, Molshazki N, Sela BA, Nitsan Z et al. Is C-reactive protein level a marker of advanced motor and neuropsychiatric complications in Parkinson's disease? J Neural Transm (Vienna). 2011;118:539-43