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Circulatory 25(OH)D and 1,25(OH)$_2$D as differential biomarkers between multiple system atrophy and Parkinson's disease patients

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ABSTRACT

Background and purpose: There is sufficient evidence to support vitamin D's noncalcemic effects and the role of vitamin D deficiency in the development of a wide range of neurological disorders. This study aimed to evaluate whether serum 25(OH)D and 1,25(OH) 2 D could be used as biomarkers to differentiate between healthy subjects (HS), multiple system atrophy (MSA) and Parkinson's disease (PD) patients of both genders.

Methods: A total of 107 subjects were included in this study, divided into three groups: 1- HS ($n = 61$), 2- MSA patients ($n = 19$), and 3- PD patients ($n = 27$). The patients were assessed using UMSARS II, UPDRS III, H&Y, MMSE and MoCA rating scales. The levels of 25(OH)D and 1,25(OH) 2 D in serum were determined using the radioimmunoassay technique.

Results: The levels of 25(OH)D and 1,25(OH) 2 D in HS were 26.85 +/− 7.62 ng/mL and 53.63 +/− 13.66 pg/mL respectively. 25(OH)D levels were lower in both MSA and PD by 61% and 50%, respectively ($P = 0.0001$ vs. HS). 1,25(OH) 2 D levels were lower in MSA by 29%($P = 0.001$ vs HS). There was a correlation between 25(OH)D and 1,25(OH) 2 D in MSA and PD, but not in HS. 1,25(OH) 2 D regressed with MMSE ($\beta = 0.476$, $P = 0.04$, $R\ 2 = 0.226$) in MSA, and with UPDRS III ($\beta = -0.432$, $P = 0.024$, $R\ 2 = 0.187$) and MoCA ($\beta = 0.582$, $P = 0.005$,$R\ 2 = 0.279$) in PD. 25(OH)D displayed considerable differentiative strength between HS and MSA (Wald = 17.123, OR = 0.586, $P = 0.0001$; AUC = 0.982, sensitivity and Youden index = 0.882, $P = 0.0001$) and PD (Wald = 18.552, OR = 0.700, $P = 0.0001$; AUC = 0.943, sensitivity = 0.889, YI = 0.791, $P = 0.0001$). 1,25(OH) 2 D distinguished MSA from PD (Wald 16.178, OR = 1.117, P = 0.0001; AUC = 0.868, sensitivity = 0.926, Youden index =0.632, P = 0.0001). H&Y exhibited the highest sensitivity, AUC, and significant distinguishing power between MSA and PD.

Conclusions: Serum 25(OH)D and 1,25(OH) 2 D could be useful biomarkers for MSA and PD. 25(OH)D and H&Y provided the highest sensitivity and group classification characteristics.

1. Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases that affect the older population. Slowness of movement, resting tremor, rigidity, and postural instability are all symptoms of PD [1]. Interestingly, there appears to be a link between PD and low levels of vitamin D in the blood, and vitamin D supplement appears to help with PD treatment [2]. Genetic studies have suggested that Nurr1 gene [3], toll-like receptor [4], gene related to lipid disorders [5], vascular endothelial factor [6], tyrosine hydroxylase [7], and angiogenin [8] are all been involved as linkage between vitamin D deficiency and PD. Calcitriol (1,25-dihydroxycholecalciferol, 1,25(OH)$_2$D) modulates inflammatory cytokine expression and is used to treat PD [9,10].

Multiple system atrophy (MSA) is a sporadic, progressive, and fatal

Abbreviations: 25(OH)D, 25-hydroxyvitamin D_3; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D_3 (Calcitriol); H&Y, Hoehn &Yahr rating scale; MMSE, Mini mental state examination; MoCA, Montreal Cognitive Assessment; UMSARS, Unified MSA Rating Scale; UPDRS, Unified PD Rating Scale.; PD, Parkinson's disease; MSA, Multiple system atrophy.

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neurodegenerative atypical parkinsonian disorder characterized by oligodendroglial and neuronal synucleinopathy. Clinically, MSA is characterized by autonomic dysfunction, parkinsonism, cerebellar ataxia, and pyramidal signs in any combination. Parkinsonian features predominate the parkinsonian subtype (MSA-P), whereas cerebellar ataxia predominates in cerebellar type (MSA-C) [11–14].

The clinical signs of MSA and PD overlap, making differential diagnosis difficult. Both present with parkinsonism and autonomic dysfunction, as well as REM sleep disorder, cognitive impairment and depression [15]. Furthermore, there have been no biomarkers available to distinguish the two entities. There has been no comprehensive and comparative study, as to our knowledge, employing both 25(OH)D (Calcidiol) and 1,25$(OH)_2$D (Calcitriol) as biomarkers and associating them with various rating scales for evaluating MSA and PD patients. In this study, we assayed serum concentrations of 25(OH)D and 1,25$(OH)_2$D in MSA and compared them to those in healthy subjects (HS) and PD. To anticipate the correlation and regression a of 25(OH)D and 1,25$(OH)_2$D with the scaling systems used in this study, and to expose their possible biomarker characteristics to aid diagnosis, correlation, regression, and Receiver operating characteristic (ROC) were performed.

2. Patients and methods

2.1. Participants

A total of 107 individuals were enrolled in this study, including 61 HS controls, 19 MSA and 27 PD patients at the Fukuoka University Hospital in Fukuoka, Japan. The ethical permission was provided by the Ethical Committee of Fukuoka University Hospital (BIR No. 2018 M030). Table 1 shows the demographic characteristics of the patients as well as the duration of their illness. The study complies with the Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subject issued by the World Medical Association. Prior to being included in the study, a signed formal consent to participate in the study was obtained from each subject or relatives. The participants were assessed and evaluated clinically as in our previous study [16]. Diagnostic Criteria for MSA [12] and Movement Disorder Society Clinical Diagnostic Criteria for PD [17] were used to diagnose MSA and PD respectively. Hoehn & Yahr (H&Y), UPDRS III, PD staging and scoring instruments [18] and UMSARS part II, MSA [13] were used to evaluate the patients. All patients and controls were free from hepatic and renal dysfunction. Participants who were already taking vitamin D supplementation were not enrolled in the study.

Table 1
Demographic characters, serum 25(OH)D, 1,25$(OH)_2$D, and the classifier scales in MSA and PD patients.

	HS	MSA	PD
Total number(n)	n = 61	n = 19	n = 27
Women	*n* = 28	*n* = 10	*n* = 18
Men	*n* = 33	n = 9	*n* = 9
Age (year)	74.4 ± 7.7	61.3 ± 8.8	67.8 ± 6.6
Women	74.5 ± 6.2	58.9 ± 8.3	67.0 ± 7.7
Men	74.4 ± 8.6	63.9 ± 9.0	69.3 ± 3.6
Disease duration (month)	X	61.6 ± 42.6	85.6 ± 60.1
25(OH)D (ng/mL)	26.85 ± 7.62	10.53 ± $3.82^{0.0001}$	13.36 ± $4.76^{0.0001}$
1,25$(OH)_2$D (pg/mL)	53.63 ± 13.66	38.02 ± $13.41^{0.001}$	59.27 ± 15.08
UMSARS II/ UPDRS III	–	24.18 ± 8.98	24.00 ± 8.40
H&Y	–	3.79 ± 0.92	2.48 ± $0.58^{0.0001}$
MMSE	–	26.26 ± $3.33^{0.011}$	28.48 ± 1.42
MoCA	–	22.42 ± $3.7^{0.004}$	25.22 ± 2.98

Statistical significance values for 25(OH)D and 1,25$(OH)_2$D are for comparisons with HS; in the case of the classifier scales the comparisons are between MSA and PD. The comparisons for detecting gender-related differences between women and men HS, MSA and PD are given in the supple 1.
HS; Healthy subjects, MSA; Multiple system atrophy, PK; Parkinson's disease.
UMSARS: Unified MSA rating scale; UPFRS: Unified PD rating scale; H&Y: Hoehn and Yarn scale. MMSE:Mini-Mental State Examination; MOCA: Montreal cognition assay. Significance values are for PD vs. MSA.

2.2. Sample preparation

Before vitamin D assay, peripheral blood samples were collected, centrifuged at 1500 rpm for 20 min to obtain serum, and stored at −80 °C before vitamin D assay. The total serum concentration of 25(OH)D was determined using a radioisotope assay utilizing 25(OH)D ^{125}I RIA Kit (DiaSorin Inc. MN, USA) and a 1,25$(OH)_2$D RIA Kit (Immunodiagnostic systems Ltd., Boldon, England), according to the manufacturer's instruction.

2.3. Statistical analyses

Data on age, 25(OH)D and 1,25$(OH)_2$D concentrations were analyzed using one way ANOVA to detect differences among the groups (HS, MSA and PD), and examined for Linearity and homogeneity. When ANOVA revealed a significant difference, the data were subjected to Tukey's multiple comparisons post hoc to identify the source of the difference. In addition, two- way ANOVA was applied to examine significant differences in factors and between-groups interactions. Gender differences were also compared within each group. On the other hand, the rating scales were analyzed by the nonparametric Mann-Whitney-*U* test.

Spearman's correlation coefficient (r_s) was used to assess bivariate correlations. The linear regression analysis was conducted to evaluate the extent of influence of the independent predictors 25(OH)D, 1,25$(OH)_2$D, age, disease duration and gender on the dependent variables (MMSE, UMSARS, H&Y, and MoCA) in MSA and PD. The following parameters were calculated: unstandardized (USC) and standardized (SC) coefficients *B* and beta (β) respectively, R-squared (R^2). The predictors were tested with univariate and multivariate logistic regression analyses to assess the contribution of each predictor alone and in combination to classify the groups (HS vs. MSA and PD; MSA vs. PD). The followings were calculated: *B*: logistic regression coefficient, odd ratio (OR), correct classification accuracy rate and Wald value (significance of predictor contribution). Receiver operating characteristic (ROC) was applied to test the strength (area under ROC curve, AUC) and sensitivity of predictors' performance-dependent classification of groups. The following parameters were also calculated: specificity, and Youden index (YI). The results are presented as means ± SD except otherwise indicated. Statistical significance was defined as *p* values less than 0.05. All statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Serum 25(OH)D and 1,25$(OH)_2$D concentrations rating scales in PD and MSA patients

The respective mean serum concentrations of 25(OH)D and 1,25$(OH)_2$D were 26.85 ± 7.62 ng/ml and 53.63 ± 13.66 pg/mL in HS, 10.53 ± 3.82 ng/ml and 38.02 ± 13.41 pg/ml in MSA, and 13.36 ± 4.76 ng/ml and 59.27 ± 15.08 pg/ml in PD (Table 1). A significant difference for between groups was detected for 25(OH)D ($P = 0.0001$) and 1,25$(OH)_2$D ($P = 0.0001$). Two-way ANOVA did not detect any significant group x age or group x gender interaction. Post hoc comparison revealed that in the PD patients only 25(OH)D was lower ($P = 0.0001$) than HS (for 1,25$(OH)_2$D $P = 0.199$), whereas in MSA both 25(OH)D ($P = 0.0001$) and 1,25$(OH)_2$D ($P = 0.001$) were lower than HS (Fig. 1). The ratio of 25(OH)D: 1,25$(OH)_2$D was 500 in the HS, decreased to 278 in MSA and to 225 in PD. Fig. 1 further reveals that while there was no

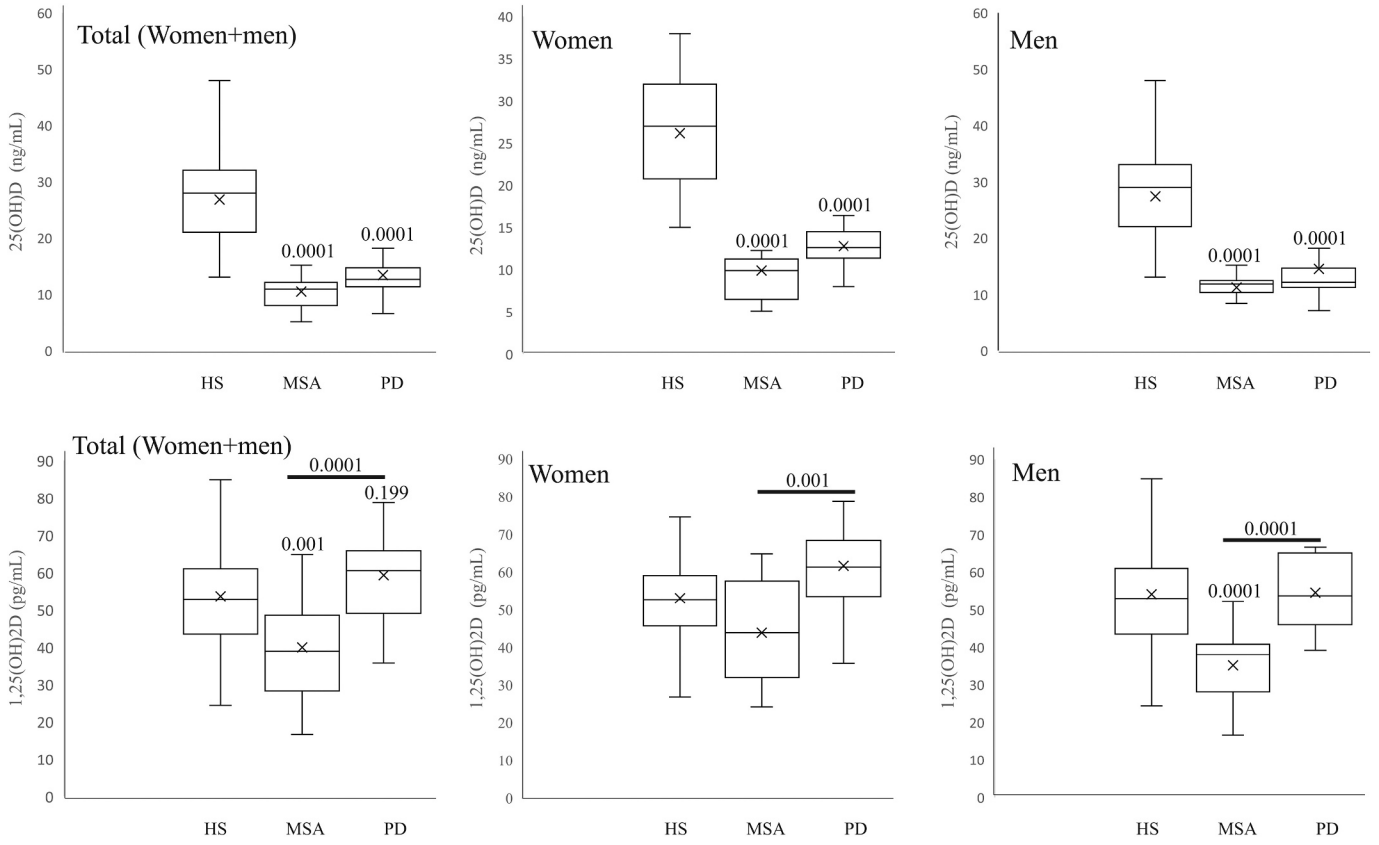

Fig. 1. Box plot of 25(OH)D and 1,25(OH)$_2$D values in women and men separately, as well as both genders together (Total). The 25th and 75th percentiles are indicated on the lower and upper edges of the boxes, respectively. The median and means are indicated by the horizontal lines and (x symbol) inside the boxes, respectively. The minimum and maximum values are shown by the bottom and upper whiskers, respectively. The levels of statistically significant comparison of each MSA and PD with HS are indicated over each point. For 25(OH)D, there was no significant difference between MSA and PD for each gender. In case of 1,25(OH)$_2$D, the significance values between MSA and PD are displayed over the horizontal bars. In addition, no significant differences in 25(OH)D and 1,25(OH)$_2$D between women and men were detected in each HS, MSA, and PD.

significant difference in 25(OH)D levels between MSA and PD, MSA had lower level of 1,25(OH)$_2$D ($P = 0.0001$). Furthermore, when the genders were separated, the results revealed that the level of 25(OH) in MSA and PD patients was lower than HS ($P = 0.0001$) in both women and men, but there was no significant difference between MS and PD in both genders. Only the level of 1,25(OH)$_2$D in men MSA was lower than that of HS ($P = 0.0001$). MSA had a lower level of 1,25(OH)$_2$D than PD in both women ($P = 0.001$) and men ($P = 0.0001$).

MSA and PD did not differ in terms of disease duration (62–85 months) or UMSARS II/UPDRS III (~24) scores. However, MSA patients' H&Y scores were greater than PD patients'($P = 0.0001$), although MSA patients had lower MMSE ($P = 0.011$) and MoCA ($P = 0.004$) scores than PD patients (Table 1). For 25(OH)D, 1,25(OH)$_2$D, and the evaluation scales within each group, there were no significant gender-dependent variations (supplementary 1).

When the groups within each gender were compared, it was found that in women there was a significant difference in 25(OH)D levels between HS and each of the MSA and PD ($P = 0.0001$). Furthermore, there were significant differences between MSA and PD in terms of 1,25(OH)$_2$D ($P = 0.001$), UMSARS II vs UPDRS III ($P = 0.05$), H&Y ($P = 0.0001$) and MoCA ($P = 0.009$). However, there were no significant differences in disease duration or MMSE.

Male patients, on the other hand, showed no significant differences in MMSE, UMSARS II vs UDPRS III or MoCA. However, there were significant differences in 25(OH)D and 1,25(OH)$_2$D between HS and each of MSA and PD ($P = 0.0001$), as well as for disease duration ($P = 0.042$) and H&Y ($P = 0.024$) between MSA and PD (supplementary 2).

3.2. Correlations and regressions among the predictors and outcomes

3.2.1. Correlations between serum 25(OH) D and 1,25(OH)$_2$D concentrations and the rating scales in HC, PD and MSA patients

In the HS, age had no significant correlation with 25(OH)D ($r_s = -0.029$; $P = 0.823$), but it did have significant negative correlation with 1,25(OH)$_2$D ($r_s = -0.308$; $P = 0.016$), indicating that 1,25(OH)$_2$D decreases with advanced age. Furthermore, only a nonsignificant negative correlation was found between 25(OH)D and 1,25(OH)$_2$D ($r_s = -0.234$; $P = 0.069$).

When the total values of both genders were analyzed in MSA patients, Fig. 2 shows that 25(OH)D was positively correlated with 1,25(OH)$_2$D, but negatively with UMSARS II and H&Y. On the other hand, 1,25(OH)$_2$D displayed positive correlation with MMSE. Fig. 2 further shows that 25(OH)D was significantly and positively correlated with 1,25(OH)$_2$D and negatively correlated with H&Y scores in the PD patients. The highest correlation of 1,25(OH)$_2$D was the positive correlation detected with MoCA scores, followed by negative correlation with UPDRS III. Furthermore, in both MSA and PD, there was no significant correlation between 25(OH)D and MMSE or MoCA. 1,25(OH)$_2$D was not significantly correlated with H&Y (in both MSA and PD) or with MMSE (in PD) (supplementary 3).

The correlation among the predictors were also evaluated in each disease group according to the gender. In women MSA patients, there was no significant correlation between 25(OH)D and other predictor, but there was a correlation between 1,25(OH)$_2$D and disease duration ($r_s = +0.778$; $P = 0.008$) and UMSARS II ($r_s = +0.727$; $P = 0.001$). However, 25(OH)D was positively correlated with 1,25(OH)$_2$D ($r_s =$

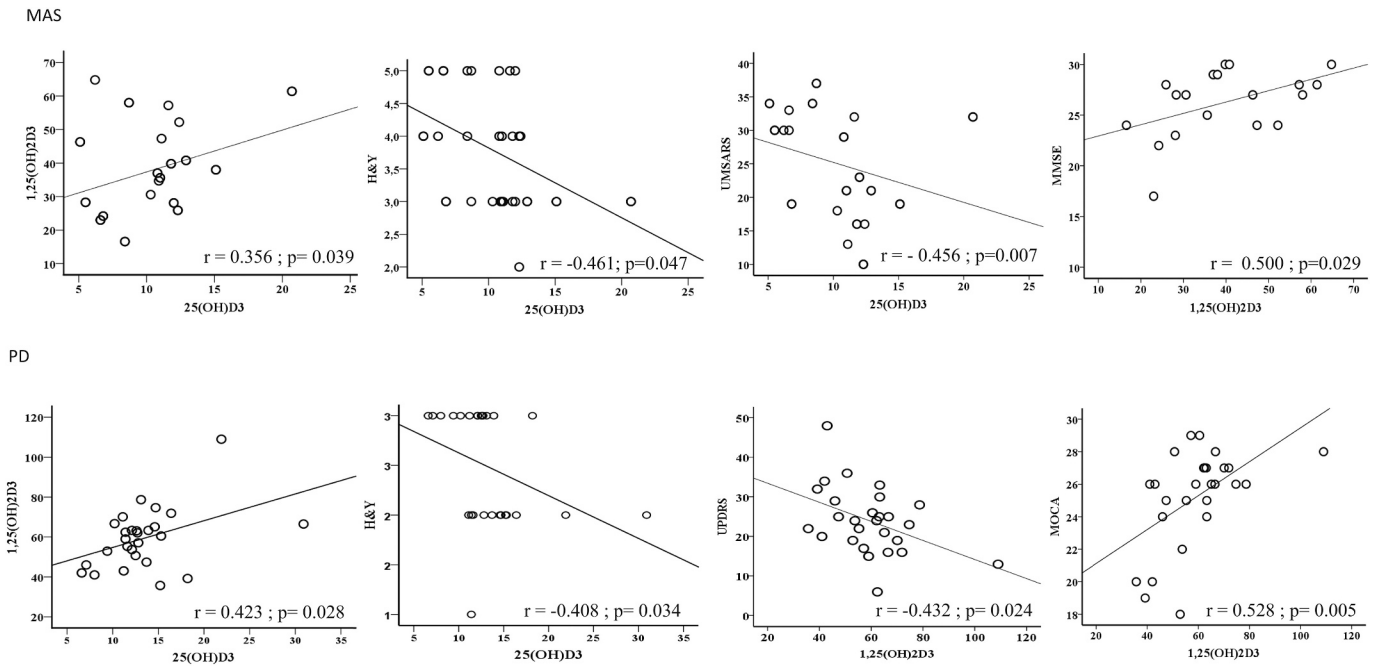

Fig. 2. Scatter plots depicting representative significant correlations of 25(OH)D and $1,25(OH)_2D$ with each other and with staging scales in MSA and PD patients. The correlation values are calculated using Spearman's correlation-tow tailed values.

0.684; $P = 0.005$) and MMSE ($r_s = +0.742$; $P = 0.022$) in men with MSA. Both 25(OH)D ($r_s = -0.723$; $P = 0.002$) and $1,25(OH)_2D$ ($r_s = -0.900$; $P = 0.0001$) were negatively correlated with UMSARS II. In PD patients, on the other hand, the only significant correlation detected in women was a positive correlation between 25(OH)D and $1,25(OH)_2D$ ($r_s = +0.721$; $P = 0.001$). Furthermore, there was no significant correlation

Table 2
Linear regression of dependent and independent variables in MSA and PD patients.

	Dependent outcome	Independent predictor	USC SC $B \pm$ SE	β	t-value	Sig.	R^2
MSA	MMSE	25(OH)D	0.278 ± 0.205	0.313	1.357	0.193	0.098
		$1,25(OH)_2D$	0.112 ± 0.05	0.476	2.229	0.04*	0.226
		Gender	−1.767 ± 1.515	−0.272	−1.166	0.26	0.074
		Disease duration	−0.014 ± 0.019	−0.179	−0.75	0.463	0.032
	UMSARS II	25(OH)D	−0.597 ± 0.355	−0.289	−1.681	0.103	0.084
		$1,25(OH)_2D$	0.15 ± 0.102	0.254	1.461	0.154	0.064
		Gender	−4.6 ± 2.725	−0.29	−1.688	0.101	0.084
		Disease duration	0.093 ± 0.041	0.482	2.268	0.03*	0.232
	H&Y	25(OH)D	−0.107 ± 0.053	−0.435	−1.993	0.063	0.189
		$1,25(OH)_2D$	0.005 ± 0.016	0.084	0.348	0.732	0.007
		Gender	−0.233 ± 0.43	−0.13	−0.542	0.595	0.017
		Disease duration	0.007 ± 0.005	0.345	1.514	0.148	0.119
	MOCA	25(OH)D	0.247 ± 0.232	0.25	1.064	0.302	0.062
		$1,25(OH)_2D$	0.071 ± 0.061	0.273	1.171	0.258	0.075
		Gender	−0.589 ± 1744	−0.082	−0.338	0.74	0.007
		Disease duration	−0.016 ± 0.021	−0.179	−0.752	0.462	0.032
PD	MMSE	25(OH)D	0.029 ± 0.06	0.098	0.494	0.626	0.01
		$1,25(OH)_2D$	0.011 ± 0.019	0.116	0.583	0.565	0.013
		Gender	0.444 ± 0.586	0.15	0.758	0.455	0.022
		Disease duration	0.004 ± 0.005	0.156	0.789	0.438	0.024
	UPDRS III	25(OH)D	−0.485 ± 0.339	−0.275	−1.428	0.166	0.075
		$1,25(OH)_2D$	−0.241 ± 0.101	−0.432	−2.398	0.024*	0.187
		Gender	6.167 ± 3.274	0.353	1.884	0.071	0.124
		Disease duration	0.061 ± 0.025	0.439	2.446	0.022*	0.193
	H&Y	25(OH)D	−0.043 ± 0.023	−0.354	−1.889	0.07	0.125
		$1,25(OH)_2D$	−0.011 ± 0.007	−0.296	−1.549	0.134	0.088
		Gender	0.278 ± 0.235	0.23	1.182	0.248	0.053
		Disease duration	0.003 ± 0.002	0.268	1.391	0.177	0.072
	MoCA	25(OH)D	0.1 ± 0.124	0.16	0.808	0.426	0.025
		$1,25(OH)_2D$	0.104 ± 0.034	0.528	3.11	0.005*	0.279
		Gender	−1.167 ± 1.218	−0.188	−0.958	0.347	0.035
		Disease duration	0.009 ± 0.01	0.173	0.879	0.388	0.03

B and β are the unstandardized (USC) and standardized (SC) coefficients respectively. SE: standard error of *B*; it is analogous to the standard deviation for a mean. Sig: significance of an individual independent predictor effect on the dependent variable. R^2: R-square, the square of β and is the correlation between the independent predictor and dependent variable.

between 25(OH)D and 1,25$(OH)_2$D in men PD patients, and the latter was only marginally and negatively correlated with UPDRS III ($r_s = -0.603$; $P = 0.086$), but positively with MoCA ($r_s = +0.607$; $P = 0.083$). (supplementary 4).

3.3. Regression analyses

3.3.1. Linear regression analysis

Table 2 shows the extent and direction of effect (SC β) of the predictors 25(OH)D, 1,25$(OH)_2$D, gender and disease duration on the scales reported for both MSA and PD. In MSA, 1,25$(OH)_2$D exerted significant effect ($P = 0.04$) on MMSE score, with USC coefficient $B = +0.112$ and SC β = +0.476 suggesting a corresponding increase of MMSE score for every unit (pg/mL) and one SD of 1,25$(OH)_2$D respectively. The R^2 value indicates that 1,25$(OH)_2$D shared positive 22.6% of the variation in MMSE. Moreover, the disease duration exerted significant effect ($P = 0.03$) on UMSARS II. The USC $B = +0.093$ indicates this unit increase of UMSARS II for every month-long disease duration; SC β = +0.482 indicates this SC increase of UMSARS II for every SC of the disease duration which appears to share positive 23% of the variation in UMSARS II. On the other hand, in case of PD every unit increase of 1,25$(OH)_2$D appears to lead to 0.241 decrease ($P = 0.024$) of UPDRS III, and every SC to 0.432 SC decrease of UPDRS III, with 18.7% share of 1,25$(OH)_2$D in UPDRS III variation. Moreover, every additional month of disease duration increases ($P = 0.022$) UPDRS with an additional 0.439 SC increase for every SC of the duration, and positive 19.3% share in UPDRS III variation. 1,25$(OH)_2$D is also positively associated with MoCA ($P = 0.005$) as its every unit increase elevates MoCA by 0.104 score, and its every SC increase leads to 0.528 SC increment in MoCA, with a 27.9% positive share in variation of MoCA. The gender predictor displayed no significant effect on the dependent parameters in either MSA or PD patients.

3.3.2. Univariate and multivariate logistic regression analyses

The strength and odd ratios of the predictors were evaluated for their contribution to differentiating the study groups compared to each other (Table 3). Univariate analysis of each predictor alone revealed no predictive value for the gender in all comparisons. On the other hand, for HS vs. MSA, univariate analysis detected the highest predictive value for age ($B = -0.196$; Wald = 24.372; OR = 0.822; $P = 0.0001$), followed by 25(OH)D ($B = -0.535$; Wald = 17.123; OR = 0.586; $P = 0.0001$), and 1,25$(OH)_2$D ($B = -0.089$; Wald = 17.912; OR = 0.915; $P = 0.0001$). Multiple regression showed that combination of age and 25(OH)D rendered either one no longer a significant independent predictor, whereas on combination of age and 1,25$(OH)_2$D their significant predictive values were maintained. When PD was compared to HS, it was found that in addition to age, 25(OH)D (but not 1,25$(OH)_2$D) displayed significant predicting strength ($B = -0.357$; Wald = 18.552; OR = 0.700; $P = 0.0001$), maintained on combination with age. Moreover, in case of MSA vs. PD, age ($P = 0.003$), 25(OH)D ($P = 0.023$) and 1,25$(OH)_2$D ($P = 0.0001$) displayed significant predictor property. Also, MMSE displayed a significant positive predictor capability when tested alone ($B = +0.444$; Wald = 6.448; OR = 1.559, $P = 0.011$), indicating that higher MMSE provides better distinction between MSA and PD. Moreover, H&Y was strong negative predictor when tested alone ($B = -2.869$; Wald = 8.207; OR = 0.057; $P = 0.004$) or with the other predictors ($B = -6.051$; Wald = 4.176; $P = 0.041$), but its OR decreased to 0.002.

Table 3
Logistic regression analyses for contribution of the individual and combined predictors to HS-MSA-PD categorization.

Group	Predictors	$B \pm$ SE	Wald	Sig.	O.R.
HS vs. MSA (Univariate)	Age	−0.196 ± 0.040	24.372	0.0001*	0.822
	Gender	−0.401 ± 0.430	0.866	0.352	0.670
	25(OH)D	−0.535 ± 0.129	17.123	0.0001*	0.586
	1,25$(OH)_2$D	−0.089 ± 0.021	17.912	0.0001*	0.915
HS vs. MSA (Multivariate)	Age	−0.766 ± 0.425	3.243	0.072	0.465
	25(OH)D	−2.131 ± 1.246	2.923	0.087	0.119
	Age	−0.269 ± 0.057	22.375	0.0001*	0.764
	1,25$(OH)_2$D	−0.163 ± 0.040	16.716	0.0001*	0.849
HS vs. PD (Univariate)	Age	−0.120 ± 0.037	10.697	0.001*	0.887
	Gender	−0.857 ± 0.482	3.160	0.075	0.424
	25(OH)D	−0.357 ± 0.083	18.552	0.0001*	0.700
	1,25$(OH)_2$D	0.028 ± 0.017	2.813	0.094	1.028
HS vs. PD (Multivariate)	Age	−0.144 ± 0.060	5.706	0.017*	0.866
	25(OH)D	−0.366 ± 0.092	15.861	0.0001*	0.694
	Age	0.119 ± 0.040	8.821	0.003*	1.127
	Gender	−0.457 ± 0.535	0.730	0.393	0.633
	25(OH)D	0.178 ± 0.078	5.151	0.023*	1.194
	1,25$(OH)_2$D	0.110 ± 0.027	16.178	0.0001*	1.117
MSA vs. PD (Univariate)	Disease duration	0.009 ± 0.006	2.089	0.148	1.009
	UMSARS II/ UPDRSIII	−0.014 ± 0.032	0.188	0.665	0.986
	H&Y	−2.869 ± 1.002	8.207	0.004*	0.057
	MMSE	0.444 ± 0.175	6.448	0.011*	1.559
	MOCA	0.261 ± 0.106	6.007	0.014*	1.298
MSA vs. PD (Multivariate)	Age	0.429 ± 0.239	3.233	0.072	1.536
	25(OH)D	−0.585 ± 0.371	2.483	0.115	0.557
	1,25$(OH)_2$D	0.419 ± 0.229	3.346	0.067	1521
	H&Y	−6.051 ± 2.961	4.176	0.041*	0.002
	MMSE	0.642 ± 0.643	0.996	0.318	1.899
	MOCA	−0.060 ± 0.401	0.022	0.881	0.942

Wald: reflects the strength (weight) of the predictor. *B*: is the slope of the regression, measures association between a predictor and an outcome, and represents rate of change in the dependent variable as independent changes. OR: is the odd (probability) ratio, obtained by exponentiation of the *B* coefficient. The OR > 1 represents the odds that an outcome will occur due to the relevant predictor, whereas OR < 1 is the likelihood of lower outcome. Gender: women vs. men.

3.3.3. Receiver operating characteristic (ROC) analysis

Table 4 shows that the independent predictors 25(OH)D and 1,25$(OH)_2$D and dependent (rating scales) predictors displayed distinct differentiating, diagnostic sensitivity. 25(OH)D displayed the highest sensitivity and Youden Index (YI) for differentiating HS vs. MSA (AUC = 0.982, $P = 0.0001$,sensitivity = 0.882,YI = 0.882) and HS vs. PD (AUC = 0.943, $P = 0.0001$, sensitivity = 0.902, YI = 0.791). On the other hand, when comparing MSA vs. PD, 1,25$(OH)_2$D showed the highest AUC (0.868, $P = 0.0001$, sensitivity =0.926 and YI =0.632).

The highest cut offs were detected for 1,25$(OH)_2$D in HS vs. MSA (41.05) and MSA vs. PD (40.90). For MSA vs. PD, H&Y had the highest AUC (0.865) and sensitivity (one against zero specificity). Fig. 3A shows that the AUCs of 25(OH)D and 1,25$(OH)_2$D are both above the diagonal

Table 4
Bivariate Receiver Operating Characteristic (ROC) analysis of the predictors and study groups.

Group	Predictor	AUC ± SE	Sig.	Cut off	Sensitivity	Specificity	Y.I.
HS vs MSA	25(OH)D	0.982 ± 0.011	0.0001*	12.95	0.882	1.000	0.882
HS vs MSA	1, 25$(OH)_2$D	0.795 ± 0.05	0.0001*	41.05	0.706	0.836	0.542
HS vs PD	25(OH)D	0.943 ± 0.027	0.0001*	16.70	0.889	0.902	0.791
HS vs PD	1, 25$(OH)_2$D	0.611 ± 0.065	0.097	58.89	0.556	0.689	0.245
MSA vs PD	25(OH)D	0.717 ± 0.066	0.004*	12.45	0.556	0.824	0.380
MSA vs PD	1, 25$(OH)_2$D	0.868 ± 0.044	0.0001*	40.90	0.926	0.706	0.632
MSA vs PD	H&Y	0.865 ± 0.056	0.0001*	3.50	1.000	0.579	0.579
MSA vs PD	MMSE	0.717 ± 0.082	0.013*	28.50	0.667	0.737	0.404
MSA vs PD	MOCA	0.751 ± 0.076	0.004*	24.50	0.741	0.789	0.530

AUC: area under the (ROC) curve; Sig.: significance of AUC. YI: Youden Index.

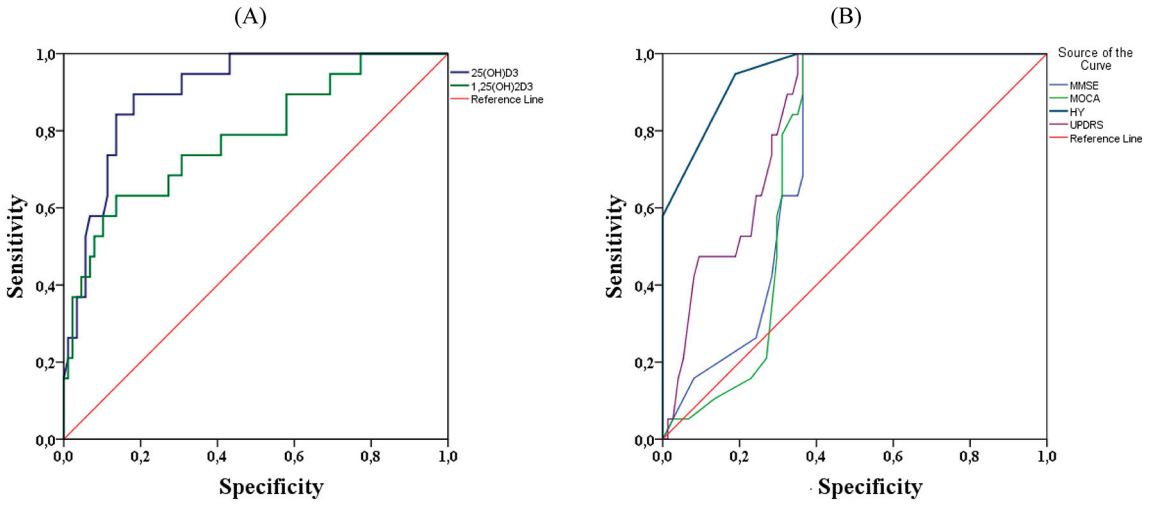

Fig. 3. Receiver-operating characteristic curve for 25(OH)D/1,25$(OH)_2$D (A) and assessment scales (B) applied to PD and MSA patients. The diagonal reference line represents the ROC curve at the 0.5 threshold (sensitivity = specificity).

reference line, but the AUC of 25(OH)D is much higher and closer to the upper-left corner, showing that 25(OH)D has a higher sensitivity, AUC and diagnostic strength than 1,25$(OH)_2$D. Furthermore, as compared to other scaling systems, H&Y had higher sensitivity and AUC that extended far above the reference line and close to the upper-left corner (Fig. 3B).

4. Discussion

In this study, we report differential reduction of 25(OH)D and 1,25$(OH)_2$D biomarkers, as well as changes in the scales systems in MSA and PD. In MSA, both biomarkers were lower than HS, whereas only 25(OH)D was lower in PD. The correlation, regression and prediction power of 25(OH)D, 1,25$(OH)_2$D, and rating scales to validate clinical diagnosis and distinguish between HS, MSA and PD were all different.

The pathological hallmark of PD is the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, as well as Lewy bodies, leading in a dopamine deficit in the basal ganglia. [21,22]. On the other hand, the accumulation of glial cytoplasmic and nuclear inclusions rich in α-synuclein in frontal and temporal regions of demented MSA patients [23] points to cognitive deterioration as a common trait in some MSA patients [24]. There is a growing body of evidence suggesting vitamin D's significance in brain development, cognition, and involvement in neurological disorders including PD. Low 25(OH)D level have been linked to neuronal injury [25], an increased risk of dementia [26], and motor impairment [27].

Vitamin D (cholecalciferol) is a seco-steroid hormone that is generated in the skin by UV irradiation of 7-dehydrocholesterol, transported to the liver by vitamin D binding protein, and hydroxylated to 25(OH)D by CYP2R1 in the endoplasmic reticulum. 25(OH)D is the major form of vitamin D in circulation, and when transported to the kidney it is converted to the active form 1,25$(OH)_2$D by mitochondrial CYP27B1 in the proximal tubules. It's worth noting that concentration of 1,25$(OH)_2$D is unrelated to that of its precursor (25(OH)D). This isn't unexpected, and it could be owing to their different kinetics and regulation, as well as the fact that the relationship between 25(OH)D (pre-hormone) and 1,25$(OH)_2$D (adaptive hormone) is far from that between a substrate and its product. Furthermore, 1,25$(OH)_2$D can be produced outside of the kidneys under endocrinological control, and it is synthesized, controlled, and converted in different ways in a variety of diseases. While a high vitamin D dose increases 25(OH)D levels in the blood, 1, 25$(OH)_2$D levels decrease as vitamin D doses increase. This could be owing to the fact that 1,25$(OH)_2$D has a negative feedback effect on CYP27B1, lowering the synthesis of both its own and its precursor 25(OH)D [28].

Vitamin D levels in the blood are linked to its concentration and activity in the brain [29]. Both vitamin D receptors (VDR) and the enzyme that converts 25(OH)D to active vitamin D (1,25$(OH)_2$D) is detected in the brain [30,31], particularly in the hypothalamus and dopaminergic neurons of the substantia nigra [32]. Additionally, the activated microglial cells can also convert 25(OH)D to 1,25$(OH)_2$D [33]. Although both 25(OH)D and 1,25$(OH)_2$D are ligands for VDR, 1,25$(OH)_2$D has hundreds of times higher affinity for the receptor [34]. Low 25(OH)D levels have been linked to higher overall UPDRS scores in PD [35], whereas higher plasma 25(OH)D levels have been linked to better cognition [36] and motor function [37]. One of the possible causes of low 25(OH)D is insufficient sun exposure [39]. This notion, on the other hand, may seem reasonable in immobile patients with advanced stages of MSA or PD who have been diagnosed for a long time. In this study, we detected lower 25(OH)D and 1,25$(OH)_2$D in MSA, but only lower 25(OH)D in PD (relative to HS). The latter result is in line with the lower 25

(OH)D levels seen in PD [38]. We previously observed a connection between low serum 25(OH)D levels and MMSE in Alzheimer's disease and Mild Cognitive Impairment [16].

The lipophilicity of 25(OH)D and its high circulating level (>500 times higher than that of $1,25(OH)_2D$) may explain the greater association of 25(OH)D levels with PD. This facilitates neuronal uptake of 25 (OH)D, increase of intracellular glutathione concentration [40], and induction of tyrosine hydroxylase, dopamine synthesis, and protection of dopaminergic neurons [41], as well as reduction of microglia-mediated neuroinflammation and oxidative stress in PD [42,43]. As a result, it's possible that if the patient's 25(OH)D level drops, the patient will be deprived of 25(OH)D's above-mentioned activities, and the dopaminergic neurons will be damaged, potentially leading to the development of PD or worsening of an existing disease.

It has been reported that there is no correlation between 25(OH)D and the cognitive performance [44]. In this study, 25(OH)D was correlated with UMSARS II in MSA, however there was no correlation between 25(OH)D and MMSE or MoCA in PD patients. In PD, the MMSE and MoCA scores were greater than in MSA. Low serum $1,25(OH)_2D$ levels in MSA could be the result of malfunction of the conversion pathway from 25(OH)D to $1,25(OH)_2D$ (CYP27B1 malfunction) or an exacerbated negative feedback loop on its synthesis with no ability to recover, whereas in PD, the pathway could restore the feed-back system between 25(OH)D and $1,25(OH)_2D$ and keep serum $1,25(OH)_2D$ at normal levels. It's worth noting that in MSA but not PD, $1,25(OH)_2D$ was reduced and correlated with MMSE. This finding could account for the rapid deterioration of MSA, higher H&Y but lower MMSE and MoCA in MSA as compared to PD. It could also mean that targeting $1,25(OH)_2D$ for MSA treatment could be beneficial therapeutic approach. $1,25(OH)_2D$ and H&Y could also be conjugated with other biomarkers for MSA diagnosis and differentiation from PD such as α-synuclein, neurofilament light-chain protein, and total tau contents of glial cytoplasmic inclusions [45].

The UPDRS III is the most reliable and frequently used scale for determining the severity of PD. It predicts physical performance measures with a significant balance component. On the other hand, there has been a notable dearth of specific validated measures to assess functional impairment and disability in MSA, as well as to compare MSA to PD. The UPDRS III does not account for MSA's complicated motor dysfunction. UMSARS II, on the other hand, is a multimodal scale that includes sections for both impairment and disability. It was developed to encompass all features of MSA, including motor impairment (Part II). This section is unique to MSA and correlates with motor but not non-motor elements of the disease [13]. Both scales had a high weight in predicting MSA or PD, although the correlations to 25(OH)D and $1,25(OH)_2D$ were different. We found that UMSARS II was correlated with 25(OH)D, whilst UPDRS III was found to be correlated with $1,25(OH)_2D$. When data from women and men were separately evaluated, it was found that UMSARS II in MSA was correlated with $1,25(OH)_2D$ in women and both 25(OH)D and $1,25(OH)_2D$ in men, but UPDRS III was found to be non-significantly correlated with $1,25(OH)_2D$ in PD. There was no statistical difference between UMSARS II and UPDRS III in MSA and PD respectively. This could be owing to the scales' specificity for MSA or PD, or the stages of MSA and PD.

Vitamin D supplementation has been shown to have an inverse relationship with PD [27] and to reduce the worsening of the H&Y and the UPDRS III in PD patients [46]. H&Y was higher in MSA than PD in this study, and it was correlated with 25(OH)D in both MSA and PD, but the correlation was eliminated when the genders were separated. This finding indicates that H&Y has a higher relative diagnostic and distinguishing value, as well as the necessity of taking gender into account when clinically evaluating rating scales. There is a gender difference in the progression and clinical aspects of Parkinson's disease. Men are twice as likely as women to get Parkinson's disease [47]. Women also have diverse symptoms and respond to pharmacological treatments differently than men [48]. However, no gender-related effect was seen in this study for 25(OH)D, $1,25(OH)_2D$, or the scales, but women's MMSE scores were higher than men's, but not significantly.

5. In conclusion

25(OH)D levels were lower in MSA and PD patients compared to HS, whereas $1,25(OH)_2D$ levels were solely lower in MSA patients. There was no difference between the genders, but there was a difference within the genders. H&Y and 25(OH)D were found to be highly relevant and differentiating. Low MMSE and MoCA, as well as high H&Y, distinguish MSA and PD, and could be used in conjunction with 25(OH)D and $1,25(OH)_2D$ biomarkers.

Author statement

All of the authors have approved the revised manuscript and agree with re-submission to your esteemed journal.

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Appendix A. Supplementary data

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