

Study of pTau217 and GFAP Levels in Iraqi Male and Female Parkinson's Patients

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Abstracts

Background: Parkinson's disease is a neurological condition that strikes an individual and gradually progresses, resulting in both motor and non-motor symptoms. The severity and progression of can be predicted by biochemical biomarkers, such as glial fibrillary acidic protein and degradation products, which are released into the cerebrospinal fluid and serum when astrocytes are injured. Astrocytic injury is suspected based on elevated levels of and its degradation products, which are indicative of neurodegenerative diseases. is a neurodegenerative disorder characterized by the accumulation of alpha-synuclein. Accumulating evidence suggests that Tau protein plays a major role in the pathological mechanisms of Parkinson's disease.

Objectives: This study aimed to evaluate the levels of Tau and human glial fibrillary acidic protein GFAP in patients with Parkinson's disease PD and subsequently compare them with those in the control group.

Methods: This case-control study included 80 participants. This included 40 patients and 40 normal healthy controls with their gender distribution. Subsequently, the 80 participants were divided into four categories: G1, Control Male n=20; G2, Control Female n=20; G3, Parkinson Male patients n=20; and G4, Parkinson Female patients n=20. An enzyme-linked immunosorbent assay was used to ascertain the quantities of pTau217 and GFAP. Total calcium is measured more often because it is easier and widely available, whereas ionized calcium requires special handling and equipment. In most patients, total calcium levels provide sufficient clinical information. Total calcium levels were measured using automated clinical chemistry analyzers. Statistical significance was defined as a P-value of 0.05 or less. Receiver operating characteristic (ROC) experiments were conducted on pTau217 and GFAP.

Results: Results showed a significant decrease in 25-hydroxyvitamin D in the patient groups compared to the control groups. In addition, G3 showed a significant (P-value 0.002) decrease compared to G1. G4 showed a significant decrease compared to G2 (P-value <0.001). The results also showed a significant decrease in total calcium levels in G3 compared to G1 (P-value <0.001), while a non-significant decrease was found in G4 compared to G2 (P-value = 0.025). Results showed a significant increase in pTau217 and GFAP in G3 and G4 compared to G1 and G2 (P-value <0.001).

Conclusions: Determining vitamin D levels in patients with Parkinson's disease is important because deficiency is common and may worsen bone health, muscle strength, balance, and overall function. Testing allows appropriate supplementation and prevention of complications, particularly falls and fractures. The pTau217 and GFAP biomarkers could open new prospects for early diagnosis, monitoring the course of the disease, and personalized treatment strategies.

Keywords: Biomarker, calcium, GFAP, Parkinson's Disease, pTau217, vitD3.

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Introduction

Parkinson's disease (PD) is the second most common neurological illness, affecting over 6 million people globally. The biggest risk factor for PD is aging. It is a strange fact that males are afflicted with this condition at a higher rate than females. Intraneuronal inclusions in the form of Lewy bodies and Lewy neurites, as well as cell loss in the substantia nigra and other brain areas, are the pathogenic features of Parkinsonism. Aggregated and misfolded α -synuclein species are the main constituents of Lewy bodies. PD is identified as a synucleinopathy (1,2).

While indirect immunohistochemistry confirmation for Tau inclusion has not yet been observed, direct immunological chemical tests of partly purified Lewy bodies have shown human phosphorylated (pTau217) inclusions in nigral neurons (3). However, several studies have provided mounting evidence of tau pathology in the brains of individuals with PD. Genomic research has also linked the microtubule-associated protein Tau (MAPT) to the sporadic form of PD (4,5).

The astrocyte marker human glial fibrillary acidic protein (GFAP) has been shown to be expressed at higher levels in the elderly brain and in response to brain injury or degeneration of the Central Nervous System (CNS). It is highly recommended that the relevant literature be re-examined, leading to an in-depth discussion as to how this early work may relate to a particular isoform or to GFAP in general, given the discovery of distinct isoforms that may have distinct functions. Transcriptomics has improved our understanding of gene transcription regulation, RNA splicing, protein function, and their impact on mRNA and protein expression in both healthy and adult brains, as well as in illness (6).

The 25-hydroxyvitamin D is a class of fat-soluble ketosteroids that can be found in certain foods or synthesized in the dermis when exposed to sunlight. After 25-hydroxyvitamin D is metabolized in the liver, it is further broken down by the kidneys and other organs into its active form, 25-hydroxyvitamin D, also known as calcitriol (7). While this was initially identified as a component linked to total calcium homeostasis and metabolism, it has now been linked to autoimmune disorders and neurodegenerative illnesses, including PD (8). The rationale for this study was the need for reliable biomarkers for the early detection of Parkinson's disease. Therefore, this study was designed to evaluate GFAP and pTau217 as potential biomarkers for PD and provide an overview of the evidence regarding their utility. Additionally, it aimed to compare the levels of these parameters between male and female patients with PD.

Patients and Methods

Samples will be collected from patients attending Baquba Teaching Hospital between Nov. 2023 and Apr. 2024. This study represents a case-control study.

The subjects were four groups with a total of 80 subjects. The study included 40 patients and 40 healthy controls with similar sex distribution. G1, Male Control n=20; G2, Female Control n=20; G3, male Parkinson's patients n=20; and G4, female Parkinson's patients n=20. Age ranged from (25 to 65) in all study groups. The inclusion criteria were a confirmed diagnosis of Parkinson's disease according to the UK Parkinson's Disease Society Brain Bank criteria. The exclusion criteria were the presence of other neurodegenerative disorders and autoimmune diseases.

The ages of the subjects ranged from 25 to 65 years. Each patient was evaluated through a face-to-face interview using a structured questionnaire which comprised their detailed medical history including certain direct questions. All patient cases were diagnosed by hospital doctors. Five milliliters of venous blood were drawn from every participant. The concentrations of serum 25-hydroxyvitamin D [25(OH)D], GFAP, and Tau were detected using the ELISA method (with kits from the USA, América, My Bio Source). Total calcium is measured more often because it is easier, and widely available, while ionized calcium requires special handling and equipment. In most patients, total calcium provides sufficient clinical information. Total calcium levels were measured by automated clinical chemistry analyzers, by using kits from LINEAR company, Spanish.

Statistical Analysis: Version 26 of the Statistical Package for Social Sciences (SPSS) was used to analyze the data. The information is displayed as mean, and standard deviation. Percentages and frequencies were used to display categorical data and tested using the Chi-squared test. Differences between two-group means were compared using the independent sample *t*-test. All analyzed parameters using Pearson's coefficient (*r*) relation were explained. A *P*-value of less than 0.05 was regarded significant. Receiver operating characteristic ROC curve analysis was performed to evaluate the diagnostic performance of GFAP AND pTau217.

Results

The results showed a significant decrease in 25-hydroxyvitamin D in patient groups compared to the control groups. In addition, G3 (19.35 ± 1.78 ng/mL) showed a non-significant (*P*-value 0.002) decrease compared to G1 (27.75 ± 2 ng/mL). While G4 (19.05 ± 1.75 ng/mL) showed a significant decrease compared to G2 (30.75 ± 1.88 ng/mL) (*P*-value <0.001).

Results also showed a significant decrease in total calcium levels in G3 (9.03 ± 0.16 mg/dL) compared to G1 (9.03 ± 0.16 mg/dL), (*P*-value <0.001) while non-significant decrease found in G4 (7.49 ± 0.2) comparing to G2 (8.81 ± 0.20 mg/dL), (*P*-value 0.025).

Table 1: Levels of 25-hydroxyvitamin D and total calcium in study groups

Parameter	G1	G2	G3	G4	P-value G1&G3	P-value G2&G4
25-hydroxyvitamin D (ng/mL)	27.75 ± 2	30.75 ± 1.88	19.35 ± 1.78	19.05 ± 1.75	0.002*	<0.001**
Total calcium (mg/dL)	9.03 ± 0.16	8.81 ± 0.20	7.25 ± 0.27	7.49 ± 0.2	<0.001**	0.025*

G1: Control Males, G2: Control Females, G3: Parkinson Males, G4: Parkinson Females. ** significant (P-value < 0.001).

Table (2) presented levels of pTau217 and GFAP in all studied groups. Results showed a significant increase in pTau217 and GFAP in G3 and G4 compared to G1 and G2 (PP-value <0.001), respectively. Results were (4.05 ± 0.11 pg./mL) and

(3.98 ± 0.11 pg./mL) in G1 and G2 for pTau217 and (10.5 ± 0.33 pg./mL) while (11.14 ± 0.31 pg./mL) for G3 and G4. The GFAP levels were (1.39 ± 0.06 pg./mL) and (1.4 ± 0.04 pg./mL) for G1 and G2.

Table 2: Tau and GFAP Levels in all studied groups.

Parameter	G1	G2	G3	G4	P-value G1&G3	p-value G2&G4
Tau (pg./mL)	4.05 ± 0.11	3.98 ± 0.11	10.5 ± 0.33	11.14 ± 0.31	<0.001**	<0.001**
GFAP (pg./mL)	1.39 ± 0.06	1.40 ± 0.04	2.76 ± 0.06	2.86 ± 0.08	<0.001**	<0.001**

G1: Control Males, G2: Control Females, G3: Parkinson Males, G4: Parkinson Females. ** significant (P-value < 0.001).

Receiver Operating Characteristic (ROC) study for Tau and GFAP

Determination of the ROC curve for the Tau protein revealed 92.11% sensitivity and 90.37% specificity with an AUC value of 0.949 (P ≤ 0.001). In conclusion, these results validate Tau as an essential biomarker of neurofibrillary pathology in PD, and of its excellent discriminative power between patients with the disease and healthy controls. Increased Tau

levels reflect neurofibrillary pathology and duration of the disease. Lastly, GFAP demonstrated excellent diagnostic performance with a sensitivity of 94.28%, specifically 91.65%, and AUC of 0.974 (P ≤ 0.001). These findings are graphically represented in Table (3) and Figure (1). GFAP was one of the most sensitive parameters reflecting astrocytic activation and gliosis, in this investigation. It is robust in performance.

Table (3) Diagnostic performance of Tau, and GFAP based on ROC analysis

Parameter	Sensitivity (%)	Specificity (%)	Cut- value	Curve off	Area	Std. Error	P-value	Asymptotic 95% Confidence Interval	
								Lower Bound	Upper Bound
Tau (pg./mL)	92.11	90.37	10.12	0.949	0.021	<0.001	0.908	0.991	
GFAP (pg./mL)	94.28	91.65	3.07	0.974	0.014	<0.001	0.947	1.000	

*Significant diagnosis with (P value ≤ 0.001).

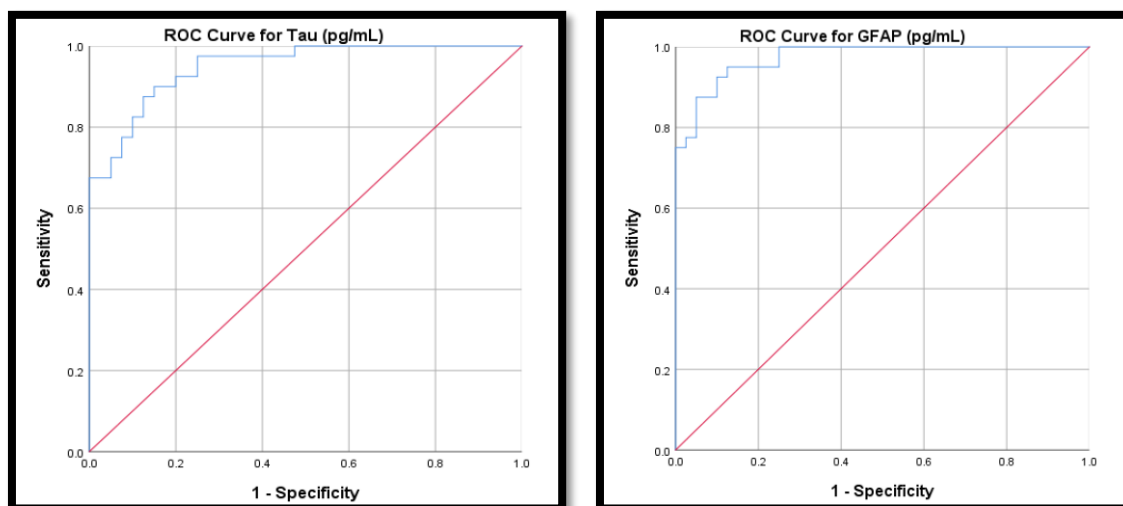


Figure (1) ROC curves of GFAP, and Tau for differentiating study groups.

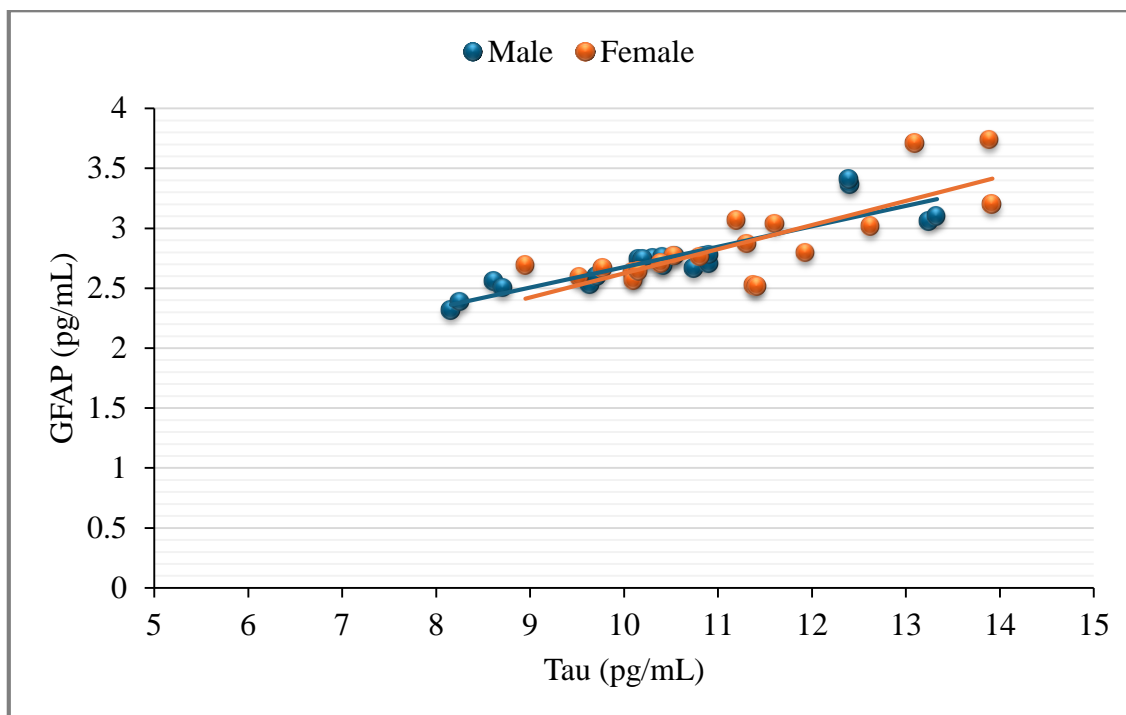


Figure 2: Correlation between Tau and GFAP Levels in Male and Female Parkinson's Disease Patients.

Table (4) Correlation of Tau with GFAP in the studied groups.

Parameter	GFAP (pg./mL)							
	G1		G2		G3		G4	
	r 1	P-value	r 2	P-value	r 3	P-value	r 4	P-value
Tau(pg./mL)	0.388	0.091	0.469	0.037*	0.894	<0.001**	0.793	<0.001**

G1: Control Male, G2: Control Female, G3: Parkinson Male, G4: Parkinson Female, r: Pearson correlation values, p: p-values, *non-Significant (P-value <0.05), Significant (P-value < 0.001).

The Tau versus GFAP levels in all male and female patients with PD are shown in Figure 2. There was a significant positive correlation in G2 and G1, with relation coefficients $r_2 = 0.469$ and $r_1 = 0.388$, and a high significant positive correlation coefficient for G4 ($r_4 = 0.793$) and G3 ($r_3 = 0.894$). These results show that Tau concentration is also positively related to GFAP between the sexes. The weaker association in females may indicate that there are differences between the sexes in the dynamics of biomarkers, which could reflect progression or underlying neuropathological pathways.

Discussion

The results of the present study show that the lack of 25-hydroxyvitamin D is more correlated with the status of neurodegenerative disease than with sex. Therefore, it is likely to be clinically beneficial to check the level of 25-hydroxyvitamin D in patients with PD. The revised data are, in turn, consistent with recent findings that have implicated low 25-hydroxyvitamin D levels in neurodegenerative pathologies through mechanisms involving calcium homeostasis, oxidative stress, and neuroinflammation (9).

Owing to PD patients being largely inactive, rigidity and bradykinesia, in addition to walking problems, might result in a decreased time of exposure to sunlight, leading to a decrease in the synthesis of 25-hydroxyvitamin D and, consequently, lower total

calcium absorption. The author reported a significant decrease in 25-hydroxyvitamin D levels for PD patients, compared to normal control subjects in the setting of this controversy (10). Moreover, the author suggested that the lack of dopaminergic neurons in the lower region of the brain stem and cortex may lead to disruption of control and coordination in swallowing among PD patients with PD. Clinical evidence has shown that dysphagia in patients with PD may result in serious complications, including dehydration, malnutrition, and deficiency of serum trace elements (e.g., calcium) (11).

The results of the current study revealed that Tau protein levels were highly elevated in PD compared to the control groups, indicating its presence as a hallmark component that can be used for discrimination. In accordance with the current study findings, a recent study indicated that plasma Tau is an attractive candidate biomarker for the differential diagnosis of distinct neurodegenerative diseases (12). The brain's multifunctional protein Tau, therefore, would appear to be one of the most important proteins linked to the pathology of PD (13). Tau hyperphosphorylation appears to play a major role in the abnormal aggregation and metabolism of Tau, with implications for neurodegeneration. This phosphorylation may occur at different sites. Therefore, the levels of some p-Tau isoforms may reflect the disease state or even predict it,

distinguishing PD from other neurodegenerative diseases (14, 15).

In this connection, the results of a recent study reached the same conclusion that higher blood p-Tau levels were related to PD. The extracellular vesicles of patients with PD at different stages were compared with those of sex- and age-matched healthy controls by the group of the authors. This group found p-Tau and amyloid beta to be significantly higher in vesicles from the PD group, as well as no difference for amyloid beta (16). The authors reported a similar study (N = 45 PD; 20 HC) with newly diagnosed patients with PD who were also assayed for plasma p-Tau using new immunoassay technology as well as GFAP. Alpha-synuclein plasma levels were the only levels that significantly differed between patients with PD and healthy controls (HC) (17).

The present findings can help broaden the understanding of the clinical use of plasma biomarkers in PD by investigating an expanded panel of common blood markers with a clinical follow-up (18,19). Recently, increasing focus has been placed on Tau and GFAP as promising prognostic markers in PD (20, 21).

Previous studies have found that GFAP is a marker of astrocytic activation and gliosis, which is more clearly related to neurodegenerative pathology. Since significantly higher GFAP levels in patients with PD than in controls make GFAP a potential biomarker for disease severity (22, 23).

Very recently, in 80 PD patients with mild cognitive impairment and 40 without) and 40 healthy subjects, the authors reported that increased plasma GFAP levels predicted the progression from preclinical to the clinical phase of PD at the three-year follow-up. The area under the curve of ROC was approximately 0.9. More broadly, the cohort observed that higher GFAP levels predicted PD versus PD with normal cognition, and PD with mild cognitive impairment as well as healthy controls. Increased GFAP was strongly associated with cognitive dysfunction in the Mini-Mental State Examination (measured by MMSE) (24). The same group is another study using both GFAP performing a composite ROC curve with AUC close to 0.86, to distinguish PD patients from other groups and healthy controls in their sample of 103 patients with PD and 37 with HC (25). The group also found that GFAP, could be used to differentiate PD patients from REM sleep phase disorder patients with an ROC curve AUC of 0.76. This group contained 109 patients and 37 controls (26).

Conclusions

Determining vitamin D levels in patients with Parkinson's disease is important because deficiency is common and may worsen bone health, muscle strength, balance, and overall function. Testing allows appropriate supplementation and prevention of complications, particularly falls and fractures. This study assessed GFAP and pTau217 levels as potential biomarkers for Parkinson's disease. Both GFAP and pTau217 levels were significantly elevated in patients

with Parkinson's disease compared to the control groups, and there were strong positive correlations between Tau and GFAP across all groups. ROC analysis demonstrated good diagnostic accuracy in distinguishing patients with PD from healthy controls. Notably, the correlations were stronger in men, suggesting possible sex-related differences in biomarker expression. These results support the potential utility of GFAP and pTau217 in the diagnosis and monitoring of PD. Further investigation in larger, multicenter cohorts is needed to confirm their role in the early detection and disease progression.

Authors' declaration

We confirm that all Figures and Tables in the manuscript belong to the current study. In addition, figures and images that do not belong to the current study have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's Approval-Ethical Clearance: The project was approved by the local ethical committee in (Baquba Teaching Hospital) according to code number (54) on (5/ 4/ 2023).

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Authors' contributions

Study conception and design: (R. M. Al-Azawee). Literature search: (R. M. Al-Azawee, A. H. Al-Qazzaz, A. M. Al-Saedy, Z. M. Al-Rubaei). Data acquisition: (R. M. Al-Azawee, A. H. Al-Qazzaz, A. M. Al-Saedy, Z. M. Al-Rubaei). Data analysis and interpretation: (R. M. Al-Azawee, A. H. Al-Qazzaz, A. M. Al-Saedy, and Z. M. Al-Rubaei). Manuscript preparation: (R. M. Al-Azawee A. H. Al-Qazzaz A. M. Al-Saedy, Z. M. Al-Rubaei). Manuscript editing and review: (R. M. Al-Azawee, Ammar H. Al-Qazzaz, A. M. Al-Saedy, Z. M. Al-Rubaei).

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دراسة مستويات GFAP و pTau217 لدى مرضى باركنسون العراقيين من الذكور والإناث
راند محمود العزاوي / وزارة التربية والتعليم / المديرية العامة للتربية والتعليم في محافظة ديالى
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الخلاصة:

خلفية البحث: مرض باركنسون هو حالة عصبية تتطور وتؤدي إلى ظهور أعراض حركية وغير حركية. ويمكن التنبؤ بشدة المرض وتطوره من خلال المؤشرات الحيوية الكيميائية، مثل البروتين الحمضي اللبني (GFAP). يُفرز بروتين GFAP ونواتج تحلله في السائل النخاعي الشوكي ومصل الدم عند إصابة الخلايا النجمية. ويُشير ارتفاع مستويات بروتين GFAP ونواتج تحلله إلى إصابة الخلايا النجمية، مما يدل على وجود مرض تنكسي عصبي. مرض باركنسون هو اضطراب تنكسي عصبي يتميز بتراكم بروتين ألفا-سينوكلين. وتشير الأدلة المتراكمة إلى أن بروتين تاو يلعب دورًا في الآليات المرضية لمرض الباركنسون.

الاهداف: تهدف هذه الدراسة الى تقييم مستويات GFAP و Tau في مرض باركنسون ومقارنتها لاحقًا بمستويات المجموعة الضابطة. **المرضى وطرق العمل:** الدراسة تمثل المجموعة المرضية والمجموعة الضابطة على 80 مشاركًا، شملت 40 مريضا و 40 شخصا سليما كمجموعة ضابطة. مع توزيعهم حسب الجنس بعد ذلك، قُسموا المشاركون إلى أربع مجموعات. المجموعة الأولى (G1): ذكور أصحاء ن=20، المجموعة الثانية (G2): إناث أصحاء ن=20، المجموعة الثالثة (G3): مرضى باركنسون من الذكور ن=20، والمجموعة الرابعة (G4): مرضى باركنسون من الإناث ن=20. استُخدم اختبار المقايسة المناعية الإنزيمية المرتبطة (ELISA) لتحديد كميات إنزيمي GFAP و pTau217 بقياس الكالسيوم الكلي بشكل أكثر شيوعًا لأنه أسهل ومتوفر على نطاق واسع، بينما يتطلب الكالسيوم المتأين معالجة ومعدات خاصة. يوفر الكالسيوم الكلي معلومات سريرية كافية لمعظم المرضى. وقد تم قياس مستويات الكالسيوم الكلي باستخدام أجهزة تحليل الكيمياء السريرية الآلية. يُعتبر الفرق ذا دلالة إحصائية عند قيمة P تساوي 0.05 أو أقل. أُجريت تجارب منحنى ROC على إنزيمي GFAP و pTau217. **النتائج:** أظهرت النتائج انخفاضًا ملحوظًا في مستوى 25-هيدروكسي فيتامين د لدى مجموعات المرضى مقارنةً بمجموعات الضابطة بالإضافة إلى ذلك، لوحظ انخفاض غير دال احصائيا (P=0.002) في المجموعة G3 مقارنةً بالمجموعة G1، بينما أظهرت المجموعة G4 انخفاضًا دالا احصائيا مقارنةً بالمجموعة G2 (P<0.001) كما أظهرت النتائج انخفاضًا ملحوظًا في مستويات الكالسيوم الكلي في المجموعة في المجموعة G3 مقارنةً بالمجموعة G1 (P<0.001) بينما لوحظ انخفاض غير دال احصائيا في المجموعة G4 مقارنةً بالمجموعة G2 قيمة (P=0.025) . أظهرت النتائج زيادة ملحوظة في مستويات pTau217 و GFAP في المجموعتين G3 و G4 مقارنةً بالمجموعتين G1 و G2 قيمة (P<0.001) . **الاستنتاجات:** يُعد تحديد مستويات فيتامين د في مرض باركنسون أمرًا بالغ الأهمية، لأن نقصه شائع وقد يؤدي إلى تدهور صحة العظام وقوة العضلات والتوازن والوظائف العامة. كما يُنصح إجراء الفحص تناول المكملات الغذائية المناسبة والوقاية من المضاعفات، وخاصة السقوط والكسور. يمكن ان تفتح المؤشرات الحيوية لمستويات GFAP و pTau217 افاقا جديدة للتشخيص المبكر، ومراقبة مسار المرض، واستراتيجية العلاج.

مفتاح الكلمات: المؤشرات الحيوية، كالسيوم، GFAP، مرض باركنسون، pTau217، 25-هيدروكسي فيتامين. D3